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Stable CD8\(^+\) T Cell Memory during Persistent Trypanosoma cruzi Infection\(^1\)

Lisa M. Bixby and Rick L. Tarleton\(^2\)

CD8\(^+\) T cell responses to persistent infections caused by intracellular pathogens are dominated by resting T effectors and T effector memory cells, with little evidence suggesting that a T central memory (T\(_{\text{CM}}\)) population is generated. Using a model of *Trypanosoma cruzi* infection, we demonstrate that in contrast to the T effector/T effector memory phenotype of the majority of T. cruzi-specific CD8\(^+\) T cells, a population of cells displaying hallmark characteristics of T\(_{\text{CM}}\) cells is also present during long-term persistent infection. This population expressed the T\(_{\text{CM}}\) marker CD127 and a subset expressed one or more of three other T\(_{\text{CM}}\) markers: CD62L, CCR7, and CD122. Additionally, the majority of CD127\(^{\text{high}}\) cells were KLRG1\(^{\text{low}}\), indicating that they have not been repetitively activated through TCR stimulation. These CD127\(^{\text{high}}\) cells were better maintained than their CD127\(^{\text{low}}\) counterparts following transfer into naive mice, consistent with their observed surface expression of CD127 and CD122, which confer the ability to self-renew in response to IL-7 and IL-15. CD127\(^{\text{high}}\) cells were capable of IFN-\(\gamma\) production upon peptide restimulation and expanded in response to challenge infection, indicating that these cells are functionally responsive upon Ag re-encounter. These results are in contrast to what is typically observed during many persistent infections and indicate that a stable population of parasite-specific CD8\(^+\) T cells capable of Ag-independent survival is maintained in mice despite the presence of persistent Ag. The Journal of Immunology, 2008, 181: 2644–2650.

The high antigenic load and ongoing TCR stimulation that often characterize persistent viral infections have been shown to negatively impact the antiviral CD8\(^+\) T cell response leading to immune exhaustion. Antiviral CD8\(^+\) T cells during chronic lymphocytic choriomeningitis virus infection, for example, show signs of functional impairment including loss of cytokine production and proliferation and susceptibility to physical deletion (1). In contrast, studies in acute resolving bacterial and viral infection models have demonstrated that once established, stable CD8\(^+\) T cell memory is maintained independently of Ag (2, 3) by the homeostatic turnover of Ag-specific CD8\(^+\) T cells mediated by the cytokines IL-7 and IL-15 (4–6). CD8\(^+\) T cell memory during persistent infections, however, is less easily defined, in part due to the numerous infections which differ in regard to duration of chronic infection, antigenic load, and pathogen tropism.

CD8\(^+\) memory cells have been broadly classified into T central memory (T\(_{\text{CM}}\))\(^3\) and T effector memory (T\(_{\text{EM}}\)) subsets. Although these subsets were initially defined based on high (T\(_{\text{CM}}\)) and low (T\(_{\text{EM}}\)) expression of the lymph node-homing molecules CD62L and CCR7 (7), a variety of additional markers have subsequently been used to discriminate between these two subsets of cells (8). Surface expression of IL-7R\(\alpha\) (CD127) and IL-15R\(\beta\) (CD122), receptors that confer responsiveness to IL-7 and IL-15, respectively, is a hallmark of stable CD8\(^+\) T cell memory (3) and identify memory CD8\(^+\) T cell precursors destined to become long-lived T\(_{\text{CM}}\) cells (9) capable of self-renewal and rapid recall to Ag (10). In contrast, CD8\(^+\) T cells in chronic infections are maintained primarily as Ag-dependent, short-lived T\(_{\text{E}}\) cells (3, 11–14) generally fail to express CD127 and CD122 and therefore proliferate poorly in response to IL-7 and IL-15 (3, 11, 12).

