Persistence and Function of Central and Effector Memory CD4$^+$ T Cells following Infection with a Gastrointestinal Helminth[1]

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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 CD62L^{high} central memory T cells (T_{CM}) and CD62L^{low} effector memory T cells (T_{EM}) 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$^+$ T_{CM} and T_{EM} populations persisted in the absence of chronic infection, and, critically, both populations were able to transfer protective immunity to naive recipients. CD62L^{high}CD4$^+$ T_{CM} were not apparent early after infection, but emerged following clearance of primary infection, suggesting that they may be derived from CD4$^+$ T_{EM}. Consistent with this theory, transfer of CD62L^{low}CD4$^+$ T_{EM} into naive recipients resulted in the development of a population of protective CD62L^{high}CD4$^+$ T_{CM}. 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.**

Mucosal surfaces, such as the respiratory and gastrointestinal (GI)[3] 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 (T_{CM}) express high levels of CD62L and can migrate through secondary lymphoid tissues, whereas effector memory T cells (T_{EM}) 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}) or T_{EM} could be detected once the parasites were eliminated. However, *Leishmania*-reactive CD4$^+$ T_{CM} 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 T_{EM}, 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$^+$ T_{CM} and T_{EM}. In addition, both *Trichuris*-responsive CD4$^+$ T_{CM} and T_{EM} are efficient at conferring resistance to secondary *Trichuris* infection. Lastly, these results demonstrate that in addition to expanding the T_{EFF} pool,

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[3] Abbreviations used in this paper: GI, gastrointestinal; Th2, Th type 2; RELM, resistin-like molecule; T_{CM}, central memory T cell; T_{EM}, effector memory T cell; T_{EFF}, effector T cell; mLN, mesenteric lymph node; pos, positive; DC, dendritic cell.

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$CD62L^{low}$ T_{EM} can also repopulate the $CD62L^{high}$ T_{CM} population, thereby replenishing the pathogen-specific T_{CM}. Taken together, these studies show that distinct subsets of memory $CD4^+$ 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

T. 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). Deposited 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 $CD8^+$ T cell depletions, 500 μg of anti-CD8 mAb (H35) was administered i.p. 24–48 h before sacrifice, which routinely depleted >98% of $CD8^+$ lymphocytes. For $CD4^+$ T cell depletions during infection, 1 mg of anti-CD4 mAb (GK1.5) was given i.p. on days 0, 1, 3, 6, and 9 postinfection and depleted >98% of $CD4^+$ 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^{-5} M 2-ME. Cells were plated at 5×10^6/ml in 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-4Rα mAb (clone M1; BD Pharmingen) was added to cultures to enhance detection of IL-4. Cell-free supernatants were harvested after 48 h, and cytokine analysis was conducted by sandwich ELISA using paired mAb to detect IL-4 (11B11 and BVD6-24G2.3), IL-5 (TRFK-5 and TRFK-4), and IL-13 (R&D Systems) as described previously (24).

Analysis of goblet cell and RELMβ responses

Segments of cecum were removed, washed in sterile PBS, and fixed for 24 h in 4% paraformaldehyde. Tissues were processed routinely and paraffin embedded using standard histological techniques. For detection of intestinal goblet cells, 5-μm sections were cut and stained with Alcian blue-periodic acid-Schiffs reagent. Isolation of proteins from fecal samples was performed as described previously (8). Equal amounts of protein (20 μg) were analyzed by SDS-PAGE and immunoblotted for RELMβ with a polyclonal rabbit anti-murine RELMβ Ab (PeproTech). Relative band intensities were quantified using ImageJ software (National Institutes of Health, ⟨http://rsb.info.nih.gov/ij/⟩).

$CD4^+$ T cell purification and adoptive transfer

Naive or immune (between 60 and 120 days postinfection) BALB/c Thy1.1, Thy1.2, or eGFP/IL-4 reporter mice were depleted of $CD8^+$ T cells by injection with anti-CD8 mAb 1 and 3 days before sacrifice (>98% effective). Cells were isolated from draining mLN and spleen, and RBC were lysed in hypotonic solution. In some experiments, $CD4^+$ T cells were purified using a T cell purification column (R&D Systems) according to the manufacturer's recommendations. In some experiments, $CD4^+$ T cells were further separated based on expression of CD62L by MACS columns (Miltenyi Biotec) with 95–98% purity of $CD62L^{high}$ and $CD62L^{low}$ fractions. There were no phenotypic or functional differences in the cells isolated from animals that were between 60 and 120 days postinfection. $CD4^+$ T cells were stained with CFSE (Molecular Probes) as described previously (25, 26). A total of 10×10^6 CFSE-labeled $CD4^+$ T cells was transferred via the retro-orbital plexus into naive congenic recipients. Mice were infected 24 h or 3 wk later with *Trichuris*.

