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Cutting Edge: Dysfunctional CD8\(^+\) T Cells Reside in Nonlymphoid Tissues During Chronic Trypanosoma cruzi Infection\(^1\)

Jennifer K. Leavey and Rick L. Tarleton\(^2\)

Chagas disease is caused by persistent Trypanosoma cruzi infection in muscle tissue that ultimately results in chronic inflammation and tissue destruction. It is unclear why T. cruzi is cleared from some tissues but persists in others, despite an active inflammatory response. In this study, we show that the majority of CD8\(^+\) T cells present in muscle tissue express memory and effector cell surface markers but have sharply attenuated effector function compared with their splenic counterparts. The dysfunction of CD8\(^+\) T cells in the muscle tissue suggests a mechanism by which T. cruzi can persist in that location and cause inflammatory damage. The Journal of Immunology, 2003, 170: 2264–2268.

Trypanosoma cruzi is a protozoan parasite and the causative agent of Chagas disease, a chronic condition that is characterized by parasite persistence and inflammation in muscle tissue, particularly the heart and gut. Primary infection is generally followed by a mild acute phase with relatively high parasite burden, in which a wide variety of cell types and tissues are infected (reviewed in Ref. 1). Because T. cruzi amastigotes reside and replicate intracellularly, cell-mediated immunity, in particular the CD8\(^+\) T cell compartment, is an indispensable component of the immune response to T. cruzi (2, 3). During chronic T. cruzi infection, large numbers of CD8\(^+\) T cells infiltrate muscle tissue. Disease pathology and tissue damage are believed to be a result of this lymphocytic infiltrate.

Recently, an emerging dogma has developed delineating memory T cells localized to nonlymphoid tissues from those in secondary lymphoid tissues. In contrast to memory T cells in secondary lymphoid tissues, memory T cells found outside of lymphoid tissues are thought to be poised effector cells capable of direct ex vivo effector function (4, 5). This theory provides an attractive explanation for the inflammation-mediated muscle damage in Chagas disease: CD8\(^+\) T cells located in the muscle are chronically activated and thereby cause damage. However, this does not explain why small numbers of T. cruzi fail to be cleared from and thus persist in the tissues of chronically infected hosts. It is possible that the T cells in the muscle have aberrant effector function and are unable to clear the parasite while still mediating inflammatory damage. Dysfunctional CD8\(^+\) T cells have been observed in a number of chronic disease models (6–10).

In this study, we have characterized the phenotype and assessed the functional capacity of CD8\(^+\) T cells in the muscle of mice chronically infected with T. cruzi. In contrast with what has been previously observed with effector memory T cells from nonlymphoid tissues, the muscle-resident CD8\(^+\) T cells do not exhibit effector function. The dysfunction of these cells may be due to the persistence of T. cruzi in that location, inhibitory components of the muscle tissue itself, or a combination of both.

**Materials and Methods**

**Mice**

C57BL/6, B6.SJL, and male C3H/HeSnJ mice were purchased from The Jackson Laboratory (Bar Harbor, ME) and bred in the University of Georgia animal facility. Female C57BL/6 and B6.SJL mice were infected with 10\(^6\) blood-form Brazil strain trypomastigotes i.p. at 6–12 wk of age. Male C3H/HeSnJ mice were infected in the same manner with 10\(^6\) trypomastigotes of the Sylvio X10/4 strain.

**Cell lines and parasites**

Blood-form trypomastigotes of the Brazil strain of T. cruzi were maintained by passage every other week in C3H/HeSnJ mice. Tissue culture-derived trypomastigotes of the Brazil or Sylvio X10/4 clone were generated by passage in vero cell or bovine skeletal muscle monolayers, respectively. All cells were maintained in RPMI 1640 with 10% FBS and standard supplements.