We investigated the effects of persistent parasite Ag on the maintenance of CD8\(^+\) T cells during chronic infection with the protozoan parasite *Trypanosoma cruzi*. *T. cruzi* is the causative agent of Chagas’ disease and is responsible for >50,000 deaths a year in Latin America (15). Although acute infection is controlled by host immune responses, parasites persist at very low levels in certain tissues (16, 17). The use of tools such as MHC class I tetramers has allowed for the ex vivo detection and characterization of parasite-specific CD8\(^+\) T cells during *T. cruzi* infection (18). This infection therefore provides an opportunity to study the effects of persistent Ag on the parasite-specific CD8\(^+\) T cell response and, more specifically, to determine whether stable memory cells capable of Ag-independent persistence are generated during this infection. We report that consistent with the persistent nature of *T. cruzi* infection, the majority of parasite-specific CD8\(^+\) T cells during chronic infection are CD62L\(^{\text{low}}\)CD127\(^{\text{low}}\)CD122\(^{\text{low}}\) and proliferate poorly to homeostatic cytokines. However, a minor population of parasite-specific CD8\(^+\) T cells also exists that displays characteristics of T\(_{\text{CM}}\) cells. These CD127\(^{\text{high}}\) Ag-specific CD8\(^+\) T cells produce IFN-\(\gamma\) following peptide restimulation, are better maintained in the absence of Ag than their CD127\(^{\text{low}}\) counterparts, and expand substantially following challenge. These data demonstrate that a stable memory CD8\(^+\) T cell population capable of Ag-independent survival is maintained for >1 year in an environment where Ag persists.

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\(^3\) Abbreviations used in this paper: T\(_{\text{CM}}\), T central memory; T\(_{\text{E}}\), T effector; T\(_{\text{EM}}\), T effector memory; TCT, tissue culture trypmastigote; SC, spleen cell; CR2P, cruzipain; GFT, \(\beta\)-galactofuranosyltransferase; FMO, fluorescence minus one.
Materials and Methods

Mice, parasites, and infections
C57BL/6d and C57BL/6-Tg(Actb-EGFP)10shb/J mice were purchased from The Jackson Laboratory and C57Bl/6-Ly5.2 mice were purchased from the National Cancer Institute at Frederick (Frederick, MD). Mice were maintained at the University of Georgia animal facility in microisolator cages under specific pathogen-free conditions. For the T. cruzi infections, tissue culture trypomastigotes (TCT) of the Brazil strain or CL strain of T. cruzi were obtained from passage through vero cells. Mice were infected either i.p. with 1000 TCT or with 2000 TCT via footpad injection and sacrificed by CO2 inhalation. In some experiments, animals were challenged with 10,000 Brazil strain TCT. All animal protocols were approved by the University of Georgia Institutional Animal Care and Use Committee.

T cell phenotyping

RBCs in single-cell suspensions of spleen cells (SC) were lysed in a hypotonic ammonium chloride solution and washed to staining buffer (2% BSA and 0.02% azide in PBS (PAB)). In some cases, mouse peripheral blood was obtained by retro-orbital venipuncture, collected in sodium citrate, and washed in PAB. SC and whole blood were incubated with tetramer-PE and the following labeled Abs: anti-CD262L, anti-CD8 allophycocyanin-Cy7 (BD Biosciences), anti-CD127 allophycocyanin (BD Biosciences), anti-CD122 Alexa Fluor 488, and streptavidin Alexa Fluor 405 (Caltag Laboratories/Invitrogen). Cells were also stained with anti-CD4, anti-CD11b, and anti-B220 Tricolor (Caltag Laboratories/Invitrogen) for use as an exclusion channel. Cells were stained for 45 min at 4°C in the dark, washed twice in PAB, and fixed in 2% formaldehyde. The ELC-Ig chimera was used for detecting CCR7 expression and was a gift from Dr. K. Klondowsk (University of Georgia, Athens, GA). For CCR7 detection, cells were incubated at 37°C for 1 h, stained with ELC-Ig for 45 min, and washed. Cells were then stained with goat anti-human IgA Alexa Fluor 488 (Invitrogen) for 30 min, washed, and stained with surface markers as indicated above. For whole blood, RBCs were lysed after surface staining in a hypotonic ammonium chloride solution and washed twice in PAB. At least 500,000 lymphocytes were acquired using a CyAn flow cytometer (DakoCytomation) and analyzed with FlowJo software (Tree Star). MHC I tetramers TSKB20 (ANYKFTLV/Kb) and TSKB74 (VNYDFTLVI/Kb) were synthesized at the Tetramer Core Facility (Emory University, Atlanta, GA).

