CD8⁺ T Cells Specific for Immunodominant Trans-Sialidase Epitopes Contribute to Control of Trypanosoma cruzi Infection but Are Not Required for Resistance

Charles S. Rosenberg, Diana L. Martin and Rick L. Tarleton

J Immunol 2010; 185:560-568; Prepublished online 7 June 2010;
doi: 10.4049/jimmunol.1000432
http://www.jimmunol.org/content/185/1/560
CD8+ T Cells Specific for Immunodominant Trans-Sialidase Epitopes Contribute to Control of Trypanosoma cruzi Infection but Are Not Required for Resistance

Charles S. Rosenberg,*† Diana L. Martin,*‡,1 and Rick L. Tarleton*‡

CD8+ T cells are essential for controlling Trypanosoma cruzi infection. During Brazil strain infection, C57BL/6 mice expand parasite-specific CD8+ T cells recognizing the dominant TSKB20 (ANYKFTLV) and subdominant TSKB74 (VNYDFTLV) trans-sialidase gene (TS)-encoded epitopes with up to 40% of all CD8+ T cells specific for these epitopes. Although this is one of the largest immunodominant T cell responses described for any infection, most mice fail to clear T. cruzi and subsequently develop chronic disease. To determine if immunodominant TS-specific CD8+ T cells are necessary for resistance to infection, we epitope-tolerized mice by high-dose i.v. injections of TSKB20 or TSKB74 peptides. Tolerance induction led to deletion of TS-specific CD8+ T cells but did not prevent the expansion of other effector CD8+ T cell populations. Mice tolerized against either TSKB20 or TSKB74, or both epitopes simultaneously, exhibited transient increases in parasite loads, although ultimately they controlled the acute infection. Furthermore, BALB/c mice tolerized against the TSDK14 peptide effectively controlled acute T. cruzi infection. These data are consistent with the hypothesis that development of high-frequency CD8+ T cell populations focused on TS-derived epitopes contributes to optimal control of acute infection but is not required for the development of immune resistance. The Journal of Immunology, 2010, 185: 560–568.
unclear what the selective advantage for expansion of the TS gene family is if they provide numerous targets for adaptive immunity (20, 21). Some have proposed that TS genes participate in immune evasion, promoting the chronic nature of T. cruzi infection (12, 17, 19, 22–26). The strong immunodomination by TS-derived epitopes results in the out-competition of other epitope-specific CD8+ T cell populations. However, the significance of the tight focusing of the CD8+ T cell response on only a few of the vast array of possible parasite-derived epitopes is not known.

Herein, we explore the role of immunodominant CD8+ T cells in immune resistance to T. cruzi infection. Although infection with this parasite elicits one of the strongest immunodominant CD8+ T cell responses documented, in vivo and in vitro studies indicate that the focus of the adaptive immune response to T. cruzi infection is remarkably plastic.

Materials and Methods

Mice and parasites

C57BL/6 and BALB/c mice were obtained from the National Cancer Institute at Frederick (Frederick, MD) and kept under specific pathogen-free conditions at the Coverdell Center animal facility (University of Georgia, Athens, GA). For T. cruzi infections, 8-wk-old female mice were infected i.p. with 1 × 10^5 trypomastigotes of the Brazil strain. Tryptamastigotes were maintained in tissue culture by serial passage through Vero cells. Mice were euthanized by CO2 inhalation. The University of Georgia of Institutional Animal Care and Use Committee approved all of the animal use protocols.

Peptide treatments

Peptides were synthesized by SigmaGenosys (St. Louis, MO) or GenScript (Piscataway, NJ). Peptides used were the H-2Kb–restricted TSKB20 (ANYKFTLV), TSKB74 (VNYDFTLV), and OVA257–264 (SIINFEKL) peptides or the H-2Kd–restricted peptides TSKD14 (IYNVGQVSI) and listeriolysin O peptide (LLO37–46) (GYKDGNEYI). Lyophilized peptide was suspended in DMSO at a concentration of 100 mg/ml and stored at −20°C. Stock peptide was diluted to the desired concentration in sterile saline (PBS) for i.v. injection (each mouse received 10 μl per injection). Peptide-treated mice initially received 300 μg peptide on day −7 and 100 μg on days −4 and −1. Mice were infected on day 0 and injected with 100 μg peptide weekly until the end of the experiment. An equal quantity of peptide was injected whether mice received one or two peptides simultaneously. Tolerized mice were sacrificed 7 d after final peptide treatment.