Flow cytometry and intracellular cytokine staining

Cells were stained with fluorochrome-conjugated mAbs against CD4 (RM4-5), CD44 (IM7), CD62L (MEL14), IL-4 (11B11), and Thy1.1 (OX-7) or isotype-specific control Abs (eBioscience) before acquisition on a FACSCalibur flow cytometer (BD Pharmingen). Briefly, cells were isolated from the draining mLN and spleen and were analyzed for expression of surface markers directly ex vivo without further activation or for intracellular cytokines following 4 h of pharmacologic stimulation with PMA (50 ng/ml) and ionomycin (500 ng/ml) in the presence of Brefeldin A (10 μg/ml). Cells were washed in staining buffer (PBS containing 0.1% BSA and 0.1% sodium azide) and incubated with Fc block (50 μg/ml 2.4G2 and 500 μg/ml rat Ig) before incubation with specific fluorochrome-conjugated mAbs. Cells were washed in staining buffer and fixed in 2% paraformaldehyde (Electron Microscopy Services). 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 $CD4^+$ 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 $CD4^+$ T cells are critical for primary worm expulsion (28), it is not known whether memory $CD4^+$ 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 postprimary infection) 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. 1*A*). Rechallenge of immune mice with *Trichuris* resulted in accelerated worm expulsion (Fig. 1*B*), undetectable levels of *Trichuris*-specific IFN-γ (data not shown), but early increased production of IL-4, IL-5, and IL-13 (Fig. 1*C*). Associated with elevated expression of type 2 cytokines, rechallenged immune mice exhibited enhanced goblet cell hyperplasia (Fig. 1*D*; 1°, 160 ± 12 goblet cells/20 crypts; 2°, 425 ± 20 goblet cells/20 crypts) and rapid expression and secretion of RELMβ (Fig. 1*E*), a goblet cell-specific molecule associated with expulsion of helminth infections (8). Both the rapid expulsion and enhanced immune response were lost when $CD4^+$ T cells were depleted from immune mice (Fig. 1, *B–E*). Furthermore, we found that $CD4^+$ T cells are not only required, but are also sufficient to transfer immunity to naive mice. Transfer of $CD4^+$ T cells from immune, but not naive, mice resulted in increased goblet cell hyperplasia (Fig. 1*F*; naive cells, 225 ± 15 goblet cells/20 crypts; immune cells, 645 ± 32 goblet cells/20 crypts), expression of RELMβ (Fig. 1*G*), and rapid clearance of worms from the GI tract (Fig. 1*H*). Consistent with the spontaneous type 2 cytokine production by cells isolated from mice infected for 60 days (Fig. 1*A*), we also observed increased RELMβ expression in unchallenged mice that received cells from immune animals (Fig. 1*G*). Thus, long-term mucosal immunity

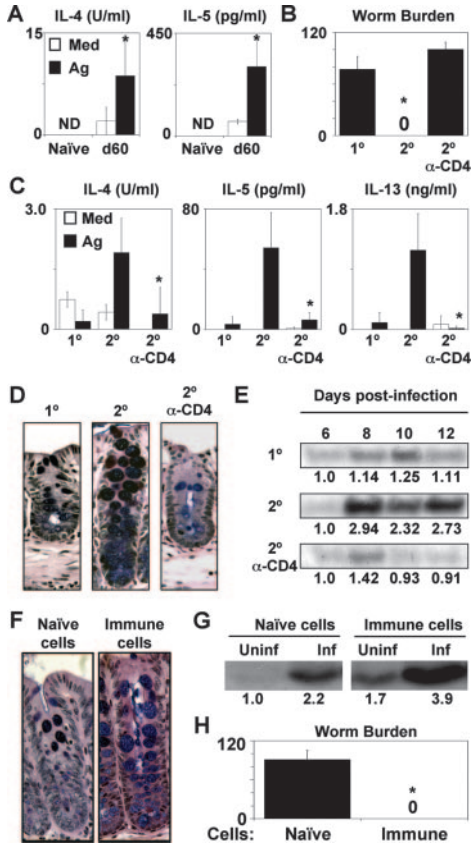

FIGURE 1. CD4$^+$ T cells mediate immunity to *Trichuris*. *A*, Cells isolated from the mLN of naive or long-term infected (day 60) BALB/c mice were restimulated in vitro with medium alone (Med) or *Trichuris* Ag, and supernatants were analyzed for IL-4 and IL-5 by ELISA. Results are presented as mean ± 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 of three. *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 three 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*).

against *Trichuris* infection is dependent upon memory CD4$^+$ T cells that persist in the absence of chronic infection and mediate rapid protective immunity.

Rapid expansion and effector differentiation of Trichuris*-specific memory CD4$^+$ T cells following rechallenge*

To visualize the proliferation and function of *Trichuris*-responsive memory CD4$^+$ T cells following rechallenge, CFSE-labeled CD4$^+$ T cells from naive or immune mice were adoptively transferred into congenic naive recipients that were subsequently infected with *Trichuris*. At day 12 postinfection, CD4$^+$ T cells from immune mice responded more robustly to infection by proliferating, demonstrating that *Trichuris*-specific memory CD4$^+$ T cells have the capability to expand rapidly following re-exposure to infection (Fig. 2*A*). Phenotypic analysis revealed that the *Trichuris*-responsive CD4$^+$ T cells (CFSE^{dim}) were predominantly CD62L^{low}, a characteristic of T_{EFF} or T_{EM} (Fig. 2*B*, *left panels*). Furthermore, consistent with the expanded population of *Trichuris*-specific CD62L^{low} cells, there was an increased frequency of effector cytokine-positive (IL-4^{pos}) cells in the mLN of mice receiving CD4$^+$ T cells from immune donors (Fig. 2*B*, *right panels*). Accelerated IL-4 production was associated with the enhanced goblet cell responses and rapid worm expulsion observed following adoptive transfer of *Trichuris*-responsive memory CD4$^+$ T cells (Fig. 1, *F–H*).