Lymphocyte proliferation

Splenocytes isolated from chronically infected mice were incubated with CFSE (Molecular Probes/Invitrogen) at a final concentration of 1 μM for 3 min at room temperature. CFSE labeling was quenched with an equal volume of horse serum and the cells were washed three times with RPMI 1640 containing 10% FBS. CFSE-labeled cells were cultured in RPMI containing 10% FBS for 72 h at 37°C with IL-7 (15 ng/ml; BD Biosciences), IL-15 (15 ng/ml; eBioscience), or medium alone. An anti-IL-15 Ab (eBioscience) was added to some cell cultures to block endogenous IL-15 activity. Following culture, splenocytes were washed twice in PAB and stained with TSKB20/Kb PE, anti-CD8 Alexa Fluor 405 (Caltag Laboratories/Invitrogen), and exclusion channel Abs.

Intracellular cytokine staining

Splenocytes isolated from naive or chronically infected mice were sorted as described below, cultured with feeder cells from GFP expressing mice [C57BL/6-Tg(Actb-EGFP)10shb/J], and stimulated with T. cruzi peptides (1 μM) for 5 h at 37°C in the presence of brefeldin A (GolgiPlug; BD Biosciences). T. cruzi peptides used in this study were TSKB20 (ANYKFTLVI), TSKB18 (ANYDFTLVI), CRZP25 (PSYRSSVPL), CRZP9 (VPLNCNRPL), GFT 16 (AAMSRHPPL), and GFT 17 (RGFDEGNGL) (18). Cells were surface stained with anti-CD8 Alexa Fluor 405 (Caltag Laboratories/Invitrogen) and intracellular cytokine staining was performed with anti-IFN-γ allophycocyanin with a Cytofix/Cytoperm kit (BD Biosciences) in accordance with the manufacturer’s instructions. At least 250,000 lymphocyte events were acquired and analyzed as above.

In vivo BrdU incorporation assay

BrdU (Sigma-Aldrich) solution (0.8 mg/ml) was made fresh every 2 days and given to mice for 12 days starting on the day of challenge. SC from mice were RBC lysed and washed twice in PAB. Cells were surface stained for 45 min at 4°C in the dark with TSKB20/Kb PE and the following labeled Abs: anti-CD8 Alexa Fluor 405 (Caltag Laboratories/Invitrogen), anti-CD45.2 allophycocyanin, and anti-CD127 PE-Cy7 (eBioscience). An exclusion channel was used as described above. Cells were permeabilized and stained with a BrdU flow kit according to the manufacturer’s instructions (BD Biosciences). Briefly, cells were permeabilized twice and treated with DNase to expose incorporated BrdU. Cells were then stained intracellularly with anti-BrdU FITC, washed twice, and fixed in 2% formaldehyde. At least 2 × 10^6 lymphocyte events were acquired as above.

CD8+ T cell sorting and adoptive transfers

SC from naive or chronically T. cruzi-infected mice were washed twice in PBS following RBC lysis. CD8+ T cells were negatively selected through magnetic sorting (CD8a+ T cell isolation kit; Miltenyi). For in vitro peptide restimulation experiments, single-cell suspensions enriched for CD8+ T cells were stained with anti-CD127 PE and cells were sorted into CD127low and CD127high populations. For adoptive transfer experiments, single-cell suspensions enriched for CD8+ T cells were stained with anti-CD127 PE (eBioscience) and anti-CD44 allophycocyanin (BD Biosciences) and cells were sorted into CD44highCD127high and CD44lowCD127low populations. Sorting was performed on a MoFlo cell sorter (DakoCytomation). In brief, 1.3 × 10^6 CD44highCD127high or CD44lowCD127low cells were transferred i.v. into naive congenic mice. Mice were infected 21 days after transfer with 10,000 Brazil strain T. cruzi parasites and administered BrdU in drinking water for 12 days to assess proliferation rates in vivo. Mice were sacrificed 12 days postchallenge infection and splenocytes were stained for BrdU and surface markers as described above.

