Homeostatic Division Is Not Necessary for Antigen-Specific CD4⁺ Memory T Cell Persistence

Evann Corbo-Rodgers, Karla R. Wiehagen, Elizabeth S. Staub and Jonathan S. Maltzman

*J Immunol* 2012; 189:3378-3385; Prepublished online 5 September 2012;
doi: 10.4049/jimmunol.1201583
http://www.jimmunol.org/content/189/7/3378

**References**

This article cites 55 articles, 29 of which you can access for free at:
http://www.jimmunol.org/content/189/7/3378.full#ref-list-1

**Subscription**

Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

**Permissions**

Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**

Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Homeostatic Division Is Not Necessary for Antigen-Specific CD4+ Memory T Cell Persistence

Evann Corbo-Rodgers,*,† Karla R. Wiehagen,*,† Elizabeth S. Staub,† and Jonathan S. Maltzman*,†

CD4+ memory T cells are generated in response to infection or vaccination, provide protection to the host against reinfection, and persist through a combination of enhanced survival and slow homeostatic turnover. We used timed deletion of the TCR-signaling adaptor molecule Src homology 2 domain-containing phosphoprotein of 76 kDa (SLP-76) with MHC:peptide tetramers to study the requirements for tonic TCR signals in the maintenance of polyclonal Ag-specific CD4+ memory T cells. SLP-76–deficient I-Aβ:gp61+ cells are unable to rapidly generate effector cytokines or proliferate in response to secondary infection. In mice infected with lymphocytic choriomeningitis virus (LCMV) or Listeria monocytogenes expressing the LCMV gp61–80 peptide, SLP-76–deficient I-Aβ:gp61+ cells exhibit reduced division, similar to that seen in vitro-generated CD44hi and endogenous CD4+CD44hi cells. Competitive bone marrow chimera experiments demonstrate that the decrease in homeostatic turnover in the absence of SLP-76 is a cell-intrinsic process. Surprisingly, despite the reduction in turnover, I-Aβ:gp61+ Ag-specific memory cells persist in normal numbers for >30 wk after LCMV infection in the absence of SLP-76. These data suggest the independent maintenance of a population of Ag-specific CD4+ memory T cells in the absence of SLP-76 and normal levels of homeostatic division.


Abbreviations used in this article: AgSp, Ag-specific; cHET, conditional heterozygous; cKO, conditional knockout; HSC, hematopoietic stem cell; LCMV, lymphocytic choriomeningitis virus; Lm-gp61, Listeria monocytogenes expressing gp61; MHC II, MHC class II; MP, memory phenotype; p.i., postinfection; SLP-76, Src homology 2 domain-containing phosphoprotein of 76 kDa; sp, self-peptide; TCM, central memory T cell; TEM, effector memory T cell.

Copyright © 2012 by The American Association of Immunologists, Inc.
Deletion of SLP-76 from AgSp CD4+ memory T cells does not alter cell surface phenotype

To investigate the role of SLP-76–dependent signals in CD4+ AgSp memory homeostasis, we compared mice in which SLP-76 can be deleted by drug administration (SLP-76<sup>flox/null</sup>CreT2<sup>R26R<sub>YFP</sub></sup>) with Cre-expressing controls (SLP-76<sup>flox</sup>/CreT2<sup>R26R<sub>YFP</sub></sup>), termed cKO and cHET, respectively (22). In this system, tamoxifen administration induces Cre recombinase activity, deletion at the SLP-76 locus, and expression of a YFP reporter that is detectable by flow cytometry. Tamoxifen-treated cKO cells lack SLP-76, whereas cHET cells retain one functional allele. To generate SLP-76–deficient AgSp CD4+ memory T cells, cHET and cKO mice were first infected with either LCMV-Armstrong or Lm-gp61 (26). Tamoxifen administration 30 d postinfection (p.i.) induces deletion of SLP-76 and expression of YFP (Fig. 1A).

We first isolated CD4<sup>+</sup>CD44<sup>hi</sup>I-Ab<sup>+</sup>gp61<sup>+</sup>YFP<sup>+</sup> splenocytes and subjected them to real-time quantitative PCR analysis for SLP-76 expression to determine the extent of deletion. SLP-76 mRNA was nearly undetectable in AgSp YFP<sup>+</sup> cKO cells (Fig. 1B), indicating efficient deletion of SLP-76 in I-A<sup>+</sup>-gp61<sup>+</sup> AgSp memory T cells. cKO YFP I-A<sup>+</sup>gp61<sup>+</sup> cells are heterogeneous in SLP-76 deletion and exhibit intermediate levels of SLP-76 mRNA compared with the cHET control (data not shown). These data show that CreT2 efficiently deletes SLP-76 from the CD4<sup>+</sup> AgSp memory compartment and suggest that YFP expression can be used as a surrogate for SLP-76 absence in cKO AgSp CD4<sup>+</sup> T cells.