CD4$^+$ T_{CM} and T_{EM} persist after worm expulsion

The congenic adoptive transfer system described above allows tracking of memory CD4$^+$ T cells in vivo; however, identification

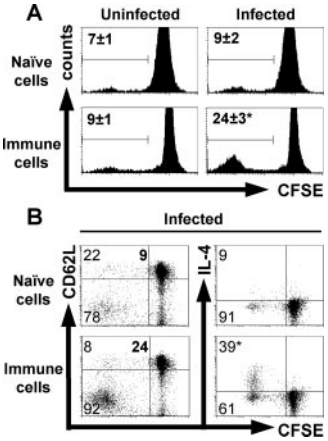

FIGURE 2. *Trichuris*-specific memory CD4$^+$ T cells rapidly expand and produce IL-4 following rechallenge. CD4$^+$ T cells were isolated from the mLN and spleen of naive or immune BALB/c Thy1.1 mice, labeled with CFSE, and 10×10^6 cells were transferred into naive congenic recipients that were infected with *Trichuris* 24 h later. *A*, Proliferation of CD4$^+$Thy1.1$^+$ cells in the mLN on day 12 postinfection was visualized by flow cytometry. Numbers represent percentage of donor CD4$^+$ T cells that diluted CFSE. *B*, Expression of CD62L on donor CD4$^+$ T cells in the mLN on day 12 postinfection was analyzed by flow cytometry directly ex vivo without additional activation, whereas cytokine production was determined after a 4-h pharmacologic stimulation. Numbers in bold represent percentage of donor CD4$^+$ T cells that diluted CFSE, whereas numbers in *upper* and *lower left corners* represent percentage of proliferated cells (CFSE^{dim}) that express high or low levels of CD62L (*left panels*) or IL-4 (*right panels*). Results are from one animal of three per group and are representative of three independent experiments. ∗, $p < 0.01$ between mice receiving naive cells or immune cells.

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^{pos} $CD4^+$ 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. 3*A*). These cytokine-positive cells displayed an effector phenotype ($CD62L^{low}$ or $CD44^{high}$), demonstrating the development of a primary effector Th2 response after infection with *Trichuris* (Fig. 3*B*). Following pathogen clearance (beyond day 17 postinfection), the frequency of eGFP/IL-4^{pos} $CD4^+$ T cells did not decrease over time (Fig. 3*A*, day 35 and day 45), demonstrating that a population of $CD4^+$ T_{EM} was maintained in the absence of persistent infection. Furthermore, the absolute number of eGFP/IL-4^{pos} $CD4^+$ cells (total, $CD62L^{high}$ and $CD62L^{low}$ 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. 3*C*). 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 T_{EM}. The majority of persistent eGFP/IL-4^{pos} $CD4^+$ T cells expressed high levels of CD44 or CD62L, characteristic of T_{EM} and consistent with the maintenance of an Ag-specific effector response (Fig. 1*A*). We also observed the emergence of a population of cells competent to produce IL-4 expressing high levels of CD62L (Fig. 3*B*, day 35 and day 45, *middle panels*). Therefore, IL-4-competent cells with the phenotype of either T_{CM} ($CD62L^{high}$) or T_{EM} ($CD62L^{low}$) persisted following sterile cure of *Trichuris* infection.

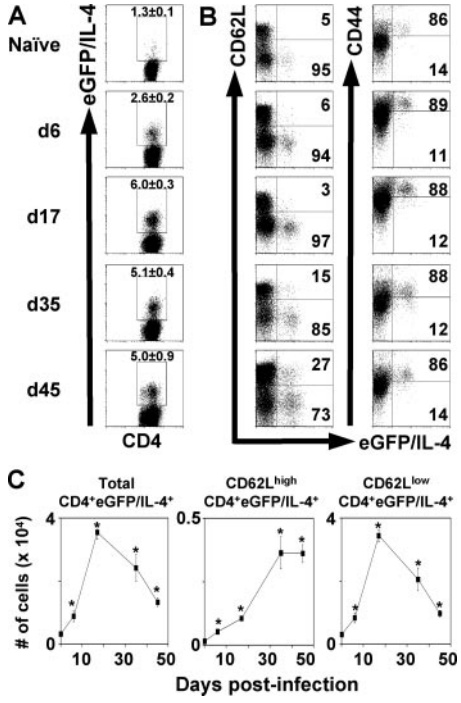

FIGURE 3. Central and effector memory $CD4^+$ T cells persist in the mLN after pathogen clearance. *A*, BALB/c eGFP/IL-4 reporter mice were infected with *Trichuris*, and on days 6, 17, 35, and 45 postinfection cells isolated from the mLN were analyzed for expression of CD4. The mean frequency ±SD of eGFP/IL-4^{pos} $CD4^+$ 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^{pos} 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^{pos} $CD4^+$ cells and eGFP/IL-4^{pos} $CD4^+CD62L^{high}$ and eGFP/IL-4^{pos} $CD4^+CD62L^{low}$ subsets following infection with *Trichuris*. Results are representative of two independent experiments. *, $p < 0.01$ between naive (day 0) and infected mice.