Results

Presence of a heterogeneous CD127high T, cruzi-specific

CD8+ T cell population during chronic infection

We have recently shown that T. cruzi infection elicits a strong CD8+ T cell response that is highly focused on epitopes encoded by genes belonging to the trans-sialidase (ts) family (18). To examine the phenotypic characteristics of parasite-specific CD8+ T cells, we analyzed the longitudinal expression of markers of Ag-independent memory on TSKB20-specific CD8+ T cells following infection of C57BL/6 mice with the Brazil strain of T. cruzi (Fig. 1A). As previously described (18), TSKB20-specific CD8+ T cells expanded and contracted during acute infection reflecting the peak parasitemia observed at ~30 dpi in this model and these cells were maintained during the chronic phase. A significant fraction of TSKB20-specific T cells expressed CD127 early in the acute infection, but this population represented <2% of all the TSKB20-specific cells by day 64 postinfection. From this low point, the fraction of TSKB20-specific cells expressing CD127 increased steadily to nearly 24% by ~1 year postinfection (Fig. 1, A and C). The frequency of TSKB20-specific cells expressing CD122 (Fig. 1A) and CCR7 (Fig. 1B) also increased as the infection progressed, but remained at <5% even at 400 days postinfection.

The pattern of expression of CD127 and CD122 on CD8+ T cells specific for the subdominant TSKB74 epitope was similar to that for TSKB20/Kb+ cells (Fig. 1C), indicating that the memory phenotype does not vary among different T cell populations within the dominance hierarchy. Of the TSKB20-specific CD127high cells, nearly one-third coexpressed CD62L and ~60% of the CD127high TSKB20/Kb+ cells lacked expression of the killer cell lectin-like receptor G1 (KLRG1) (Fig. 1D), a molecule induced by repetitive Ag stimulation that marks CD8+ T cells with impaired proliferative capacities (19). Collectively, these results indicate a heterogeneous population of parasite-specific CD8+ T cells with the phenotype of long-lived, Ag-independent TCM cells persists in mice with chronic T. cruzi infection.
A subset of T. cruzi-specific CD8⁺ T cells from chronically infected mice proliferate in response to IL-7 or IL-15

The observed frequencies of CD127⁺ and CD122⁺ Ag-specific CD8⁺ T cells from chronically T. cruzi-infected mice predict that only a subset of the T. cruzi-specific T cell population should respond to IL-7 or IL-15. To test this prediction, splenocytes were cultured with IL-7 and IL-15 and proliferation was measured on the basis of dilution of CFSE stain. Because we have previously observed that endogenous IL-15 in these spleen cell cultures contributes to background proliferation in the negative control (medium only) conditions, graded doses of an anti-IL-15 Ab were added to block endogenous IL-15 in cultures. The effects of endogenous IL-15 on the proliferation of TSB20-specific cells from chronically infected mice could be blocked in a dose-dependent manner with anti-IL-15-blocking Ab, and this blocking effect could be partially overcome by addition of exogenous IL-15 (Fig. 2). Likewise, a fraction of the TSB20-specific cells in chronically infected mice responded to IL-7 (Fig. 2), thus confirming that CD127 and CD122 expression correlated with the ability of these T cells to functionally respond to the respective ligands.

CD127⁺ and CD122⁺ T cells produce IFN-γ in response to T. cruzi peptides encoding both immunodominant and subdominant epitopes

Although CD8⁺ T cells specific for the immunodominant TSB20 and TSB74 epitopes (18) in chronically infected mice contain a relatively low number of CD127⁺ cells, we hypothesized that CD8⁺ T cells specific for subdominant epitopes might contain a higher fraction of CD127⁺ T cells. Immunodominant responses may achieve their respective frequencies due to a greater amount and presentation of cognate Ag throughout the infection, and the abundance of Ag likely contributes to the maintenance of these cells as primarily TEm or TEM populations. Lower frequency CD8⁺ T cells specific for scarce or sparsely presented Ags may encounter Ag less frequently and thus contain a higher proportion of stable TCM-like cells. To test this hypothesis, we examined the responses of the relatively rarer T. cruzi-specific CD8⁺ T cells specific for...
sorted controls did not produce IFN-γ in response to any of the T. cruzi peptides. Surprisingly, the majority of responding CD8+ T cells to the CRZP/GFT pool of peptides was in the CD127low population, as was also the case for the ts pool of peptides. However, we did observe the presence of a small population of IFN-γ-producing CD8+ T cells in both peptide pools in the CD127high fraction, indicating that regardless of the epitope specificity and possibly the level of Ag exposure, the majority of T. cruzi-specific cells in persistently infected mice are of the CD127low phenotype. Importantly, these data also confirm that T. cruzi-specific CD127high T cells are functionally responsive and capable of being activated to effector function.