T cell phenotyping

For ex vivo lymphocyte phenotyping, spleens were removed and dissociated by rubbing between two glass slides in a medium of hypotonic ammonium chloride to lyse RBCs. Cell numbers were determined on a Z2 Coulter particle count and size analyzer (Beckman Coulter, Fullerton, CA). A total of 5 × 10^6 washed splenocytes were suspended for staining in PBS with 1% BSA and 0.05% sodium azide (both from Sigma-Aldrich, St. Louis, MO), TSKB20/Kb, TSKB74/Kb, and TSKD14/Kd tetramers were synthesized at the Tetramer Core Facility (Emory University, Atlanta, GA) and were labeled with PE (Molecular Probes, Carlsbad, CA). Abs used were CD8 Pacific Blue, CD4 PE-Cy5, CD127 PE-Cy7, FoxP3 PE (eBioscience, San Diego, CA), CD11b PE-Cy5, B220 PE-Cy5, CD25 allophycocyanin (CalTag Laboratories, Burlingame, CA), CD44 allophycocyanin, CD11a FITC, and CD62L PE-Cy5 (BD Biosciences, San Jose, CA). Cells were stained at 4°C for 30 min, washed with PBS with 1% BSA and 0.05% sodium azide and fixed in 2% formaldehyde. The eBioscience intracellular staining kit was used for FoxP3 staining. At least 500,000 cells were collected for each sample on a CyAn ADP using Summit, version 4.3.

FIGURE 1. Repetitive i.v. administration of peptide depletes epitope-specific CD8+ T cells during acute T. cruzi infection. A. Protocol used to induce epitope-specific tolerance using high-dose peptide administration. Mice were injected with 100 μl peptide on indicated days before and after infection with 1000 Brazil trypomastigotes. The primary injection was with 300 μg peptide, and 100 μg was administered in the following treatments. Tolerized mice were allowed to rest for 7 d after final treatment before the experimental endpoint. Spleens of peptide-treated mice were assayed for the presence of epitope-specific CD8+ T cells at 21 d postinfection with Brazil strain T. cruzi. B. Splenocytes from OVA257–264-, TSKB20-, and TSKB74-treated B6 mice were stained for CD4 and TSKB20/Kb or TSKB74/Kb tetramers. Histograms are gated on CD8+ cells that were CD4−CD11b− B220+. Numbers indicate percentage of tetramer+ cells of total CD8+ T cells. Data are from individual mice and are representative of five experiments. C. D. The total numbers of TSKB20/Kb (C) or TSKB74/Kb (D) CD8+ T cells per spleen were calculated. Data are mean ± SEM and are cumulative from three separate experiments (n = 4−11 per group). *p < 0.05 compared with OVA Tx group.
T cell stimulation and intracellular cytokine staining

A total of $1.5 \times 10^6$ splenocytes were stimulated in 96-well round-bottom tissue culture plates (Costar, Corning, NY) at 37˚C for 5 h in the presence of 1 μM peptide and brefeldin A. A, Representative intracellular IFN-γ staining at 21 d postinfection. Histograms are gated on CD8+ CD4− lymphocytes and numbers indicate percentage of cytokine-producing CD8+ T cells. Data are from individual mice and are representative of five experiments. B and C, The percentage of CD8+ T cells producing IFN-γ in response to TSKB20 (B) or TSKB74 (C) peptide stimulation over the course of acute infection. Data are mean ± SEM from one experiment (n = 4–5 per group) and are representative of five experiments. *p < 0.05 compared with OVA Tx group. D, Naive splenocytes were pulsed with 1 μM TSKB20, 1 μM TSKB74, or no peptide and then labeled with high, medium, or low concentrations of CFSE, respectively. At 28 d postinfection, equal numbers of each population were cotransferred i.p. into mice then detected in the spleens at 16 h. Numbers indicate the percentage of specific lysis measured for individual mice. E, Data are mean ± SEM and are cumulative of two in vivo CTL experiments (n = 6–7 per group). *p < 0.05 compared with OVA Tx group.