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

Several previous studies on memory $CD8^+$ T cells have shown that T_{CM} provide more effective and rapid immunity than T_{EM} (16, 18, 19). In contrast, $CD4^+$ T_{EFF} or T_{EM} confer more rapid protective immunity to *L. major* infection than T_{CM} (20, 30, 31). To determine whether the $CD4^+$ T_{CM} or T_{EM} 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 $CD62L^{high}$ and $CD62L^{low}$ fractions, labeled with CFSE, and transferred into naive congenic recipients that were subsequently infected with *Trichuris*. Examination of the activated ($CD44^{high}$) $CD62L^{high}$ vs $CD62L^{low}$ donor cells in the mLN 12 days after infection revealed that there was equivalent proliferation of both subsets (Fig. 4*A*, 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. 4*B*), RELMβ production (Fig. 4*C*, *inset*), and mediated rapid worm expulsion (Fig. 4*C*). Thus, unlike

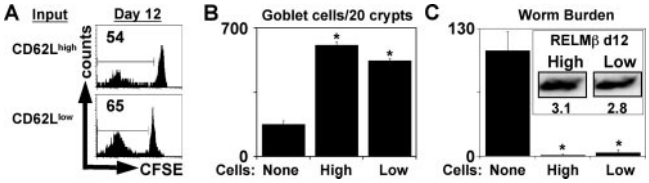

FIGURE 4. Central or effector memory $CD4^+$ T cells mediate immunity to rechallenge. Cells isolated from the mLN and spleen of immune BALB/c Thy1.1 mice were separated based on high or low expression of CD62L. Purified fractions were labeled with CFSE, transferred into naive congenic recipients, and 24 h later the recipient mice were infected with *Trichuris*. *A*, Proliferation of $CD44^{high}CD4^+Thy1.1^+$ cells in the mLN on day 12 postinfection was analyzed by flow cytometry. Numbers on histograms represent percentage of donor $CD44^{high}CD4^+Thy1.1^+$ cells that proliferated and are from one representative animal of three from three independent experiments. *B*, Goblet cells were quantified 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 $CD62L^{high}$ (High) or $CD62L^{low}$ (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 $CD62L^{high}$, or mice receiving $CD62L^{low}$ 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^{pos} $CD62L^{high}$ T_{CM} arise after pathogen clearance (Fig. 3*B*, *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 (10×10^6 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^{pos} (Fig. 5*A*, *lower left panel*, 1.4%), consistent with the results presented above (Fig. 3*B*). Upon transfer into congenic recipients, $CD62L^{high}$ cells gave rise to both $CD62L^{high}$ and $CD62L^{low}$ populations after challenge with *Trichuris* (Fig. 5*A*, *middle histogram*). The cells retaining high expression levels of CD62L remained predominantly eGFP/IL-4^{neg} (Fig. 5*A*, *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. 5*A*, *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^{pos} before transfer (Fig. 5*B*, *lower left panel*, 15%). Upon transfer and rechallenge, there was significant expansion of $CD62L^{low}$ cells that expressed eGFP/IL-4 (Fig. 5*B*, *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. 5*B*, *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*.

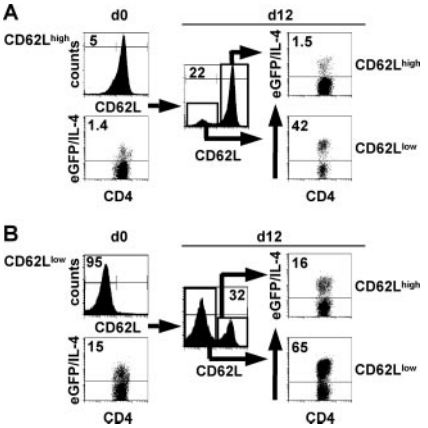

FIGURE 5. Relationship between central and effector memory $CD4^+$ T cells. $CD4^+$ T cells isolated from the mLN and spleen of immune BALB/c eGFP/IL-4 reporter mice were separated into $CD62L^{high}$ (*A*) or $CD62L^{low}$ (*B*) fractions, and 10×10^6 cells of each fraction were transferred into congenic recipients. Twenty-four hours later, the recipients were infected with *Trichuris*. Before transfer and on day 12 postinfection, donor $CD4^+Thy1.2^+$ 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.