**Maintenance and expansion of CD127highCD8+ T cells from chronically infected mice**

Preservation following pathogen clearance and rapid expansion following secondary Ag exposure are hallmarks of Ag-independent memory T cells. To further examine these characteristics in the CD127highCD8+ T cell population from chronically infected mice, splenocytes from naive and chronically infected mice were enriched for CD8+ T cells and then sorted into CD44highCD127high and CD44highCD127low groups (Fig. 4A). We chose to sort on CD44high cells to focus on Ag-experienced CD8+ T cells. Equal numbers of sorted cells were transferred into CD45.1+ congenic naive mice, and mice were rested to determine the maintenance of these cells in the absence of Ag. Monitoring the peripheral blood of recipient mice for CD45.2+ donor cells at 20 days after transfer revealed that the CD127high donor populations from naive and chronically infected mice were better maintained at this time than the CD127low donor populations (Fig. 4B). Phenotypic analysis revealed that the CD127high transferred cells retained their original CD127high phenotype in the absence of Ag. In addition, the donor cells in the recipients of CD127low cells were now ~40% CD127high, indicating either the preferential retention or expansion of the minor contaminating CD127high population in these recipients or the conversion of CD127low into CD127high cells (Fig. 4B).

To determine the in vivo proliferative capacity of the transferred cell populations, the mice that received donor cells were infected with T. cruzi at 21 days after transfer and administered BrdU in drinking water. Examination of the CD8+ T cell population in the

**FIGURE 2.** A subset of TSKB20 tetramer CD8+ T cells from chronically infected mice proliferate in response to the cytokines IL-7 and IL-15. Splenocytes from mice chronically infected with T. cruzi at 154 dpi were labeled with CFSE and cultured in the presence of 15 ng/ml IL-7 or IL-15 or with medium alone for 72 h. An anti-IL-15-blocking Ab was added to cultures at the indicated concentrations. The concentration of 0.78 μg/ml was previously shown to block 100% of 50 ng/ml IL-15 activity. Cells were stained with the TSKB20/Kb tetramer and for surface expression of CD4/CD11b/B220. Proliferation of CD4/CD11b/B220+ TSKB20-specific CD8+ T cells was assessed by CFSE dilution. Numbers indicate the percentage of TSKB20+ cells, and CD127low T cells are functionally responsive. Splenocytes from naive and chronically infected mice were pooled, enriched for CD8+ T cells, and then sorted into CD127high and CD127low groups (sort purity was ~97% for both populations). IFN-γ production by purified CD127high and CD127low CD8+ T cells was assessed following a 5-h incubation with a pool of ts peptides (TSKB20, TSKB74) or a pool of non-ts peptides (CRZP5 and CRZP9, GFT16 and GFT17) in the presence of GFP+ feeder cells. Splenocyte cultures from naive (top panel) and chronically infected mice (bottom panel) were stained with anti-CD8 and anti-IFN-γ. Numbers indicate the percentage of CD8+ IFN-γ-producing cells for each condition. All plots are gated on GFP-negative cells. Data are representative of two independent experiments.