In vivo cytotoxicity assay

Spleen cells from naive mice were incubated for 1 h at 37˚C with 10 μM peptide or media alone and then labeled with different concentrations of CFSE (Molecular Probes) as described (8) to produce CFSE high, medium, and low populations. Equal numbers of CFSE-labeled cells were transferred i.p. into recipients, and after 16 h, splenocytes were isolated and CFSE-labeled cells were detected by flow cytometry. Percentage of specific killing was determined using the equation: $1 - (\frac{\text{CFSE}_{\text{high}} \text{ naive}}{\text{CFSE}_{\text{medium}} \text{ naive}}) \times (\frac{\text{CFSE}_{\text{high}} \text{ infected}}{\text{CFSE}_{\text{medium}} \text{ infected}}) \times 100\%$.

Real-time PCR

Mouse hind leg muscles were collected, and popliteal lymph nodes were removed as well as extraneous adipose tissue prior to DNA extraction as described (27). Extracted DNA was analyzed by real-time PCR essentially as described (27). PCR reactions consisted of IQ SYBR Green Supermix (Bio-Rad, Hercules, CA) and primers specific for T. cruzi or mouse genomic DNA (27). An iQ5 Multi-Color Real-Time PCR Detection System was used with iQ5 Standard Edition Optical System software, version 2 (both Bio-Rad). T. cruzi equivalents were calculated as the quantity of T. cruzi satellite DNA divided by the quantity of mouse TNF-α DNA in each sample.

Statistical analysis

Statistical significance was calculated using a two-tailed Student t test.

Results

Repetitive i.v. administration of peptide results in depletion of epitope-specific CD8+ T cells during acute T. cruzi infection

To determine the importance of immunodominant TS peptide-specific CD8+ T cell responses in T. cruzi infection, we first developed a system in which immunodominant TS-specific CD8+ T cell responses were ablated. Because the TSKB20 and TSKB74 epitopes (and cross-reactive epitopes) are encoded by numerous TS genes (>200 in the case of TSKB20/21 (8)), it was not feasible to generate gene knockout parasites that do not express either Ag. We instead induced immune tolerance to these epitopes by administration of high doses of soluble peptide i.v. (Fig. 1A), a method previously used to induce and maintain epitope-specific tolerance in models of viral infection (28–32).
Depletion of tetramer$^+$ CD8$^+$ T cells by repetitive peptide treatment also resulted in epitope-specific immune tolerance as assessed by the failure to produce IFN-γ in response to stimulation with peptide ex vivo (Fig. 2A–C). Additionally, both TSKB20- and TSKB74-tolerized mice were deficient in peptide-specific cytotoxicity in vivo at 28 d postinfection (Fig. 2D, 2E). Compared to OVA257–264-treated mice, TSKB20-treated mice killed the majority of TSKB74-pulsed target cells but not TSKB20-pulsed targets, and TSKB74-treated mice efficiently killed most TSKB20-loaded targets but not TSKB74-loaded targets (Fig. 2D, 2E). Although peptide-treated mice exhibited essentially background levels of tolerizing epitope-specific CD8$^+$ T cells (Figs. 1, 2A–C), low levels of tolerizing epitope-specific cytotoxicity were apparently maintained in vivo (Fig. 2D, 2E). Nevertheless, this residual killing was less than that previously observed for very subdominant $T. cruzi$-specific CD8$^+$ T cells (8, 37). Notably, CD8$^+$ T cells from OVA257–264-treated mice produced IFN-γ after stimulation with TSKB20 or TSKB74 peptide but did not produce IFN-γ after stimulation with OVA257–264 peptide (Fig. 2A), indicating that the peptide treatment does not stimulate T cell priming in the face of infection-induced inflammation. From these experiments, we conclude that peptide treatments prevented the normally robust expansion of functional CD8$^+$ T cells specific for the tolerizing peptide.

