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Persistence and Function of Central and Effector Memory CD4+ T Cells following Infection with a Gastrointestinal Helminth

Colby Zaph,* Kathryn A. Rook,* Michael Goldschmidt,* Markus Mohrs, † Phillip Scott,* and David Artis2*†

Immunity in the gastrointestinal tract is important for resistance to many pathogens, but the memory T cells that mediate such immunity are poorly characterized. In this study, we show that following sterile cure of a primary infection with the gastrointestinal parasite Trichuris muris, memory CD4+ T cells persist in the draining mesenteric lymph node and protect mice against reinfection. The memory CD4+ T cells that developed were a heterogeneous population, consisting of both CD62Lhigh central memory T cells (TCM) and CD62Llow effector memory T cells (TEM) that were competent to produce the Th type 2 effector cytokine, IL-4. Unlike memory T cells that develop following exposure to several other pathogens, both CD4+ TCM and TEM populations persisted in the absence of chronic infection, and, critically, both populations were able to transfer protective immunity to naive recipients. CD62LhighCD4+ TCM were not apparent early after infection, but emerged following clearance of primary infection, suggesting that they may be derived from CD4+ TEM. Consistent with this theory, transfer of CD62LlowCD4+ TEM into naive recipients resulted in the development of a population of protective CD62LhighCD4+ TCM. Taken together, these studies show that distinct subsets of memory CD4+ T cells develop after infection with Trichuris, persist in the GALT, and mediate protective immunity to rechallenge. The Journal of Immunology, 2006, 177: 511–518.

UCOSAL surfaces, such as the respiratory and gastrointestinal (GI) tracts, are primary entry points for many infectious agents, and studies with viral, bacterial, and parasitic pathogens have shown that protective immunity to rechallenge develops at these sites (1–6). For instance, re-exposure to the GI nematode Trichuris muris leads to rapid immune-mediated expulsion of a secondary infection (7). In common with other GI helminths, immunity to a primary infection with Trichuris is dependent upon CD4+ Th type 2 (Th2) cells that develop in the GALT, produce IL-4 and IL-13, and mediate physiological changes in the GI tract (including alterations in epithelial cell turnover, goblet cell hyperplasia, and expression of resistin-like molecule (RELM)β associated with clearance of the worms and sterile immunity (8–12). However, whereas many of the factors that orchestrate immunity to a primary infection with GI pathogens such as Trichuris are well defined, those that regulate T cell memory and immunity to rechallenge have not been analyzed.

Memory T cells are heterogeneous and have been separated into at least two distinct subsets based upon phenotype, function, and migratory pattern (4, 13–15). Central memory T cells (TCM) express high levels of CD62L and can migrate through secondary lymphoid tissues, whereas effector memory T cells (TEM) express low levels of CD62L and accumulate at extralymphoid sites. Although memory T cell subsets have been characterized most extensively using models of CD8+ T cell memory (16–19), the development and maintenance of memory CD4+ T cells is less well understood. We recently found that following infection with the protozoan parasite Leishmania major, no CD4+ effector T cells (T Eff) could be detected once the parasites were eliminated. However, Leishmania-reactive CD4+ TCM developed, persisted in the absence of chronic infection, and mediated immunity to rechallenge (20). In contrast, memory CD4+ T cells that persist following clearance of viral infection in sites draining the lung are enriched for TEM, as measured by both surface phenotype and cytokine production (21, 22). Thus, the mechanisms associated with the development and persistence of memory CD4+ T cell responses following exposure to different pathogens remain unclear.

In this study, we functionally characterize for the first time the CD4+ T cell memory response that develops following exposure to the intestinal helminth parasite, Trichuris. Unlike memory CD4+ T cells that develop following infection with several other pathogens, sterile immunity to Trichuris is characterized by the persistence of both CD4+ TCM and TEM. In addition, both Trichuris-responsive CD4+ TCM and TEM are efficient at conferring resistance to secondary Trichuris infection. Lastly, these results demonstrate that in addition to expanding the T Eff pool,
CD62L<sup>low</sup> T<sub>EM</sub> can also repopulate the CD62L<sup>high</sup> T<sub>CM</sub> population, thereby replenishing the pathogen-specific T<sub>CM</sub>. Taken together, these studies show that distinct subsets of memory CD4<sup>+</sup> T cells develop after infection, persist in the GALT, and mediate protective immunity to rechallenge.