**Isolation of lymphocytes from nonlymphoid tissues and adoptive transfer**

Before tissue removal, mice were perfused with 10 ml of PBS with 10 U/ml heparin. In most experiments, muscle-derived lymphocytes were prepared by incubation of heart or skeletal muscle tissue with 0.4% collagenase II (Sigma-Aldrich, St. Louis, MO) in PBS with 5% FBS for 1 h at 37°C. Lymphocytes were obtained from lung, liver, and some skeletal muscle samples by teasing tissues apart and vigorously pushing through a 40-μm nylon mesh screen. For cell transfer experiments, 5 × 10\(^5\) chronic (day 90 postinfection) B6.SJL spleen cells in PBS were transferred i.v. into naive or chronic (day 365 postinfection) C57BL/6 mice.

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Intracellular cytokine analysis and cell sorting

Single-cell RBC-depleted suspensions of spleen, muscle, lung, or liver were prepared and cultured overnight or for 5 h in 96-well flat-bottom tissue culture plates that had been previously coated with 30 µg/ml anti-CD3 (145-2C11) or anti-CD28 (37.51). Brefeldin A (1 µg/ml) was added for the last 5 h of culture. Cells were then stained for intracellular and cell surface Ags using BD PharMingen (San Diego, CA) Abs and CytoFix/Cytoperm kit in accordance with the manufacturer's recommendations. For cell sorting, spleen and muscle preparations were stained with anti-CD8 FITC in PBS with 2% FBS. CD8+ T cells were sorted by FACS to >98% purity.

CTL assay

A redirected 51Cr release assay to measure total CTL activity was performed as follows. Log-phase P815 cells were radioactively labeled with 1.5 h at 37°C in 200 µl of Na235CrO4 (Amer sham Pharmacia, Piscataway, NJ) with 5% FBS after which they were washed thoroughly. Effector CTL were isolated directly ex vivo from the spleen or muscle tissue 11 days after the challenge of chronically T. cruzi-infected (day 365 postinfection) mice with 105 Brazil strain T. cruzi trypomastigotes. Effector cells were added to the target cells at various ratios and some wells included 3 µg/ml anti-CD3 (145-2C11) to cross-link the FcR-bearing P815 cells with the T cells. After 4–6 h, culture supernatants were counted in a Packard (Meriden, CT) Cobra II gamma counter. Spontaneous 51Cr release from target cells was <15% of total lysis.

Results

CD8+ memory T cells develop in muscle tissue during T. cruzi infection

We first sought to characterize the phenotype of CD8+ T cells in muscle tissue over the course of infection to search for clues that would explain why these cells populate this location but do not clear T. cruzi from it. Leg muscle (hamstring and quadriceps) and spleen from C57BL/6 mice infected with 103 Brazil strain T. cruzi trypomastigotes were obtained at various times after infection. The isolated cells were analyzed by flow cytometry for the presence of T cell memory and activation markers. At day 84 postinfection, the CD8+ T cells located in the muscle were not markedly different from those in the spleen (Fig. 1A). A majority of CD8+ T cells from both tissues expressed the activation/memory markers CD44, CD11a, and Ly-6C, and lacked expression of the naive cell marker CD62L. During the acute phase of T. cruzi infection (days 20–40), nearly all of the relatively few CD8+ T cells in the muscle tissue expressed CD44, CD11a, and Ly-6C, and lacked expression of CD62L (Fig. 1B). Memory CD8+ T cells were also abundant in the spleen. By day 34 postinfection, ~70% of splenic CD8+ T cells expressed a memory phenotype (CD11a+CD44+Ly-6C+CD62L−). In comparison, <20% of CD8+ T cells had this phenotype in naive animals. As the animals progressed into the chronic phase of infection, the percentage of CD8+ T cells in both the muscle and spleen that expressed memory markers gradually decreased; however, effector/memory phenotype cells were in the majority of CD8+ T cells throughout the infection.