**Peptide-tolerized mice lack epitope-specific CD8$^+$ T cell effector functions**

Although the evidence above indicates that the decreased tetramer$^+$ populations and functions were epitope-specific, we considered the possibility that the high dose of peptide administered could induce enhanced regulatory T cell (Treg) populations capable of general immune suppression. Approximately 15% of splenic CD4$^+$ T cells in naive mice expressed the FoxP3 transcription factor, whereas the proportion of Tregs decreased in spleens after $T. cruzi$ infection (Fig. 3A, 3B). Importantly, the proportion of Tregs in spleens of all peptide-treated groups were similar over the course of acute infection (Fig. 3B), indicating that tolerization with $T. cruzi$-derived epitopes did not lead to an atypical expansion of Tregs.

**Peptide-induced tolerance does not enhance Treg populations**

At the peak of the T cell response (~3 wk postinfection; Ref. 8 and data not shown), TSKB20- and TSKB74-specific CD8$^+$ T cells in the spleens of mice injected with the respective peptides were nearly undetectable (Fig. 1B). Infected control mice injected with the irrelevant OVA257–264 peptide had normal proportions of TSKB20 and TSKB74 tetramer$^+$ CD8$^+$ T cells compared with infected mice treated with PBS + DMSO or nontreated mice (data not shown). Importantly, TSKB20-tolerized mice had a normal complement of TSKB74 tetramer$^+$ CD8$^+$ T cells, and TSKB74-injected mice had TSKB20 tetramer$^+$ CD8$^+$ T cells (Fig. 1B), demonstrating that depletion via TS-derived peptides was epitope-specific and did not prevent priming of other CD8$^+$ T cells specific for homologous TS epitopes. Peptide treatments effectively prevented expansion of peptide-specific T cells throughout the course of acute infection (Fig. 1C, 1D). Interestingly, spleens of TSKB20-treated mice had significantly more TSKB74 tetramer$^+$ CD8$^+$ T cells at the peak of expansion compared with OVA257–264-treated mice (Fig. 1D). A similar compensation in immunodominance hierarchies has been noted in virus infection with epitope-loss variants and deletion mutants (33–36) and suggests that $T. cruzi$-specific CD8$^+$ T cells with alternative specificities expand in the absence of competition by the normally dominant TSKB20-specific CD8$^+$ T cell population.

**FIGURE 3.** Peptide-induced tolerance does not enhance Treg populations. Tolerized mice were monitored for Treg populations (A, B) as well as IL-10 production (C) during infection. A, Representative intracellular staining for the FoxP3 transcription factor at 21 d postinfection. Histograms are gated on CD4$^+$ and were costained with CD25. Numbers in parentheses correspond to the percentage of cells in each quadrant. B, The percentage of FoxP3-expressing CD4$^+$ T cells in spleens over the course of infection. Data points are for individual mice and are mean ± SEM from two experiments (n = 3–12 per group). C, Splenocytes were incubated for 5 h in media alone or with plate-bound mouse CD3 mAb in the presence of brefeldin A. Histograms are gated on CD4$^+$ CD8$^+$ cells, and numbers indicate the percentage of IL-10−/and/or IFN-γ−positive cells per quadrant. Data are from individual mice and are representative of three experiments.

**FIGURE 4.** Mice tolerized against immunodominant $T. cruzi$ epitopes control acute infection. The quantity of $T. cruzi$ DNA in skeletal muscle of peptide-treated mice was detected at indicated time points by real-time PCR. Data points are for individual mice and bars are the means from five experiments (n = 11–17 per group). Several individuals were removed from the analysis because they passed the Grubbs’ outlier test (GraphPad Software, La Jolla, CA). *p < 0.05.
Production of IL-10 by CD4+ T cells has also been shown to suppress CD8+ T cell function during infections (38). Polyclonally stimulated CD4+ T cells from infected peptide-treated mice exhibited robust IFN-γ production, whereas few IL-10-producing CD4+ T cells were detectable (Fig. 3C). Both OVA257–264 and TS peptide-treated mice maintained similar populations of cytokine-producing CD4+ T cells during acute infection (Fig. 3C and data not shown). Furthermore, stimulation of splenocytes with TSKB20 or TSKB74 peptides in vitro failed to elicit IL-10 production irrespective of the in vivo peptide treatment (data not shown). Thus, we found no evidence that peptide-induced T cell tolerance was due to extrinsic T cell regulation and conclude that it likely was the result of deletion of peptide-specific CD8+ T cells.