**Materials and Methods**

**Animals**

BALB/cByJ mice were obtained from The Jackson Laboratory, BALB/c Thy1.1 mice were originally obtained from Dr. L. Turka (University of Pennsylvania, Philadelphia, PA). BALB/c eGFP/IL-4 reporter mice were generated as described previously (23). Animals were maintained in a specific pathogen-free environment at the University of Pennsylvania and tested negative for pathogens in routine screening. All experiments were conducted following the guidelines of the University of Pennsylvania Institutional Animal Care and Use Committee.

**Parasites, Ags, and infections**

*Trichuris muris* was maintained in genetically susceptible or immunocompromised animals. Between days 35 and 42 postinfection, adult worms were isolated and cultured in RPMI 1640 containing 500 U/ml penicillin and 500 μg/ml streptomycin for 24 h. *Trichuris* excretory-secretory Ag was isolated at 4 h, dialyzed, sterile filtered, and protein concentrations were determined by Bradford assay. Ag preparations were then used in lymphocyte restimulations (50 μg/ml). Deposed eggs were collected after 24 h of culture, washed three times in sterile water, incubated at room temperature for 6 wk, and stored at 4°C. Mice were infected on day 0 with 150–200 embryonated eggs, and parasite burdens were assessed on various days postinfection.

**Abs and in vivo depletions**

mAbs were prepared from ammonium sulfate precipitation of hybridoma culture supernatants or ascites and dialyzed extensively in PBS. For CD8<sup>+</sup> T cell depletions, 500 μg of anti-CD8 mAb (H53-59.7) was administered i.p. 24–48 h before sacrifice, which routinely depleted >98% of CD8<sup>+</sup> lymphocytes. For CD4<sup>+</sup> T cell depletions during infection, 1 mg of anti-CD4 mAb (GL1.5) was given i.p. on days 0, 1, 3, 6, and 9 postinfection and depleted >85% of CD4<sup>+</sup> T cells. Control mice received equivalent amounts of purified rat IgG (Sigma-Aldrich).

**Cell culture and cytokine analysis**

At necropsy, the mesenteric lymph node (mLN) was harvested, and single-cell suspensions were prepared in DMEM supplemented with 10% heat-inactivated FBS, 2 mM glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, 25 mM HEPES, and 5 × 10<sup>-5</sup> M 2-ME. Cells were plated at 5 × 10<sup>5</sup> cells/24-well culture plates in medium alone or in the presence of *T. muris* excretory-secretory Ag (50 μg/ml). In addition, 2.5 μg/ml anti-IL-4 mAb (clone M1; BD Pharmingen) was added to cultures to enhance responses. For CD4<sup>+</sup> T cell depletions from control mice following in vitro restimulation (Fig. 1F), 1 mg of anti-CD4 mAb (H11022) was maintained in genetically susceptible or immunocompromised mice in a specific pathogen-free environment at the University of Pennsylvania and tested negative for pathogens in routine screening. All experiments were conducted following the guidelines of the University of Pennsylvania Institutional Animal Care and Use Committee. For intracellular staining, cells were permeabilized with 0.5% saponin before staining. Analysis was conducted using CellQuest Pro software (version 5.1; BD Biosciences).

**Statistics**

Results represent the mean ± SD of individual animals. Statistical significance was determined by Student’s t test (when comparing two groups) or ANOVA with a post hoc test (when comparing more than two groups).