CD8+ T cells in muscle have markedly attenuated effector function when compared with their splenic counterparts

The cell surface phenotype of CD8+ T cells in the muscle during chronic T. cruzi infection does not suggest a defect in this population, which would account for the persistence of T. cruzi in that location. Therefore, we proceeded to examine the functional capacity of cells from the muscle compared with those of the spleen. Remarkably, we found a dramatic difference in the ability of muscle-derived and splenic CD8+ T cells to produce IFN-γ in response to anti-CD3 stimulation (Fig. 2A). IFN-γ production was observed in 55% of the CD8+ T cells in the spleens of chronically infected mice when incubated with plate-bound anti-CD3 Abs. In contrast, only 2% of muscle-derived CD8+ T cells produced IFN-γ under the same conditions (Fig. 2A). Although the difference in responsiveness between the spleen and muscle CD8+ T cells was not always this dramatic, in every animal tested at least 3-fold more CD8+ T cells from the spleen produced IFN-γ in response to anti-CD3 stimulation than did the CD8+ T cells from the muscle. The deficiency in the responsiveness of T cells isolated from muscle, relative to cells from spleen was not due to the use of collagenase treatment in the isolations from muscle because lymphocytes isolated from muscle by mechanical disruption behave identically (data not shown). Lymphocytes accumulate and parasites persist preferentially in skeletal muscle in C57BL/6 mice infected with the Brazil strain of T. cruzi and in the heart of C3H/HeSnJ mice infected with the Sylvio X10/4 strain (11). CD8+ T cells from the hearts of chronically infected C3H/HeSnJ mice also had impaired IFN-γ production compared with splenic CD8+ T cells from the same animals indicating that the defect is not limited to skeletal muscle (Fig. 2A). The difference in responsiveness of the spleen and muscle cell populations is even more pronounced when the analysis is focused on the memory (CD44+CD11a+) population (Fig. 2B).

As a second measure of effector activity, the total cytotoxic capacity of muscle-derived and splenic CD8+ T cells from chronically infected mice was examined using a redirected
cytotoxicity assay. Splenic CD8^+ T cells exhibited dose-dependent killing of anti-CD3-sensitized P815 cells, while the cytolytic activity of muscle-derived cells was essentially nil (Fig. 2C).

**CD8^+ T cell responsiveness in chronic T. cruzi infection is tissue specific**

CD8^+ T cells from the muscle of chronically T. cruzi-infected mice did not display characteristics commonly ascribed to anergic or apoptotic cells. For example, costimulation with anti-CD28 or accessory APC did not restore function (Fig. 3D), nor did short-term culture with or without IL-2 or IL-12 (Fig. 3C). These culture conditions have been shown to restore function of anergic cells (12–14). Additionally, the muscle-derived CD8^+ T cells were not apoptotic, because they did not bind annexin V (Fig. 3A). Lastly, the muscle-derived CD8^+ T cells lacked expression of the inhibitory receptor CD94/NKG2A (Fig. 3B), which has been shown in some systems to be up-regulated on nonfunctional CD8^+ T cells (15).

To determine whether CD8^+ T cells require constant contact with muscle tissue to remain unresponsive, CD8^+ T cells from the spleen or muscle were purified by cell sorting and tested for function. The production of IFN-γ by CD8^+ cells purified from muscle tissue remains below par in comparison to that of purified CD8^+ cells from spleen, indicating that CD8^+ T cells do not require active suppression by muscle components to be dysfunctional (Fig. 3E).

To ascertain whether the muscle environment per se could deactivate the T cells that traffic there, 50 × 10^6 spleen cells from chronically infected B6.SJL mice were transferred into either chronically infected or naive congenic C57BL/6 recipients. Seven days later, the mice were perfused with heparinized PBS, and the spleen, muscle, lung, and liver were removed for analysis. Transferred cells were detected by expression of the CD45.1. Transferred cells trafficking to the spleen and lung of either the chronic or naive recipients remained responsive to anti-CD3 stimulation. However, transferred cells obtained from the muscle or liver were poorly responsive. The endogenous CD8^+ T cells in all of the tissues of the chronically infected recipients responded with the same pattern; however, in the naive host, the IFN-γ response by endogenous CD8^+ T cells was low in all tissues, as expected (Fig. 4).