**Mice tolerated against immunodominant T. cruzi epitopes control acute infection**

*T. cruzi* Brazil strain-infected mice genetically deficient for, or depleted of, CD8+ T cells exhibit uncontrolled parasitemia and mortality by approximately 1 mo postinfection (39–41). However, neither TSKB20- nor TSKB74-tolerized mice deficient in the respective immunodominant T cell population succumbed to acute infection of up to 35 d. Because skeletal muscle is a site of *T. cruzi* persistence in this model, we measured parasite load in muscle to determine the quality of immune control of *T. cruzi* infection in tolerated mice. The level of parasites, as measured by real-time PCR, was similar between peptide-treated groups of mice throughout infection (Fig. 4), with the exception that TSKB74-treated mice had more parasites at day 21 postinfection compared with control OVA257–264-treated mice (*p* = 0.03) (Fig. 4). Although several TSKB20-tolerized mice had elevated numbers of parasites at 21 d postinfection, the group average was not statistically different (*p* = 0.4) when compared with that of OVA257–264-treated mice. We observed slightly greater cellular infiltration as well as parasitized host cells in muscle sections of individual tolerated mice exhibiting increased parasite loads (data not shown), further suggesting that depleting TSKB20- or TSKB74-specific CD8+ T cells can have a negative, though minor, impact on control of infection. Ultimately, both TS peptide-tolerized groups controlled parasite loads similar to OVA257–264-treated mice (Fig. 4), demonstrating that immune control of *T. cruzi* infection occurs despite the absence of the normal immunodominant CD8+ T cell population.

**Tolerized mice generate protective effector CD8+ T cell responses despite the absence of immunodominant CD8+ T cells**

Because the TSKB20- and TSKB74-specific CD8+ T cells can represent as much as 40% of the total *T. cruzi*-specific CD8+ T cell population in infected mice (8), we next assessed the effect of depleting the immunodominant T cells on the overall size of the responding CD8+ T cell population bearing an activated phenotype. Most CD8+ T cells in a naive spleen express the lymph node homing receptor, CD62L, and the IL-7Rα-chain, CD127, but very few of these naive T cells have an Ag-experienced phenotype (CD44hi CD11ahi) (Fig. 5A). Spleens of *T. cruzi*-infected mice genetically deficient for, or depleted of, CD8+ T cells exhibit uncontrolled parasitemia and mortality by approximately 1 mo postinfection (39–41). However, neither TSKB20- nor TSKB74-tolerized mice deficient in the respective immunodominant T cell population succumbed to acute infection of up to 35 d. Because skeletal muscle is a site of *T. cruzi* persistence in this model, we measured parasite load in muscle to determine the quality of immune control of *T. cruzi* infection in tolerated mice. The level of parasites, as measured by real-time PCR, was similar between peptide-treated groups of mice throughout infection (Fig. 4), with the exception that TSKB74-treated mice had more parasites at day 21 postinfection compared with control OVA257–264-treated mice (*p* = 0.03) (Fig. 4). Although several TSKB20-tolerized mice had elevated numbers of parasites at 21 d postinfection, the group average was not statistically different (*p* = 0.4) when compared with that of OVA257–264-treated mice. We observed slightly greater cellular infiltration as well as parasitized host cells in muscle sections of individual tolerated mice exhibiting increased parasite loads (data not shown), further suggesting that depleting TSKB20- or TSKB74-specific CD8+ T cells can have a negative, though minor, impact on control of infection. Ultimately, both TS peptide-tolerized groups controlled parasite loads similar to OVA257–264-treated mice (Fig. 4), demonstrating that immune control of *T. cruzi* infection occurs despite the absence of the normal immunodominant CD8+ T cell population.