$CD4^+$ 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 (10×10^6) 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 cells 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 $CD62L^{low}$ cells from the mLN following transfer of purified $CD62L^{low}$ cells were similar ($CD62L^{high}$, 1.9×10^4; $CD62L^{low}$, 1.3×10^4), suggesting that this is not 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

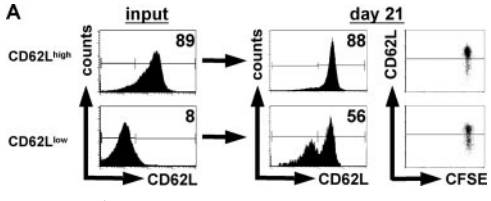

FIGURE 6. $CD4^+$ T_{EM} transition to T_{CM} in the absence of infection. $CD4^+$ T cells isolated from the mLN and spleen of immune BALB/c Thy1.1 mice were separated into $CD62L^{high}$ or $CD62L^{low}$ fractions (input), labeled with CFSE, and 10×10^6 cells were transferred into congenic recipients. Before transfer and on day 21 posttransfer, donor $CD4^+Thy1.1^+$ 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.

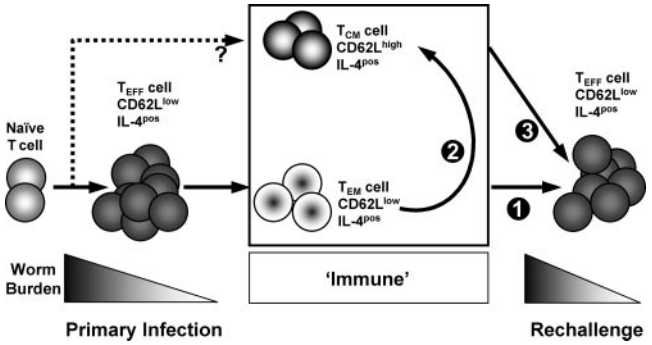

FIGURE 7. Model of CD4$^+$ T cell memory in the GALT. During a primary infection, a robust Th2 effector (T_{EFF}) cell population develops that mediates protective immunity. Following sterile cure, T_{EM} that are CD62L^{low} and IL-4^{pos} and T_{CM} that are CD62L^{high} and IL-4^{pos} persist. Upon rechallenge, T_{EM} rapidly proliferate and expand the T_{EFF} pool (1). Persistent CD62L^{low} T_{EM} also can transition into CD62L^{high} T_{CM} following clearance of a primary or secondary infection (2), although CD62L^{high} T_{CM} may also arise before sterile cure (dotted line). Following secondary infection, these T_{CM} can also rapidly give rise to T_{EFF} and contribute to the rapid immune response associated with long-term immunity to infection (3).

persistence of T_{CM} and T_{EM} in the GALT after pathogen clearance. Moreover, these memory cell populations appear to have the potential to repopulate central and effector memory cell pools in vivo.

Discussion

In this report, we show that long-term immunity to *Trichuris* is dependent upon memory CD4$^+$ T cells and identify three important aspects of CD4$^+$ T cell memory in the GI tract. First, both CD4$^+$ T_{CM} and T_{EM} develop and persist after sterile cure of *Trichuris* infection. Second, both CD4$^+$ T_{CM} and T_{EM} that arise following infection exhibit the ability to mediate protective immunity to rechallenge. Finally, similar to memory CD8$^+$ T cells, CD4$^+$ T_{CM} can derive from T_{EM} following sterile cure of infection.

Previous studies of CD4$^+$ and CD8$^+$ T cell memory following infection with *L. major*, *Listeria monocytogenes*, or lymphocytic choriomeningitis virus have suggested that IFN-γ-producing T_{EFF} or T_{EM} require persistent infection to be sustained, whereas T_{CM} can persist in the absence of chronic infection (18, 20, 30, 31). In contrast, the majority of virus-specific memory CD4$^+$ T cells that are maintained in sites draining the lung in the absence of Sendai virus infection have an activated phenotype (CD44^{high}, CD62L^{low}) and rapidly express IFN-γ, characteristics of T_{EM} (21, 22). Therefore, the identification of a population of *Trichuris*-specific IL-4-expressing CD62L^{low}CD4$^+$ T cells—a phenotype consistent with a T_{EM} population—that persists after sterile cure of primary infection is more consistent with the composition of memory CD4$^+$ T cells observed in the lung following clearance of viral infection than other infections. It is important to note that although mice that have cleared a primary *Trichuris* infection have no persistent parasites, it is not possible to conclude that there is no residual Ag that remains following sterile cure of infection. Nevertheless, upon rechallenge, this persistent T_{EM} population is able to proliferate and rapidly expand the T_{EFF} pool (Fig. 7 and Ref. 1).