To confirm that tamoxifen administration and loss of SLP-76 p.i. did not alter AgSp CD4<sup>+</sup> memory T cell populations, we analyzed I-A<sup>+</sup>-gp61<sup>+</sup> splenocytes from LCMV-infected C57BL/6, cHET, and cKO mice for relative frequency and cell surface phenotype before and 2 wk after deletion. I-A<sup>+</sup>-gp61<sup>+</sup> cells were similar in relative frequency and expressed high levels of CD44 regardless of SLP-76 expression (Fig. 1C). Lack of staining with a tetramer loaded with p5 (not shown). These data suggest that CreT2 efficiently deletes SLP-76 from the CD4<sup>+</sup> AgSp memory compartment and suggest that YFP expression can be used as a surrogate for SLP-76 absence in cKO AgSp CD4<sup>+</sup> T cells.

For some experiments we generated I-A<sup>+</sup>-gp61<sup>+</sup>CD4<sup>+</sup> memory T cells by infection with Lm-gp61 (26). Because the overall number and frequency of I-A<sup>+</sup>-gp61<sup>+</sup> cells after Lm-gp61 infection are 10-fold lower than with LCMV (27), we enriched for CD4<sup>+</sup> cells by magnetic depletion of CD8<sup>+</sup> and MHC II<sup>+</sup> cells. Following CD4<sup>+</sup> enrichment, I-A<sup>+</sup>-gp61<sup>+</sup> cells were detectable among infected mice within the CD4<sup>+</sup>CD44<sup>+</sup> population (Fig. 1D). There was mouse-to-mouse variability in the relative percentage of tetramer<sup>+</sup> cells that was not statistically different between the cKO and cHET mice. These data indicate that loss of SLP-76 during the memory phase has no immediate effect on the number of I-A<sup>+</sup>-gp61<sup>+</sup>–specific CD4<sup>+</sup> memory T cells.

To determine whether deletion of SLP-76 altered the cell surface phenotype of AgSp CD4<sup>+</sup> memory T cells, we compared cell surface protein expression among YFP<sup>+</sup> cells. YFP expression is induced in ~20–30% of cHET and cKO I-A<sup>+</sup>-gp61<sup>+</sup> memory cells after LCMV infection (Fig. 1E). The ratio of CD62L<sup>lo</sup> effector memory (T<sub>EM</sub>) to CD62L<sup>hi</sup> central memory (T<sub>CM</sub>) populations was equivalent between cHET and cKO YFP<sup>+</sup> cells (Fig. 1E). We next evaluated surface expression of CD27, a stimulatory member of the TNFR family that segregates short- versus long-lived memory populations (28). cHET and cKO YFP<sup>+</sup> AgSp cells expressed equivalent percentages of long-lived CD27<sup>hi</sup> and short-lived CD27<sup>lo</sup> populations. Finally, we examined cell surface levels of CD127, the α-chain of the IL-7R (2, 29). CD127 expression was equivalent on cHET and cKO AgSp memory cells. Cell surface phenotypes were similar following Lm-gp61 infection and SLP-76 deletion (data not shown). Taken together, these data suggest that...
deletion of SLP-76 does not phenotypically alter the memory population at this early time point.

**SLP-76–deficient memory cells do not respond to TCR-induced signals**

A hallmark of memory T cell function is the ability to respond rapidly to pathogen re-exposure by proliferation and cytokine generation. To ascertain whether SLP-76 is necessary for rapid cytokine induction in CD4+ memory T cells, we analyzed PBLs from cHET and cKO mice 3 wk after deletion (7 wk after LCMV infection) for TNF-α, IL-2, and IFN-γ production. PBLs were mixed with congenic CD45.1+ C57BL/6 splenocytes, stimulated by addition of gp61–80 peptide, and assessed for intracellular cytokine levels 4 h later. Approximately 3–4% of CD4+CD45.2+ CD44+YFP+ gated cells from cHET mice generated TNF-α and/or IL-2 (Fig. 2A). In contrast, CD4+CD45.2+CD44+YFP+ gated cells from cKO mice failed to generate detectable cytokine levels using this assay. Importantly, bypassing SLP-76 with phorbol ester and calcium ionophore generated equivalent TNF-α and IL-2 in cHET and cKO cells, confirming a functional memory T cell phenotype. Consistent with incomplete SLP-76 deletion in YFP+ cells, both cHET and cKO YFP+ CD44hi cells were capable of generating TNF-α, IL-2, and IFN-γ (data not shown).