**Tolerized mice generate normal effector CD8+ T cell populations despite the absence of immunodominant CD8+ T cells.** Spleens of peptide-treated mice were assayed for the presence of Ag-experienced effector CD8+ T cells. A, Representative staining for CD44 and CD11a expression at 21 d postinfection. Histograms are gated on CD8+ cells that were CD4+ CD11b− B220−. Similar results were obtained in three separate experiments. B, The total number of CD44hi CD11ahi CD8+ T cells per spleen was calculated. Data are mean ± SEM from one experiment (n = 4–5 per group). *p* < 0.05 compared with the OVA Tx group. C, Representative staining for CD62L and CD127 at 21 d postinfection. Histograms are gated on CD8+ cells that were CD4+ CD11b− B220−. Numbers indicate the percentage of CD8+ T cells that have lost (left) or retained (right) expression of each marker (bold lines). Shaded lines are a fluorescence-minus-one control for the indicated marker. Similar results were obtained in three separate experiments. D, Splenocytes were incubated for 5 h in media alone or with plate-bound mouse CD3 mAb in the presence of brefeldin A at 35 d postinfection. Data are the mean (± SEM) percentage of CD8+ T cells producing IFN-γ for each condition (n = 3–17 per group from three separate experiments). The percentage of CD8+ T cells capable of producing IFN-γ was also similar between groups at 14, 21, and 28 d postinfection (data not shown).
B6 mice tolerized simultaneously against both TSKB20 and TSKB74 (labeled as TS treated [tx]) showed a predictable decrease in both TSKB20- and TSKB74-specific CD8+ T cells (Fig. 6A, 6B) but interestingly had increased numbers of activated (CD44 hi CD11ahi) CD8+ T cells (Fig. 6C) and a greater percentage of CD8+ T cells capable of producing IFN-γ in response to CD3 stimulation (Fig. 6D). Few activated CD8+ T cells had decreased expression of CD3 or TCR β-chain in either control or TS peptide-tolerized mice (data not shown), excluding the possibility that the CD44hi CD11ahi CD8+ T cell population consisted of expanded TSKB20- or TSKB74-specific cells that were undetected by tetramer staining due to downregulation of the TCR. Furthermore, the TS-tolerized mice had more tissue parasites at the peak of infection (p = 0.018 at 21 d postinfection), although they had effectively controlled their parasite load by 28 d postinfection (Fig. 6D). Thus, TSKB20- and TSKB74-specific CD8+ T cells are required for optimal control of T. cruzi at the peak of the infection, but other CD8+ T cells of unknown specificity can substitute to eventually contain the acute infection.

B6 mice tolerized simultaneously against both TSKB20 and TSKB74 (labeled as TS treated [tx]) showed a predictable decrease in both TSKB20- and TSKB74-specific CD8+ T cells (Fig. 6A, 6B) but interestingly had increased numbers of activated (CD44 hi CD11ahi) CD8+ T cells (Fig. 6C) and a greater percentage of CD8+ T cells capable of producing IFN-γ in response to CD3 stimulation (Fig. 6D). Few activated CD8+ T cells had decreased expression of CD3 or TCR β-chain in either control or TS peptide-tolerized mice (data not shown), excluding the possibility that the CD44hi CD11ahi CD8+ T cell population consisted of expanded TSKB20- or TSKB74-specific cells that were undetected by tetramer staining due to downregulation of the TCR. Furthermore, the TS-tolerized mice had more tissue parasites at the peak of infection (p = 0.018 at 21 d postinfection), although they had effectively controlled their parasite load by 28 d postinfection (Fig. 6D). Thus, TSKB20- and TSKB74-specific CD8+ T cells are required for optimal control of T. cruzi at the peak of the infection, but other CD8+ T cells of unknown specificity can substitute to eventually contain the acute infection.

**Discussion**

Adaptive immunity to intracellular pathogens depends on CD8+ T cell recognition of host cells presenting foreign Ag. The consequences of Ag-specific CD8+ T cell responses focusing on a restricted versus broader set of pathogen-derived epitopes is not fully understood. The issue of immunodominance is particularly complicated for understanding immune control of protozoan parasites potentially presenting an expansive set of antigenic determinates, especially in comparison with viral pathogens where immunodominance has been extensively investigated. In this study, we addressed the role that immunodominant CD8+ T cells play in host defense against T. cruzi infection.