**Results**

**Long-term Trichuris-specific CD4<sup>+</sup> Th2 memory in the absence of persistent infection**

Following sterile cure of a primary infection with *Trichuris*, mice exhibit enhanced resistance to reinfection (7, 27). Although CD4<sup>+</sup> T cells are critical for primary worm expulsion (28), it is not known whether memory CD4<sup>+</sup> T cells develop during primary infection nor whether these cells are required for resistance to reinfection. To investigate this, we examined the immune response of immune mice (>60 days postinfection) before reinfection as well as the kinetics of the response to challenge. Before reinfection, cells isolated from immune mice did not produce detectable levels of IFN-γ or IL-13 (data not shown), but secreted significantly higher levels of Ag-specific IL-4 and IL-5 than cells from control mice following in vitro restimulation (Fig. 1A). Rechallenge of immune mice with *Trichuris* resulted in accelerated worm expulsion (Fig. 1B), undetectable levels of *Trichuris*-specific IFN-γ (data not shown), but early increased production of IL-4, IL-5, and IL-13 (Fig. 1C). Associated with elevated expression of type 2 cytokines, rechallenged immune mice exhibited enhanced goblet cell hyperplasia (Fig. 1D; 1°, 160 ± 12 goblet cells/20 crypts; 2°, 425 ± 20 goblet cells/20 crypts) and rapid expression and secretion of RELMβ (Fig. 1E), a goblet cell-specific molecule associated with expulsion of helminth infections (8). Both the rapid expulsion and enhanced immune response were lost when CD4<sup>+</sup> T cells were depleted from immune mice (Fig. 1, B–E). Furthermore, we found that CD4<sup>+</sup> T cells are not only required, but are also sufficient to transfer immunity to naive mice. Transfer of CD4<sup>+</sup> T cells from immune mice, but not naive mice, resulted in increased goblet cell hyperplasia (Fig. 1F; naive mice, 225 ± 15 goblet cells/20 crypts; immune mice, 645 ± 32 goblet cells/20 crypts), expression of RELMβ (Fig. 1G), and rapid clearance of worms from the GI tract (Fig. 1H). Consistent with the spontaneous type 2 cytokine production by cells isolated from mice infected for 60 days (Fig. 1A), we also observed increased RELMβ expression in unchallenged mice that received cells from immune animals (Fig. 1G). Thus, long-term mucosal immunity...
FIGURE 1. CD4+ T cells mediate immunity to Trichuris. A, Cells isolated from the mLN of naive or long-term infected (day 60) BALB/c mice were restimulated in vitro with medium alone (Med) or infected from the mLN of naive or long-term infected (day 60) BALB/c mice for 60 days. B, Worm burdens were determined at day 12 postinfection, and results are presented as mean ± SD of four individual mice from one representative experiment of two. B–E, Naive (1°) or immune (2°) mice were infected with Trichuris. Some immune mice were treated with anti-CD4 mAb (GK1.5) on days 0, 1, 3, 6 and 9 postinfection (2° α-CD4). B, Worm burdens were determined at day 12 postinfection, and results are presented as mean ± SD of four individual mice from one representative experiment of three. C, Supernatants were analyzed for production of IL-4, IL-5, and IL-13 by ELISA following in vitro restimulation of mLN cells isolated at day 12 postinfection with medium (Med) or Trichuris Ag. Results are presented as mean ± SD of three individual mice from one representative experiment. D, Representative cecal sections from groups of three infected (1°) or rechallenged immune (2°) mice ± anti-CD4 mAb taken on day 12 postinfection were stained for goblet cells. Results are representative of three independent experiments. E, RELMβ expression was examined in pooled fecal pellets (20 μg of total protein) from groups of three infected (1°), rechallenged immune (2°), or CD4+ T cell-depleted rechallenged immune (2° anti-CD4) mice at days 6, 8, 10, and 12 postinfection and are representative of three independent experiments. Numbers below bands represent relative band intensities. F–H, CD4+ T cells (10 × 10^6) isolated from the mLN and spleen of naive or immune (day 60–120 postinfection) BALB/c mice were transferred into naive hosts, infected with Trichuris 24 h later, and analyzed on day 12 postchallenge. F, Representative cecal sections from groups of three mice receiving cells from naive (naive cells) or immune (immune cells) mice on day 12 postinfection were stained for goblet cells. G, RELMβ expression was analyzed by immunoblotting of protein isolated from pooled fecal pellets (20 μg of total protein) from each group of three mice collected at day 12 postinfection and are representative of three independent experiments. Numbers below bands represent relative band intensities. H, Worm burdens were analyzed at day 12 postinfection, and results are presented as mean ± SD of four individual mice from one representative experiment of three. ND, Not detected. *p < 0.01 between naive mice and mice infected with Trichuris for 60 days (A), 1°, 2°, and 2° + anti-CD4 (α-CD4) (B and C), and mice receiving naive or immune cells (H).
of Trichuris-specific memory T cells requires a secondary infection and does not allow identification and characterization of persistent Trichuris-specific memory CD4⁺ T cells before rechallenge. To directly examine the nature of the memory CD4⁺ T cells that develop and persist following Trichuris infection, we used bicistronic cytokine reporter mice (23). With this reporter system, expression of eGFP identifies cells that have previously been stimulated to express IL-4 and reflects the potential of cells to produce IL-4 (23, 29). Furthermore, this system allows for the detection of IL-4 competent cells without additional antigenic or pharmacologic stimulation. Following a primary infection of BALB/c reporter mice with Trichuris, >95% of the eGFP/IL-4⁺ cells were CD4⁺ T cells (data not shown). There was a significant increase in the frequency of eGFP/IL-4⁺ CD4⁺ T cells in the mLN during the peak of the primary response, with a 2-fold increase by day 6 postinfection and a 4-fold increase by day 17 (Fig. 3A). These cytokine-positive cells displayed an effector phenotype (CD62Llow or CD44high), demonstrating the development of a primary effector Th2 response after infection with Trichuris (Fig. 3B). Following pathogen clearance (beyond day 17 postinfection), the frequency of eGFP/IL-4⁺ CD4⁺ T cells did not decrease over time (Fig. 3A, day 35 and day 45), demonstrating that a population of CD4⁺ TEM was maintained in the absence of persistent infection. Furthermore, the absolute number of eGFP/IL-4⁺ CD4⁺ cells (total, CD62Lhigh and CD62Llow populations) reflected the increased frequencies observed at all time points postinfection, with significantly higher numbers of cells in the mLN of infected animals compared with naive mice (Fig. 3C). Despite the lack of parasites, it is not possible to conclusively determine whether Trichuris Ag persists after sterile cure. Therefore, we refer to a population of effector cytokine-positive CD4⁺ T cells that persists following sterile cure of infection as TEM. The majority of persistent eGFP/IL-4⁺ CD4⁺ T cells expressed high levels of CD44 or CD62L, characteristic of TEM and consistent with the maintenance of an Ag-specific effector response (Fig. 1A). We also observed the emergence of a population of cells competent to produce IL-4 expressing high levels of CD62L (Fig. 3B, day 35 and day 45, middle panels). Therefore, IL-4-competent cells with the phenotype of either TCM (CD62Lhigh) or TEM (CD62Llow) persisted following sterile cure of Trichuris infection.