**Discussion**

In this study, we have shown that CD8^+ T cells isolated from the muscle tissue of mice that are chronically infected with T. cruzi have limited cytotoxic and IFN-γ-producing activity compared with their splenic counterparts. These deficiencies exist despite the fact that the muscle-localized CD8^+ T cells express surface markers consistent with an effector/memory phenotype. The muscle environment may directly influence the
function of cells there, because chronic spleen cells become refractory to stimulation after being transferred to naive mice, migrating to the muscle, and being isolated from that tissue. However, constant contact with the muscle environment is not required for sustained suppression of CD8$^+$ T cells isolated from that tissue, because CD8$^+$ T cells sorted from muscle tissue retain their nonresponsive phenotype. Several possible causes for the nonresponsiveness of muscle tissue were ruled out. The T cells isolated from muscle were not apoptotic, did not express the inhibitory receptor CD94/NKG2A, and did not appear to be classically anergic because their function was not restored upon culture with costimulation in the form of anti-CD28 or IL-2.

Interestingly, our findings do not complement the evolving paradigm of "effector memory" T cells to nonlymphoid tissues (5). We do not see direct ex vivo effector function of CD8$^+$ T cells from the tertiary tissues of muscle or liver in chronic *T. cruzi* infection, although we do from the lung. We have explored several possible explanations for this. First, effector memory T cells may not develop in the same way in chronic infections as they do in infections that are more readily resolved, or alternately, effector memory T cells may reside in only a subset of tertiary tissues.

To date, the bulk of evidence describing effector memory T cells has been reported in models of infectious disease where the infection was completely resolved such as vesicular stomatitis virus (5) and *Listeria monocytogenes* (16), and not in chronic disease models. Previous work on diseases characterized by persistent infection, such as lymphocytic choriomeningitis virus (6, 7), SIV (8), HIV (9), and hepatitis C virus (10), has shown that some T cells become dysfunctional or exhausted in the presence of constant antigenic stimulation. *T. cruzi* also establishes persistent infection of hosts; however, we lack evidence that the nonresponsiveness of CD8$^+$ T cells in our model is due to persistent antigenic stimulus. Chronic spleen cells become nonfunctional after being adoptively transferred and migrating to the muscle tissue of naive animals. However, transferred cells isolated from the spleen of the same naive animals retain function, which makes Ag transfer an unlikely explanation for the nonresponsiveness of the cells recovered from the muscle.

An alternative explanation for our results is that different peripheral tissues support T cell function to varying degrees. We have shown that during chronic *T. cruzi* infection, T cell effector function is strong in the lung and spleen but not in the liver or muscle. Also, activated T cells from the spleens of chronically *T. cruzi* infected mice migrate to various peripheral tissues after adoptive transfer, but the ability of these cells to respond to stimuli differed greatly based on where they migrated. These cells, which were capable of producing IFN-γ before transfer, could no longer do so when isolated from muscle or liver, but could when isolated from spleen and lung, suggesting that spleen and lung tissues encourage the maintenance of T cell responses while muscle and liver do not. T cell dysfunction in muscle tissue could be easily explained by the persistence of Ag there, but the root of T cell dysfunction in the liver is less clear. Liver-derived T cells have recently been shown to have direct ex vivo effector function (5), but the liver has long been considered a tolerogenic organ. Introduction of Ag into the portal vein results in immune tolerance, and liver allografts are particularly well tolerated (reviewed in Ref. 17).

The sum of our results may help explain why tissue damage occurs during chronic *T. cruzi* infection. We propose this model: generation of a potent immune response to *T. cruzi* clears parasites from most tissue sites; however, because the environment of the muscle is not amenable to the maintenance of *T. cruzi*-specific CD8$^+$ T cell responses, small numbers of parasites are maintained there. During the chronic phase of *T. cruzi* infection, the muscle may assume characteristics of an immune-privileged site, suppressing the action of effector T cells that migrate to the tissue and thus limiting peripheral damage. However, cumulative damage occurs when central memory cells migrate through the body, encounter residual infected cells, and exert effector function before they can be suppressed. Although further studies are needed to confirm the role of dysfunctional T cells in Chagas disease, this study provides some new clues toward explaining this paradoxical condition.

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References