**FIGURE 6.** Mice tolerant to both TSKB20 and TSKB74 are ultimately resistant to T. cruzi infection. B6 mice were injected with both TSKB20 and TSKB74 peptides (TS Tx) similar to the description in Fig. 1A. TS Tx mice received the same total quantity of peptide as OVA Tx mice (i.e., half the effective dose of each individual peptide compared with experiments described in Figs. 1–5). A, Representative tetramer staining at 21 d postinfection. Histograms are gated on CD8+ cells that were CD4+ CD11b- B220-. Numbers indicate percentage of tetramer+ cells out of CD8+ T cells. Data are from individual mice and are representative of five experiments. B, Representative intracellular staining for IFN-γ produced in response to the indicated stimulus (see Materials and Methods). Histograms are gated on CD8+ CD4+ cells. Numbers indicate percentage of IFN-γ+ CD8+ T cells. C, The total number of CD44hi CD11ahi CD8+ T cells per spleen was calculated. Data are mean ± SEM from one experiment (n = 3–5 per group). *p < 0.05 compared with the OVA Tx group (same control individuals as in Fig. 5B). D, Quantity of T. cruzi DNA in skeletal muscle of peptide-treated mice detected by real-time PCR. Data points are individual mice, and bars are the means from two experiments (n = 5–8 per group). One individual outlier was removed from the analysis. *p < 0.05.
resistance to *T. cruzi* infection by ablating epitope-specific T cells via administering high doses of peptide. We achieved significant epitope-specific tolerance against the dominant TSKB20 and subdominant TSKB74 peptides in H-2Kb-restricted B6 mice and the dominant TSKD14 peptide in H-2Kd-restricted B6 mice during acute *T. cruzi* Brazil strain infection. B6 mice tolerized to TSKB20, TSKB74, or both epitopes simultaneously, exhibited modest and transitory increases in parasite load, suggesting that these greatly expanded T cell populations contribute to control of *T. cruzi*. Although these immunodominant TS-specific CD8+ T cells represent a significant portion of the parasite-specific response, deleting them during infection ultimately had minor consequences for the outcome of infection; thus, they are not required for the acute resistance provided by the adaptive immune response to *T. cruzi*.

Immunodominant CD8+ T cells are implicated as important for control of intracellular pathogens because they represent a majority of the responding T cell pool in circulation and at sites of infection. Attempts to determine the necessity of immunodominant T cells have often relied upon experimental infections with natural mutants or engineered viruses and bacteria that lack the epitope of interest (34–36, 43–49). Tolerance induction has also been used as a means of depleting mice of epitope-specific CD8+ T cells during viral infection (30, 31, 50–55). In some cases, loss of the immunodominant CD8+ T cell population impaired viral control (31, 34, 36, 44, 47, 50–54) or enhanced disease without affecting viral load (34), whereas in other situations pathogen load was not affected (30, 43, 46, 49) or disease manifestations were ameliorated (30, 47, 52, 55). Compensation in the dominance hierarchy in the absence of immunodominant T cells occurred in many of these infection models (34–36, 43, 46–48), although often T cells recognizing minor epitopes emerge instead of enhanced subdominant responses (35, 36, 46, 47). Thus, elimination of immunodominant CD8+ T cell responses has variable results depending on the infection model employed and is not readily predictable.

The observation that TS peptide-tolerized mice are resistant to acute *T. cruzi* infection directed us to question which parasite-derived peptides protective CD8+ T cells respond to in the absence of the normally dominant responses. In our studies, both B6 and BALB/c mice depleted of the previously identified immunodominant TS-specific T cells expanded effector CD8+ T cell populations to a similar level as control mice. These responding effector CD8+ T cells had an Ag-experienced CD44hi CD11ahigh phenotype and rapidly produced IFN-γ in response to stimulation with anti-CD3 Abs, whole *T. cruzi* lysate, or *T. cruzi*-infected dendritic cells (C.S. Rosenberg, unpublished observations). However, we were unable to identify the Ag specificity of these compensating T cells in screens against previously predicted CD8+ T cell targets (8) in either mouse strain (C.S. Rosenberg, unpublished observations). A broader screen for epitope-specific responses will help to identify the focus of compensating CD8+ T cells in TS peptide-tolerized mice, although *T. cruzi*’s large proteome (>12,000 genes) may preclude a full description of all of the epitopes recognized in the mouse model.