The disparity between the persistence and function of T_{CM} and T_{EM} in different infection models may be influenced by the cytokine polarization of the CD4$^+$ T cell memory responses. *Trichuris* induces a strong type 2 response at the mucosal site of infection, whereas infection with viral, bacterial, or protozoan pathogens results primarily in potent type 1 memory responses associated with the production of IFN-γ (32–34). Previous 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 T_{EM} 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 T_{EM} 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 CD62L^{low} T_{EM}, *Trichuris*-responsive CD62L^{high}CD4$^+$ T_{CM} developed after infection, persisted after sterile cure, and could mediate immunity to rechallenge in the GI tract. CD4$^+$ T_{CM} 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 CD62L^{low} T_{EM} cells (Fig. 7 and Ref. 2), although it is possible that they arise early following primary infection (Fig. 7, dotted line). Following rechallenge, these CD62L^{high} T_{CM} can give rise to an IL-4-expressing CD62L^{low} population (Fig. 7 and Ref. 3) and contribute to the T_{EFF} pool. Previous studies with CD4$^+$ T cells have suggested that commitment to effector function is limited to T_{EM} but not T_{CM}, whereas CD8$^+$ T_{CM} 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 T_{CM} 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$^+$ T_{EM} and T_{CM} would be advantageous to the host. One possibility is that having a subset of CD4$^+$ T_{EM} repopulating the T_{CM} pool provides an intrinsic pathway to protect the repertoire of memory responses while allowing a rapid, but flexible response. Maintaining CD62L^{high} 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 T_{EM} 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 had a significant negative effect on the probability of reinfection several months later. However, human infections tend to be repeated low-dose infections, whereas the data presented in this report are from a single inoculation of parasites. Nevertheless, the demonstration here that persistent of *Trichuris*-specific memory Th2 cells can mediate rapid immunity to rechallenge, reinforces the idea that long-term antihelminth immunity—via immunization or prophylactic treatment of infection—is an attainable goal.

Together, these results provide a model of mucosal $CD4^+$ T cell memory comprised of distinct memory T cell subsets. A population of persistent, cytokine-competent T_{EM} provides a potent effector response to control infection in the GI tract. In addition, T_{CM} 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

The authors have no financial conflict of interest.