**FIGURE 3.** T. cruzi-specific CD127high and CD127low T cells are functionally responsive. Splenocytes from T. cruzi-infected (200 dpi) or aged-matched naive mice were pooled, enriched for CD8+ cells, and sorted into CD127high and CD127low groups (sort purity was ~97% for both populations). IFN-γ production by purified CD127high and CD127low CD8+ T cells was assessed following a 5-h incubation with a pool of ts peptides (TSKB20, TSKB74) or a pool of non-ts peptides (CRZP5 and CRZP9, GFT16 and GFT17) in the presence of GFP+ feeder cells. Splenocyte cultures from naive (top panel) and chronically infected mice (bottom panel) were stained with anti-CD8 and anti-IFN-γ. Numbers indicate the percentage of CD8+ IFN-γ-producing cells for each condition. All plots are gated on GFP-negative cells. Data are representative of two independent experiments.
spleen 12 days after infection revealed that donor cells in the recipients of both CD127\textsuperscript{high} and CD127\textsuperscript{low} cells from chronically infected mice had expanded substantially as revealed by the frequency of donor cells (Fig. 4C) and by BrdU incorporation (Fig. 4D). The donor CD127\textsuperscript{high} cells exhibited the highest frequency as expected based on their greater survival during the 21-day Ag-free period. Also, the majority of the initially CD127\textsuperscript{high} and CD127\textsuperscript{low} donor cells were CD127\textsuperscript{low} following challenge, indicating the conversion to a TE/TEM phenotype (Fig. 4D). Naive CD127\textsuperscript{high} donor cells had also expanded following challenge, although far fewer of these cells incorporated BrdU and converted to a CD127\textsuperscript{low} phenotype compared with chronic donor cells (Fig. 4D).

TSKB20-specific CD8\textsuperscript{+} T cells also expanded following challenge infection in the CD127\textsuperscript{high} and CD127\textsuperscript{low} chronic donor populations but were undetectable in the naive CD127\textsuperscript{high} population (Fig. 4C). Transferred TSBK20-specific and total CD8\textsuperscript{+} T cells from chronic CD127\textsuperscript{high} and CD127\textsuperscript{low} donors incorporated similar amounts of BrdU (Fig. 4D). These results indicate that CD127\textsuperscript{high/CD8\textsuperscript{+}} T cells from chronically infected mice are more efficiently maintained in naive mice than CD127\textsuperscript{low} cells; however, donor cells in the recipients of both CD127\textsuperscript{high} and CD127\textsuperscript{low} cells expand robustly following antigenic challenge.

**Discussion**

In this study, we demonstrate that Ag-independent CD8\textsuperscript{+} T cell memory can be generated and maintained despite pathogen persistence in T. cruzi infection. A heterogeneous subset of Ag-specific CD8\textsuperscript{+} T cells was detected in chronically infected mice that
expressed markers typically associated with long-term memory, including CD127, CD62L, and CCR7. These CD127high Ag-specific cells were capable of producing IFN-γ in response to T. cruzi peptides, were present in CD8+ T cell populations with distinct T. cruzi Ag specificities and frequencies, and were stably maintained when transferred to an Ag-free environment. These data are in contrast to numerous studies (3, 11, 12, 20), suggesting that persistent infections in general compromise the maintenance of long-term, Ag-independent CD8+ T cell memory.