Central or effector memory CD4⁺ T cells can mediate immunity to rechallenge

Several previous studies on memory CD8⁺ T cells have shown that TCM provide more effective and rapid immunity than TEM (16, 18, 19). In contrast, CD4⁺ TEM confer more rapid protective immunity to L. major infection than TCM (20, 30, 31). To determine whether the CD4⁺ TEM populations—defined by expression of CD62L—that develop following Trichuris infection differed in their ability to mediate protective immunity, purified cells from wild-type immune donors were separated into CD62Lhigh and CD62Llow fractions, labeled with CFSE, and transferred into naive congenic recipients that were subsequently infected with Trichuris. Examination of the activated (CD44high) CD62Lhigh vs CD62Llow donor cells in the mLN 12 days after infection revealed that there was equivalent proliferation of both subsets (Fig. 4A, histograms). The proliferation of both subsets of memory CD4⁺ T cells was associated with Th2 cytokine-dependent physiological changes in the GI tract, because both populations induced goblet cells (Fig. 4B), RELMβ production (Fig. 4C, inset), and mediated rapid worm expulsion (Fig. 4C). Thus, unlike

FIGURE 3. Central and effector memory CD4⁺ T cells persist in the mLN after pathogen clearance. A, BALB/c eGFP/IL-4 reporter mice were infected with Trichuris, and on days 6, 17, 35, and 45 postinfection cells isolated from the mLN were analyzed for expression of CD4. The mean frequency ± SD of eGFP/IL-4⁺ CD4⁺ cells from four animals per time point is shown. B, Cells isolated in A were analyzed by flow cytometry for expression of CD62L and CD44, and numbers represent frequency of eGFP/IL-4⁺ cells expressing high or low levels of CD62L or CD44 from one representative animal of four per time point. C, Absolute numbers of eGFP/IL-4⁺ CD4⁺ cells and eGFP/IL-4⁺ CD4⁺ CD62Lhigh and eGFP/IL-4⁺ CD4⁺ CD62Llow subsets following infection with Trichuris. Results are representative of two independent experiments, *p < 0.01 between naive (day 0) and infected mice.