References

1. Kim, S. K., K. S. Schluns, and L. Lefrancois. 1999. Induction and visualization of mucosal memory CD8 T cells following systemic virus infection. *J. Immunol.* 163: 4125–4132.
2. Lepage, A. C., D. Buzoni-Gatel, D. T. Bout, and L. H. Kasper. 1998. Gut-derived intraepithelial lymphocytes induce long term immunity against *Toxoplasma gondii*. *J. Immunol.* 161: 4902–4908.
3. Marzo, A. L., V. Vezys, K. Williams, D. F. Tough, and L. Lefrancois. 2002. Tissue-level regulation of Th1 and Th2 primary and memory CD4 T cells in response to *Listeria* infection. *J. Immunol.* 168: 4504–4510.
4. Masopust, D., V. Vezys, A. L. Marzo, and L. Lefrancois. 2001. Preferential localization of effector memory cells in nonlymphoid tissue. *Science* 291: 2413–2417.
5. Pope, C., S. K. Kim, A. Marzo, D. Masopust, K. Williams, J. Jiang, H. Shen, and L. Lefrancois. 2001. Organ-specific regulation of the CD8 T cell response to *Listeria monocytogenes* infection. *J. Immunol.* 166: 3402–3409.
6. Woodland, D. L. 2003. Cell-mediated immunity to respiratory virus infections. *Curr. Opin. Immunol.* 15: 430–435.
7. Wakelin, D. 1967. Acquired immunity to *Trichuris muris* in the albino laboratory mouse. *Parasitology* 57: 515–524.
8. Artis, D., M. L. Wang, S. A. Keilbaugh, W. He, M. Brenes, G. P. Swain, P. A. Knight, D. D. Donaldson, M. A. Lazar, H. R. P. Miller, et al. 2004. RELMβ/FIZZ2 is a goblet cell-specific immune-effector molecule in the gastrointestinal tract. *Proc. Natl. Acad. Sci. USA* 101: 13596–13600.
9. Bancroft, A. J., A. N. McKenzie, and R. K. Grencis. 1998. A critical role for IL-13 in resistance to intestinal nematode infection. *J. Immunol.* 160: 3453–3461.
10. Cliffe, L. J., N. E. Humphreys, T. E. Lane, C. S. Potten, C. Booth, and R. K. Grencis. 2005. Accelerated intestinal epithelial cell turnover: a new mechanism of parasite expulsion. *Science* 308: 1463–1465.
11. Else, K. J., F. D. Finkelman, C. R. Maliszewski, and R. K. Grencis. 1994. Cytokine-mediated regulation of chronic intestinal helminth infection. *J. Exp. Med.* 179: 347–351.
12. Finkelman, F. D., T. Shea-Donohue, J. Goldhill, C. A. Sullivan, S. C. Morris, K. B. Madden, W. C. Gause, and J. F. Urban, Jr. 1997. Cytokine regulation of host defense against parasitic gastrointestinal nematodes: lessons from studies with rodent models. *Annu. Rev. Immunol.* 15: 505–533.
13. Bunce, C., and E. B. Bell. 1997. CD45RC isoforms define two types of CD4 memory T cells, one of which depends on persisting antigen. *J. Exp. Med.* 185: 767–776.
14. Sallusto, F., D. Lenig, R. Forster, M. Lipp, and A. Lanzavecchia. 1999. Two subsets of memory T lymphocytes with distinct homing potentials and effector functions. *Nature* 401: 708–712.
15. Reinhardt, R. L., A. Khoruts, R. Merica, T. Zell, and M. K. Jenkins. 2001. Visualizing the generation of memory CD4 T cells in the whole body. *Nature* 410: 101–105.
16. Lang, K. S., M. Recher, A. A. Navarini, N. L. Harris, M. Lohning, T. Junt, H. C. Probst, H. Hengartner, and R. M. Zinkernagel. 2005. Inverse correlation between IL-7 receptor expression and CD8 T cell exhaustion during persistent antigen stimulation. *Eur. J. Immunol.* 35: 738–745.
17. Marzo, A. L., K. D. Klonowski, A. Le Bon, P. Borrow, D. F. Tough, and L. Lefrancois. 2005. Initial T cell frequency dictates memory $CD8^+$ T cell lineage commitment. *Nat. Immunol.* 6: 793–799.
18. Wherry, E. J., V. Teichgraber, T. C. Becker, D. Masopust, S. M. Kaech, R. Antia, U. H. von Andrian, and R. Ahmed. 2003. Lineage relationship and protective immunity of memory CD8 T cell subsets. *Nat. Immunol.* 4: 225–234.
19. Wherry, E. J., D. L. Barber, S. M. Kaech, J. N. Blattman, and R. Ahmed. 2004. Antigen-independent memory CD8 T cells do not develop during chronic viral infection. *Proc. Natl. Acad. Sci. USA* 101: 16004–16009.
20. Zaph, C., J. Uzonna, S. M. Beverley, and P. Scott. 2004. Central memory T cells mediate long-term immunity to *Leishmania major* in the absence of persistent parasites. *Nat. Med.* 10: 1104–1110.
21. Cauley, L. S., T. Cookenham, T. B. Miller, P. S. Adams, K. M. Vignali, D. A. Vignali, and D. L. Woodland. 2002. Cutting edge: virus-specific $CD4^+$ memory T cells in nonlymphoid tissues express a highly activated phenotype. *J. Immunol.* 169: 6655–6658.
22. Roman, E., E. Miller, A. Harmsen, J. Wiley, U. H. Von Andrian, G. Huston, and S. L. Swain. 2002. CD4 effector T cell subsets in the response to influenza: heterogeneity, migration, and function. *J. Exp. Med.* 196: 957–968.
23. Mohrs, M., K. Shinkai, K. Mohrs, and R. M. Locksley. 2001. Analysis of type 2 immunity in vivo with a bicistronic IL-4 reporter. *Immunity* 15: 303–311.
24. Artis, D., A. Villarino, M. Silverman, W. He, E. M. Thornton, S. Mu, S. Summer, T. M. Covey, E. Huang, H. Yoshida, et al. 2004. The IL-27 receptor (WSX-1) is an inhibitor of innate and adaptive elements of type 2 immunity. *J. Immunol.* 173: 5626–5634.
25. Zaph, C., and P. Scott. 2003. Th1 cell-mediated resistance to cutaneous infection with *Leishmania major* is independent of P- and E-selectins. *J. Immunol.* 171: 4726–4732.
26. Lyons, A. B., and C. R. Parish. 1994. Determination of lymphocyte division by flow cytometry. *J. Immunol. Methods* 171: 131–137.
27. Cliffe, L. J., and R. K. Grencis. 2004. The *Trichuris muris* system: a paradigm of resistance and susceptibility to intestinal nematode infection. *Adv. Parasitol.* 57: 255–307.
28. Koyama, K., H. Tamauchi, and Y. Ito. 1995. The role of $CD4^+$ and $CD8^+$ T cells in protective immunity to the murine nematode parasite *Trichuris muris*. *Parasite Immunol.* 17: 161–165.
29. Voehringer, D., K. Shinkai, and R. M. Locksley. 2004. Type 2 immunity reflects orchestrated recruitment of cells committed to IL-4 production. *Immunity* 20: 267–277.
30. Uzonna, J. E., G. Wei, D. Yurkowski, and P. Bretscher. 2001. Immune elimination of *Leishmania major* in mice: implications for immune memory, vaccination, and reactivation disease. *J. Immunol.* 167: 6967–6974.
31. Belkaid, Y., C. A. Piccirillo, S. Mendez, E. M. Shevach, and D. L. Sacks. 2002. $CD4^+CD25^+$ regulatory T cells control *Leishmania major* persistence and immunity. *Nature* 420: 502–507.
32. Pamer, E. G. 2004. Immune responses to *Listeria monocytogenes. Nat. Rev. Immunol.* 4: 812–823.
33. Sacks, D., and N. Noben-Trauth. 2002. The immunology of susceptibility and resistance to *Leishmania major* in mice. *Nat. Rev. Immunol.* 2: 845–858.
34. Wherry, E. J., and R. Ahmed. 2004. Memory CD8 T-cell differentiation during viral infection. *J. Virol.* 78: 5535–5545.
35. Hayashi, N., D. Liu, B. Min, S. Z. Ben-Sasson, and W. E. Paul. 2002. Antigen challenge leads to in vivo activation and elimination of highly polarized TH1 memory T cells. *Proc. Natl. Acad. Sci. USA* 99: 6187–6191.
36. Wu, C. Y., J. R. Kirman, M. J. Rotte, D. F. Davey, S. P. Perfetto, E. G. Rhee, B. L. Freidag, B. J. Hill, D. C. Douek, and R. A. Seder. 2002. Distinct lineages of T_H1 cells have differential capacities for memory cell generation in vivo. *Nat. Immunol.* 3: 852–858.
37. Swain, S. L., H. Hu, and G. Huston. 1999. Class II-independent generation of CD4 memory T cells from effectors. *Science* 286: 1381–1383.
38. Roberts, A. I., S. Devadas, X. Zhang, L. Zhang, A. Keegan, K. Greeneltch, J. Solomon, L. Wei, J. Das, E. Sun, et al. 2003. The role of activation-induced cell death in the differentiation of T-helper-cell subsets. *Immunol. Res.* 28: 285–293.
39. Zhang, X., T. Brunner, L. Carter, R. W. Dutton, P. Rogers, L. Bradley, T. Sato, J. C. Reed, D. Green, and S. L. Swain. 1997. Unequal death in T helper cell (Th)1 and Th2 effectors: Th1, but not Th2, effectors undergo rapid Fas/FasL-mediated apoptosis. *J. Exp. Med.* 185: 1837–1849.
40. Harbertson, J., E. Biederman, K. E. Bennett, R. M. Kondrack, and L. M. Bradley. 2002. Withdrawal of stimulation may initiate the transition of effector to memory CD4 cells. *J. Immunol.* 168: 1095–1102.