We next evaluated the ability of SLP-76–deficient memory cells to respond to secondary infection. cHET and cKO mice were infected with LCMV, administered tamoxifen to induce deletion, and then secondarily infected with Lm-gp61 (Fig. 2B). In this experimental design, the only Ag expressed in common is the gp61–80 epitope. Total (YFP+ plus YFP−) I-Ab:gp61+ cells from both cHET and cKO mice expanded >7-fold in peripheral blood during the course of the experiment. YFP+ cells from the cHET but not cKO mice expanded. The lack of expansion in YFP+ cKO cells, through day 8 after reinfection, suggests that SLP-76–deficient memory cells are unable to divide in response to both TCR-driven signaling and infection.

---

**FIGURE 1.** Deletion of SLP-76 after LCMV or Lm-gp61 infection does not alter phenotype or frequency. (A) For all AgSp memory experiments mice were infected at day −30, followed by pathogen clearance and memory formation. Tamoxifen was administered from day 0 to 5 to induce deletion of SLP-76. Cells were tracked based on YFP expression and tetramer binding. (B) RNA was isolated from FACS-sorted tetramer+ CD4+ CD44hiYFP+ memory T cells and used for real-time PCR analysis. Values were normalized internally to β-actin. Data are representative of two independent experiments, with two pooled mice per genotype. (C) I-Ab:gp61+CD4+ memory T cells from spleen of LCMV-infected C57BL/6, cHET, and cKO mice were obtained 2 wk after deletion. Plots show CD4+ dump- CD11b− CD11c− B220− cells. Cells used for control staining with I-Ab:CLIP were a mixture from all mice used in each experiment. (D) Spleen and lymph nodes from Lm-gp61–infected cHET and cKO mice isolated 2 wk after deletion were enriched for CD4+ cells by magnetic depletion of CD8+ and MHC II+ cells. Enriched cells were stained and gated on CD4+CD44hiI-Ab:gp61+ cells. Contour plots were gated on live, singlet cells (left) and are further gated on CD4+ T cells (right) from cHET (top) and cKO (bottom) mice. Data are representative of two to three mice per genotype in two independent experiments. (E) Histograms show overlays of I-Ab:gp61+CD4+ cells from C57BL/6 (gray), cHET (dashed), and cKO (black) mice following LCMV infection. Plots on right were further gated on YFP. Data in (C) and (E) are representative of six independent experiments with a total of 23 cHET and 14 cKO mice.

**FIGURE 2.** SLP-76–deficient LCMV AgSp memory cells do not respond to TCR signals. (A) Peripheral blood from mice 3 wk after deletion was mixed with CD45.1+ congenically marked C57BL/6 splenocytes and stimulated for 4 h in the presence of brefeldin A with gp61 peptide or PMA plus ionomycin. Plots shown were gated on CD4+ T cells from cHET and cKO mice isolated 2 wk after deletion. Plots are representative of at least two cHET and three cKO mice from two independent experiments. (B) Mice were infected with LCMV, depleted at day 30 p.i., and then secondarily infected with Lm-gp61 (2 × 10⁵ CFU). Expansion of I-Ab:gp61+ memory cells was assessed in peripheral blood 1 d prior to and 8 d after reinfection. Plots were gated on total CD4+ and CD4+YFP+ as indicated. Inset numbers are relative percentage of cells within the gated area. Data are representative of at least two cHET and three cKO mice from two independent experiments.
Loss of SLP-76 reduces homeostatic turnover in CD4\(^+\) AgSp memory T cells

Persistence of the CD4\(^+\) AgSp memory T cell pool results from a combination of increased individual cell survival and slow homeostatic turnover of the population. To assess turnover in SLP-76–deficient AgSp CD4\(^+\) T cells, mice infected with either LCMV or Lm-gp61 and deleted for SLP-76 were administered BrdU in drinking water for 2 wk (Fig. 3A). BrdU administration was started 5 d after the completion of tamoxifen to maximize the number of tetramer\(^+\) cells. BrdU\(^+\) cells were detectable in I-A\(^b\)-gp61\(^+\)CD4\(^+\) splenocytes from Lm-gp61 and LCMV-infected cHET mice (Fig. 3B, 3C, left). There was an overall reduction in BrdU incorporation detected in total (YFP\(^+\) plus YFP\(^-\)) I-A\(^b\)-gp61\(^+\) cells from cKO when compared with the cHET cells (Fig. 3B, left). A positive control for BrdU staining gated on a combination of CD11b\(^+\), CD11c\(^+\), and B220\(^+\) cells from the same sample is shown for comparison. Turnover was reduced in YFP\(^-\) (SLP-76–deficient) I-A\(^b\)-gp61\(^+\)CD4\(^+\) cells generated by Lm-gp61 infection (Fig. 3B, right). Furthermore, despite substantial mouse-to-mouse variability, there was a statistically significant reduction in the percentage of I-A\(^b\)-gp61\(^+\)YFP\(^+\) cells with BrdU incorporation isolated from LCMV-infected cKO mice when compared with cHET mice (Fig. 3C, right). Taken together, these data indicate that the basal turnover rate of AgSp CD4\(^+\) memory T cells is dependent on continued SLP-76 expression, regardless of the infectious pathogen.