FIGURE 4. Central or effector memory CD4⁺ T cells mediate immunity to rechallenge. Cells isolated from the mLN and spleen of immune BALB/c Thy1.1 mice were separated based on high or low expression of CD62L. Purified fractions were labeled with CFSE, transferred into naive congenic recipients, and 24 h later the recipient mice were infected with Trichuris. A, Proliferation of CD44highCD62Lhigh Thy1.1⁺ cells in the mLN on day 12 postinfection was analyzed by flow cytometry. Numbers on histograms represent percentage of donor CD44highCD62Lhigh Thy1.1⁺ cells that proliferated and are from one representative animal of three from three independent experiments. B, Goblet cells were quantitated by direct counting of tissue sections from mice on day 12 postinfection. Data represent mean ± SD number of goblet cells per 20 crypts from groups of three animals and is representative of three experiments. C, Worm burdens were determined microscopically at day 12 postinfection, and results are presented as mean ± SD of three individual mice from one representative experiment of three. Inset, RELMβ expression in pooled fecal pellets from groups of three mice receiving CD62Lhigh (High) or CD62Llow (Low) cells was analyzed at day 12 postinfection by immunoblotting (20 μg of total protein). Numbers refer to relative band intensities compared with mice receiving no cells (1.0; data not shown). Results are representative of three independent experiments. *p < 0.01, between mice receiving no cells, mice receiving CD62Lhigh, or mice receiving CD62Llow cells.
CD4⁺ T cell memory in other infectious diseases, both T_{CM} and T_{EM} persist after sterile cure of *Trichuris* and can mediate protective immunity against secondary infection in the gut.

**Relationship between CD4⁺ T_{CM} and T_{EM}**

Based on the data presented here, two phenotypically distinct populations of mucosal memory CD4⁺ T cells develop after infection with *Trichuris* and exhibit the characteristics of either T_{CM} or T_{EM}. CD62L_{low} IL-4-competent T_{EFF} or T_{EM} are evident throughout primary infection, whereas it appears that eGFP/IL-4 positive CD62L_{low} T_{EFF} or T_{CM} arise after pathogen clearance (Fig. 3B, *central panel*). Studies with CD8⁺ T cells have demonstrated that T_{CM} are derived from T_{EM}, whereas the origin and interrelationship of memory CD4⁺ T cell subsets is unknown (18). To address the relationship between cytokine-competent CD4⁺ T_{CM} (CD62L_{high}) and T_{EM} (CD62L_{low}) that develop following *Trichuris* infection, equal numbers (10 × 10⁶ cells) of CD62L_{high} or CD62L_{low} CD4⁺ T cells were purified from immune eGFP/IL-4 reporter mice (>60 days postinfection). Analysis of CD62L_{high} cells revealed that only a small frequency of the population was eGFP/IL-4 positive (Fig. 5A, *lower left panel*, 1.4%), consistent with the results presented above (Fig. 3B). Upon transfer into congenic recipients, CD62L_{high} cells gave rise to both CD62L_{high} and CD62L_{low} populations after challenge with *Trichuris* (Fig. 5A, *middle histogram*). The cells retaining high expression levels of CD62L remained predominantly eGFP/IL-4 positive (Fig. 5A, *upper right dot plot*, 1.5%), suggesting that effector cytokine expression does not develop in the CD62L_{high} cells directly. In contrast, a significant proportion of CD62L_{low} cells derived from CD62L_{high} donors expressed eGFP/IL-4 (Fig. 5A, *lower right dot plot*, 42%) demonstrating that upon secondary infection, CD62L_{high} T_{CM} can efficiently give rise to cytokine-positive CD62L_{low} T_{EFF}. Therefore, CD62L_{high} T_{CM} that develop during *Trichuris* infection appear to mediate protective immunity primarily by differentiating into IL-4-expressing, CD62L_{low} effector Th2 cells.

In contrast to the CD62L_{high} population, a significant proportion of the donor CD62L_{low} T_{EM} isolated from immune eGFP/IL-4 reporter mice were eGFP/IL-4 negative before transfer (Fig. 5B, *lower left panel*, 15%). Upon transfer and rechallenge, there was significant expansion of CD62L_{low} cells that expressed eGFP/IL-4 (Fig. 5B, *lower right dot plot*, 65%). Highly purified CD62L_{low} cells also gave rise to a population of CD62L_{high} cells in which there was a diminished frequency of cells competent to express IL-4 (Fig. 5B, *upper right dot plot*, 16%). Therefore, CD4⁺ CD62L_{high} T_{CM} can arise from CD62L_{low} T_{EM} following secondary challenge, suggesting that CD4⁺ T_{EM} can replenish the T_{CM} pool following rechallenge with *Trichuris*.