41. Hu, H., G. Huston, D. Duso, N. Lepak, E. Roman, and S. L. Swain. 2001. CD4$^+$ T cell effectors can become memory cells with high efficiency and without further division. *Nat. Immunol.* 2: 705–710.
42. Lee, T. D., D. Wakelin, and R. K. Grencis. 1983. Cellular mechanisms of immunity to the nematode *Trichuris muris. Int. J. Parasitol.* 13: 349–353.
43. Campbell, D. J., and E. C. Butcher. 2002. Rapid acquisition of tissue-specific homing phenotypes by CD4$^+$ T cells activated in cutaneous or mucosal lymphoid tissues. *J. Exp. Med.* 195: 135–141.
44. Lambrecht, B. N., and H. Hammad. 2003. Taking our breath away: dendritic cells in the pathogenesis of asthma. *Nat. Rev. Immunol.* 3: 994–1003.
45. Lanzavecchia, A., and F. Sallusto. 2001. Regulation of T cell immunity by dendritic cells. *Cell* 106: 263–266.
46. Mora, J. R., M. R. Bono, N. Manjunath, W. Weninger, L. L. Cavanagh, M. Rosemblatt, and U. H. Von Andrian. 2003. Selective imprinting of gut-homing T cells by Peyer's patch dendritic cells. *Nature* 424: 88–93.
47. Mora, J. R., G. Cheng, D. Picarella, M. Briskin, N. Buchanan, and U. H. von Andrian. 2005. Reciprocal and dynamic control of CD8 T cell homing by dendritic cells from skin- and gut-associated lymphoid tissues. *J. Exp. Med.* 201: 303–316.
48. Nagler-Anderson, C. 2001. Man the barrier! Strategic defences in the intestinal mucosa. *Nat. Rev. Immunol.* 1: 59–67.
49. Barber, D. L., E. J. Wherry, and R. Ahmed. 2003. Cutting edge: rapid in vivo killing by memory CD8 T cells. *J. Immunol.* 171: 27–31.
50. Kaech, S. M., E. J. Wherry, and R. Ahmed. 2002. Effector and memory T-cell differentiation: implications for vaccine development. *Nat. Rev. Immunol.* 2: 251–262.
51. Sallusto, F., J. Geginat, and A. Lanzavecchia. 2004. Central memory and effector memory T cell subsets: function, generation, and maintenance. *Annu. Rev. Immunol.* 22: 745–763.
52. Turner, J. D., H. Faulkner, J. Kamgno, F. Cormont, J. Van Snick, K. J. Else, R. K. Grencis, J. M. Behnke, M. Boussinesq, and J. E. Bradley. 2003. Th2 cytokines are associated with reduced worm burdens in a human intestinal helminth infection. *J. Infect. Dis.* 188: 1768–1775.
53. Jackson, J. A., J. D. Turner, L. Rentoul, H. Faulkner, J. M. Behnke, M. Hoyle, R. K. Grencis, K. J. Else, J. Kamgno, M. Boussinesq, and J. E. Bradley. 2004. T helper cell type 2 responsiveness predicts future susceptibility to gastrointestinal nematodes in humans. *J. Infect. Dis.* 190: 1804–1811.