Deletion of SLP-76 has no effect on persistence of CD4\(^+\) AgSp memory T cells

Because basal turnover was reduced among cKO AgSp CD4\(^+\) memory T cells generated by either infection, we hypothesized that persistence of this population would also be decreased compared with cHET cells following LCMV infection. To address this hypothesis we assessed individual C57BL/6, cHET, and cKO mice for I-A\(^b\)-gp61\(^+\) cell numbers at 2, 6, 15, and 30 wk after deletion. Importantly, the number of I-A\(^b\)-gp61\(^+\)CD4\(^+\) memory T cells was maintained 2 wk after deletion (Figs. 1C, 4A). In the C57BL/6 and cHET mice, numbers of splenic I-A\(^b\)-gp61\(^+\)CD4\(^+\) T cells decreased during the course of the experiment, consistent with previous work using peptide stimulation as a method of quantitation (30). Surprisingly, we found equivalent numbers of I-A\(^b\)-gp61\(^+\) cells in the spleens of cKO mice at each time point compared with controls. To evaluate the possibility that YFP\(^-\) SLP-76–deficient cells had outcompeted the YFP\(^+\) SLP-76–deficient cells, we further examined the relative percentage and total numbers of YFP\(^+\) cells. YFP\(^+\)I-A\(^b\)-gp61\(^+\) cell numbers showed a similar slope of decline independent of SLP-76 expression (Fig. 4B). Furthermore, the relative percentage of tetramer-specific cells that were YFP\(^+\) was maintained in the absence of SLP-76 for at least 30 wk. Taken together, these data indicate that persistence of I-A\(^b\)-gp61\(^+\) cells is not dependent on expression of SLP-76.

A major reservoir of long-lived memory cells is bone marrow (31). To address the possibility of selective maintenance of YFP\(^+\) cKO cells in the spleen due to deletion of bone marrow memory T cells, we evaluated the bone marrow 30 wk after deletion for the presence of YFP\(^+\)AgSp cells. As in the spleen, we saw an equal percentage of cKO YFP\(^+\)-I-A\(^b\)-gp61\(^+\)CD4\(^+\) memory T cells compared with the cHET cells (Fig. 4C). Analysis of liver, lung, and lymph node similarly showed no differences in persistence (data not shown). These data suggest that maintenance of SLP-76–deficient CD4\(^+\) AgSp memory T cells in the spleen is not due to altered homing or selective loss from other tissues.

The duration of TCR stimulus, extent of clonal competition, and TCR avidity can alter the ratio of T\(_{CM}\) to T\(_{EM}\) (32, 33). TCR signal strength also correlates with commitment to the T\(_{EM}\) pool (34). Therefore, we hypothesized that loss of SLP-76–dependent TCR signals would alter the maintenance of these subsets, distinguishable by differences in CD62L expression. cHET and cKO YFP\(^+\)-I-A\(^b\)-gp61\(^+\) memory T cells exhibited both T\(_{CM}\) and T\(_{EM}\) populations with equivalently high expression of IL-7R\(^\alpha\) and CD27 in both the spleen and bone marrow at 15 and 30 wk after deletion (Fig. 4D and data not shown). The high percentage of CD27 seen at week 30 suggests that most of the remaining memory cells are long-lived (28), regardless of genotype. These data suggest that long-term maintenance of CD4\(^+\) AgSp memory T cell subsets is unchanged in the absence of SLP-76.