**CD4⁺ T_{EM} transition to T_{CM} in the absence of infection**

To test whether the development of CD4⁺ CD62L_{high} T_{CM} from CD62L_{low} T_{EM} was dependent upon rechallenge or could occur in the absence of infection, equal numbers (10 × 10⁶) of purified CD62L_{high} and CD62L_{low} CD4⁺ T cells from immune mice were transferred into naive recipients in the absence of infection (Fig. 6, *left panels*). Three weeks later, CFSE^{bright} donor CD4⁺ T cells in the mLN were analyzed for expression of CD62L. Following transfer, persistent CD62L_{high}CD4⁺ T cells maintained expression of CD62L (Fig. 6, *upper right panel*). In contrast, donor CD62L_{low}CD4⁺ T cells that persisted after transfer did not remain uniformly CD62L_{low}, because a significant proportion expressing high levels of CD62L emerged (Fig. 6, *lower right histogram*). This increase could be the result of either increased death of CD62L_{low}CD4⁺ T cells or a selective outgrowth of contaminating CD62L_{high} T cells. In the absence of MHC class II tetramers or TCR transgenic T cells for helminth parasites such as *Trichuris*, at present we cannot definitively say that the loss of CD62L_{low} T cells was not a factor in the appearance of the CD62L_{high} population. However, absolute numbers of recovered CD62L_{high} and CD62L_{low} T cells from the mLN following transfer of purified CD62L_{low} cells were similar (CD62L_{high}, 1.9 × 10⁶; CD62L_{low}, 1.3 × 10⁶), suggesting that this is due to selective survival. Furthermore, analysis of CFSE-labeled cells shows that this transition occurred independent of proliferation (Fig. 6, *dot plots*), suggesting that the CD62L_{high} T_{CM} that arise following transfer of CD62L_{low} T_{EM} are not the result of expansion of a small number of contaminating CD62L_{high} T_{CM} in the donor CD62L_{low} T_{EM} population. Rather, these data indicate that T_{EM} can directly convert into T_{CM}. Thus, similar to memory CD8⁺ T cells, the transition from CD62L_{low}CD4⁺ T_{EM} to CD62L_{high}CD4⁺ T_{CM} does not require infection and may be a natural step in the development of long-lived memory CD4⁺ T cells.

Taken together, the results presented in this study support a novel model of memory CD4⁺ T cell development and maintenance at mucosal sites (Fig. 7), in which infection results in the

![FIGURE 5](image-url) Relationship between central and effector memory CD4⁺ T cells. CD4⁺ T cells isolated from the mLN and spleen of immune BALB/c eGFP/IL-4 reporter mice were separated into CD62L_{high} (A) or CD62L_{low} (B) fractions, and 10 × 10⁶ cells of each fraction were transferred into congenic recipients. Twenty-four hours later, the recipients were infected with *Trichuris*. Before transfer and on day 12 postinfection, donor CD4⁺ Thy1.2⁺ T cells were analyzed by flow cytometry for eGFP/IL-4 and CD62L expression. Histograms are gated on donor Thy1.2⁺ cells, and dot plots on the right side are gated on either CD62L_{high} or CD62L_{low} subpopulations. Results presented are from one individual animal of three and are representative of three independent experiments.

![FIGURE 6](image-url) CD4⁺ T_{EM} transition to T_{CM} in the absence of infection. CD4⁺ T cells isolated from the mLN and spleen of immune BALB/c Thy1.1 mice were separated into CD62L_{high} or CD62L_{low} fractions (input), labeled with CFSE, and 10 × 10⁶ cells were transferred into congenic recipients. Before transfer and on day 21 posttransfer, donor CD4⁺ Thy1.1⁺ T cells in the mLN were analyzed by flow cytometry for CD62L expression and dilution of CFSE. Results presented are from one individual animal of three and are representative of two independent experiments.
recent studies have demonstrated that IFN-γ-producing T cells are short-lived in vivo (35, 36), whereas Th2 cells are more amenable to surviving in adoptive hosts (37) and are more resistant to activation-induced cell death than Th1 cells (38, 39). Therefore, maintenance of Trichuris-specific TEM in the GALT may reflect the differences in the survival of Th1 and Th2 memory cells in vivo. Supporting this theory, in vitro- or in vivo-generated TCR transgenic CD4+ Th2 cells can persist for several weeks in vivo in the absence of Ag and respond rapidly to secondary stimulation by producing effector cytokines such as IL-4 (37, 40, 41). In addition, a previous study demonstrated that adoptive transfer of effector CD4+ T cells isolated from mice infected with Trichuris were able to persist for more than 40 days and mediate protective immunity to rechallenge (42). IL-4-competent CD4+ T cells with the characteristics of TEM also persisted in the absence of chronic infection with another GI helminth parasite, Nippostrongylus brasiliensis, and mediated protective immunity to rechallenge (23).