Comparison of the reference genomes for the related trypanosomatids *T. cruzi*, *Trypanosoma brucei*, and *Leishmania major* revealed massive expansion in several gene families encoding surface proteins uniquely in *T. cruzi* (12, 56). Because surface-
expressed or secreted proteins are excellent sources of epitopes for both B cell and T cell recognition (57, 58), it is hypothesized that these large gene families have expanded because of immune selective pressure (26) and likely are involved in immune evasion (19, 59, 60). The TS gene family has drastically expanded to represent upwards of 6% of the annotated \textit{T. cruzi} CL Brener genome (12), and this may underestimate by half the true number of full and partial TS sequences (D.B. Weatherly, manuscript in preparation). Several hundred TS genes encode epitopes recognized by TSKB20-specific CD8+ T cells (8), and many of these gene products are represented in the proteome of the mammalian-dwelling stages of \textit{T. cruzi} Brazil strain (22). Furthermore, distinct strains likely have distinct sets of TS genes (8), resulting in strain-variant immunodominance patterns (8, 13, 61). The benefit of carrying within an otherwise fairly compact genome a large number of genes encoding related but variable surface proteins is clear in cases where a pathogen expresses only one variant at a time, as with African trypanosomes (62). The relative benefit in terms of immune evasion of simultaneously expressing variants, some of which contain the same immunodominant epitope, is less evident. However, the fact that \textit{T. cruzi} persists in hosts despite highly functional parasite-specific immune responses suggests that its strategy of immune evasion is successful—if not entirely obvious.

Rodrigues and coworkers (13, 23) have proposed that the strong immunodominance by TS-specific T cells restricts the generation of a broader, more protective immune response and allows \textit{T. cruzi} to escape complete destruction. Although immunodominance certainly restricts the focus of the immune response, it is probably not the primary reason why the majority of \textit{T. cruzi}-infected hosts are unable to achieve sterile immunity. First, the documented immunodominant CD8+ T cells of known specificity do not account for all of the T cells responding during infection; it is possible that these T cells of as yet unknown specificity are reactive to a broader set of \textit{T. cruzi} epitopes. Second, vaccination to boost TS-specific CD8+ T cells enhances protection in mice (17, 20, 63); if immunodominance prevented immune control, then one would expect a stronger dominant response induced by prior vaccination to be deleterious for these hosts. Third, although humans and mice generate TS-specific CD8+ T cells, the strong immunodominance observed in B6 mice is somewhat anomalous compared with that observed for other mouse haplotypes (13, 42, 63) and humans (8, 64, 65). Therefore, either there are highly immunodominant CD8+ T cell responses whose specificity has yet to be identified in these hosts or immunodominance per se is not required for persistence. Finally, as shown in this study, tolerizing B6 mice against the immunodominant TS epitopes had a transient negative impact on host control of \textit{T. cruzi} replication but little influence on the ultimate outcome of acute infection. It remains to be determined if diverting the focus of the CD8+ T cell response away from these particular TS-encoded epitopes allows for the recognition of a broader set of epitopes (encoded by TS and other large gene families or perhaps a more conserved set of genes) or otherwise alters the development of chronic disease due to the long-term persistence of \textit{T. cruzi} in its host.

Because intracellular protozoan parasites do not rely on host cell machinery for gene expression, the pool of proteins readily introduced into the MHC class I presentation pathway is controlled at the level of the parasite. Dominant Ags from numerous parasites are surface-expressed and secreted proteins (6–8, 10, 66); therefore, it seems likely that these pathogens have evolved to balance the necessary function of these proteins with the possibility of them serving as targets for immune recognition. Intriguingly, protective epitopes encoded in several secreted Ags of other parasites display significant variation both within (10) and between strains (6), similar to TS genes in \textit{T. cruzi}. This variation has significant outcomes in terms of immunodominance (10) and cross-protection (6, 67). The issue of if and how variant T cell epitopes influence the outcome of these human diseases remains to be determined.

**Acknowledgments**

We thank Angel Padilla, Juan Bastamante, and Matthew Collins for technical assistance and Julie Nelson of the Center for Tropical and Emerging Global Diseases Flow Cytometry Facility at the University of Georgia. We also thank members of the Tarleton Research Group for helpful discussion.

**Disclosures**

The authors have no financial conflicts of interest.

**References**

infection in a highly susceptible mouse strain after immunization with recombinant proteins based on aminoglycoside surface protein 2. Infect. Immun. 73: 6017–6025.