Although the characteristics of CD8+ T cell memory generated during acute resolving infections have been well documented, only recently has work focused on memory responses during chronic infections, revealing that memory T cells present in chronic infections are often phenotypically and functionally distinct from memory T cells in acute resolving infections (1). Specifically, the detrimental effects of persistent Ag on pathogen-specific CD8+ T cells often reported in viral models of infection include loss of cytolytic function and cytokine activity, impaired proliferation, and eventual deletion in the most extreme circumstances (11, 12, 21, 22). Maintenance of CD8+ T cell memory is also affected, as responsiveness to IL-7 and IL-15 is impaired in antiviral CD8+ T cell responses in persistent infections (3, 20, 23), suggesting that long-term immunity may be compromised following infection resolution. Although these findings highlight important characteristics of CD8+ T cell memory during high-load chronic infections, many factors, including the frequency and duration of Ag encounter as well as the cell tropism of the pathogen, may dictate the final outcome of Ag-specific CD8+ T cells during various persistent infections. T. cruzi infection differs dramatically from models of T cell memory during high-load chronic infections, including CD127, CD62L, and CCR7. These CD127high Ag-specific cells in this model can therefore serve several functions. TCM cells exhibit a CD44highCD62Llow TEM-like phenotype during chronic infection. However, a subpopulation of TEM cells may have distinct histories of Ag encounter and/or may be at different points along a differentiation pathway, leading to the acquisition of a stable TCM phenotype. This is certainly a possibility, as studies by Kaech et al. (9) have demonstrated that acquisition of CD62L expression occurs over time and in the absence of antigen stimulation. Expression of KLRG1, a receptor indicating repetitive Ag stimulation (27) or replicative senescence (19), was also examined on the CD127high TSKB20-specific population. Approximately 60% of CD127high cells were KLRG1low, thus resembling Ag-specific CD8+ T cells from acute resolving infections and indicating that this population of T. cruzi-specific CD8+ T cells has not been recently or repeatedly activated. This is perhaps not surprising given that T. cruzi persists at very low levels in only certain tissues, making the likelihood of encountering Ag lower than that during infections characterized by high pathogen load. Recruitment of naive CD8+ T cells into the memory pool during the chronic phase of infection also is likely to contribute to the heterogeneity of these TCM populations. Indeed, Vezys et al. (28) have shown that newly generated naive CD8+ T cells primed during low-level persistent polyoma virus infection contribute to the diversity of the antiviral memory pool, and preliminary data suggest that naive priming is also occurring during chronic T. cruzi infection (L. M. Bixby and R. L. Tarleton, unpublished data).

Memory phenotype CD127highCD8+ T cells from chronically infected mice were better maintained following transfer into naive hosts than their CD127low counterparts, presumably due to their ability to receive survival signals from IL-7 through expression of CD127. Nonetheless, a smaller fraction of CD127low sorted donor cells were also maintained following transfer to an Ag-free environment. Notably, nearly half of these sorted CD127low donors did not maintain their original phenotype and were CD127high 20 days following transfer to naive mice. These data could suggest that CD127low cells are capable of recovering a CD127high phenotype, allowing their long-term survival, following removal from Ag. However, we cannot rule out the possibility that a minor contaminating CD127high population survived and expanded in these recipients. These rested donor cells in recipients of both CD127high and CD127low cells also expanded following challenge infection and showed a CD127low phenotype, consistent with a TSK or TEM phenotype expected after activation.

Collectively, our data support a model in which as Ag load is controlled during T. cruzi infection, a TCM-like population is observed that is composed of a pool of cells that rarely encounters Ag and likely includes newly recruited cells from the naive population. Although these TCM-like cells are presumably activated periodically by Ag as a consequence of parasite persistence, the TCM phenotype may restrict homing of these cells to lymphoid tissues, making their presence at sites of parasite persistence rare. TCM cells in this model can therefore serve several functions. TCM cells could provide a pool for the production of additional T/F/TEM cells when Ag does reach a draining lymph node, either during persistent infection or following reinfection. Additionally, TCM cells likely represent a subset of cells that can preserve long-term T cell memory in the case that Ag is eventually cleared. Thus, the data presented here have significant implications for long-term immunological protection in individuals that have been successfully drug treated during T. cruzi infection. Benznidazole is the principle drug available for the treatment of T. cruzi infection, although this compound has serious potential side effects and a reported variable efficacy in clearing the infection. Nonetheless, we have recently documented complete parasite clearance in mice treated with benznidazole that resulted in the emergence of a protective T. cruzi-specific, predominantly CD127high TCM population (29). These parasite-specific TCM cells present in mice following drug cure were detected for >300 days after treatment and likely arise from the minor CD127high TCM-like population we have detected in chronically infected mice and have reported on here. These findings have implications for the majority of individuals with Chagas’ disease, who are not diagnosed and therefore do not receive drug treatment until symptoms appear decades following infection.

Similar evidence has recently been presented for TCM-like cells during persistent hepatitis C and γ-herpesvirus infection (8, 25), both of which are also characterized by low Ag load. These studies
and the data presented here demonstrate that not all chronic infections uniformly result in CD8+ T cell dysfunction, including the failure to generate Ag-independent memory, and emphasize that the presence of TEm/Tcm cells is not determined by whether Ag is cleared, but individually at the T cell level based on how frequently Ag is encountered.

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