Given that virus-responsive memory CD4+ T cells that express IFN-γ can persist in lung-draining LN, commitment to distinct Th cell subsets cannot be the only explanation for the differences in persistence of memory T cells. In addition to intrinsic mechanisms that may differentially regulate the persistence and function of memory T cells, tissue-specific regulation of T cell memory may contribute to the development of Trichuris-specific memory cells. For instance, microbial and environmental stimuli and/or the presence of specialized APC populations in the gut may influence the persistence and function of memory CD4+ T cells following exposure to Trichuris. Certainly, activation of T cells by mucosal dendritic cells (DC) results in cytokine expression and homing phenotypes that are distinct from T cells primed by peripheral DCs (43–47). Therefore, it is possible that priming of CD4+ T cells by DCs in the gut and other mucosal sites such as the lung will also affect the ontogeny, survival, and function of memory T cells and the mechanisms that regulate their function upon re-exposure to infection. Furthermore, microbial stimuli from both normal and pathogenic gut flora, coupled with environmental Ags, may also constitute unique signals that affect the quality of the memory responses in the gut (48).

In addition to the persistent CD62Llow TEM memory response, CD62Lhigh TEM develop after infection, persisted after sterile cure, and could mediate immunity to rechallenge in the GI tract. CD4+ TEM develop after clearance of primary or secondary Trichuris infection, express high levels of CD62L, are able to express IL-4, and appear to derive from CD62Llow TEM cells (Fig. 7 and Ref. 2), although it is possible that they arise early following primary infection (Fig. 7, dotted line). Following rechallenge, these CD62Lhigh TEM can give rise to an IL-4-expressing CD62Llow population (Fig. 7 and Ref. 3) and contribute to the TEM pool. Previous studies with CD4+ T cells have suggested that commitment to effector function is limited to TEM but not TEM, whereas CD8+ TEM can produce effector cytokines such as IFN-γ and express effector molecules such as perforin (18, 20, 49–51). Results presented in this study demonstrate that commitment to effector cytokine expression in TEM populations is not restricted to CD8+ T cells and support the contention that common regulatory pathways may exist in the memory CD4+ and CD8+ T cell compartments.

A question that arises is why maintaining both persistent Trichuris-responsive CD4+ TEM and TEM would be advantageous to the host. One possibility is that having a subset of CD4+ TEM repopulating the TEM pool provides an intrinsic pathway to protect the repertoire of memory responses while allowing a rapid, but flexible response. Maintaining CD62Lhigh LN-homing memory T
cells allows licensing of a subset of Trichuris-responsive memory T cells to traffic through peripheral LNs, which is primarily a CD62L-dependent phenomenon, thereby facilitating recirculation to additional sites, more extensive immunological surveillance, and allowing memory cells to encounter additional survival signals that may be present at optimal concentrations in extra-GALT sites. At the same time, persistent TEM facilitate rapid immune responses upon re-exposure to infection.

Recent studies have provided compelling evidence for the importance of Th2 responses in secondary immunity to helminth infection in humans (52, 53). Jackson et al. (53) demonstrated that increased Th2 cytokine responses immediately before deworming and upon re-exposure to infection. At the same time, persistent TEM facilitate rapid immune responses to additional sites, more extensive immunological surveillance, and CD62L-dependent phenomenon, thereby facilitating recirculation to the GI tract. In addition, TEM develop in the GALT, exhibiting the ability to differentiate into effector CD4+ T cells and mediate protective immunity. These results identify novel aspects of CD4+ T cell memory in the GI tract and provide a framework to investigate the factors that regulate the maintenance of distinct memory CD4+ T cell populations at mucosal sites.

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Disclosures

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