Decreased turnover in the absence of SLP-76 is cell-intrinsic

To confirm that the decrease in homeostatic turnover was a cell-intrinsic process, we next generated competitive bone marrow chimeras. This approach ensures competition and the presence of WT cells to provide growth factors. Lethally irradiated C57BL/6 (WT)CD45.1 mice were reconstituted with bone marrow from WTCD45.2; cHETCD45.2; or cKO\(^-\)CD45.2 combined with WT\(^+\)CD45.1/CD45.2 in a 70:30 ratio. Eight weeks following reconstitution, mice were infected with either Lm-gp61 or LCMV and administered tamoxi-

FIGURE 3. AgSp memory cells require SLP-76 for turnover. (A) cHET and cKO mice were orally administered BrdU between days 10 and 24 after deletion. FACS analysis is shown of BrdU-labeled splenocytes from cHET and cKO mice (B) after Lm-gp61 or (C) after LCMV infection gated on CD4\(^+\) and negative for a dump consisting of CD11b, CD11c, and B220 (left). Samples were further gated on CD44\(^{hi}\)I-A\(^b\)-gp61\(^+\) cells (center); those initially gated dump\(^+\) cells are shown in the rightmost plot. Numbers represent the percentage of cells within each gate. BrdU gating was determined by BrdU Ab staining splenocytes from an uninfected mouse not administered BrdU. Graphs show the percentage of YFP\(^+\) cells that had incorporated BrdU. Each point represents an individual mouse. Data are compiled from two independent experiments using each pathogen using 7 cHET and 19 cKO mice in total.
Infections per time point, with 82 mice total. (A) CD4\(^+\)I-A\(^b\):gp61\(^+\) and (B) CD4\(^+\)I-A\(^b\):gp61\(^+\)YFP\(^+\) cells per spleen over time in C57BL/6 (Δ), cHET (●), and cKO (○) mice are shown. Each point represents an individual mouse compiled from at least two independent infections per time point, with 82 mice total. (C) Bone marrow was isolated from cHET and cKO mice 30 wk after deletion. The percentage of CD4\(^+\)CD44\(^hi\)I-A\(^b\):gp61\(^+\)YFP\(^+\) cells was calculated relative to total CD4\(^+\) lymphocytes. Data are compiled from two independent experiments, with at least three mice per genotype. (D) Phenotypic analysis of splenocytes at 15 and 30 wk after deletion from cHET (shaded gray) and cKO (black lines). Histograms were gated on CD4\(^+\)CD44\(^hi\)I-A\(^b\):gp61\(^+\)YFP\(^+\) cells. Dashed lines are populations from the cHET samples gated on naive CD4\(^+\)CD44\(^lo\)YFP\(^+\) (CD62L and IL-7R histograms) and dump\(^+\) (CD11b\(^+\)CD11c\(^+\)B220\(^+\))YFP\(^+\) (CD27 histograms). Data are representative of two to three independent experiments with at least four mice each.

FIGURE 4. Persistence is independent of SLP-76 in AgSp memory cells. Numbers of (A) CD4\(^+\)I-A\(^b\):gp61\(^+\) and (B) CD4\(^+\)I-A\(^b\):gp61\(^+\)YFP\(^+\) cells per spleen over time in C57BL/6 (Δ), cHET (●), and cKO (○) mice are shown. Each point represents an individual mouse compiled from at least two independent infections per time point, with 82 mice total. (C) Bone marrow was isolated from cHET and cKO mice 30 wk after deletion. The percentage of CD4\(^+\)CD44\(^hi\)I-A\(^b\):gp61\(^+\)YFP\(^+\) cells was calculated relative to total CD4\(^+\) lymphocytes. Data are compiled from two independent experiments, with at least three mice per genotype. (D) Phenotypic analysis of splenocytes at 15 and 30 wk after deletion from cHET (shaded gray) and cKO (black lines). Histograms were gated on CD4\(^+\)CD44\(^hi\)I-A\(^b\):gp61\(^+\)YFP\(^+\) cells. Dashed lines are populations from the cHET samples gated on naive CD4\(^+\)CD44\(^lo\)YFP\(^+\) (CD62L and IL-7R histograms) and dump\(^+\) (CD11b\(^+\)CD11c\(^+\)B220\(^+\))YFP\(^+\) (CD27 histograms). Data are representative of two to three independent experiments with at least four mice each.

To assess turnover, BrdU was administered to Lm-gp61– and LCMV-infected mice beginning 4 or 12 wk following deletion, respectively. cKO cells in mixed bone marrow chimeras infected with Lm-gp61 incorporated less BrdU when compared with WT competitors (Fig. 5B). There was a statistically significant reduction in BrdU incorporation 18 wk p.i. (14 wk after deletion). Similar to data in nonchimeric mice 6 wk p.i. (Fig. 3) and competitive chimeras infected with Lm-gp61 at 10 wk p.i., there was a substantial reduction in turnover detectable in cells lacking SLP-76 when compared with cells expressing SLP-76 at this late time point (Fig. 5E).

To address whether increased competition found in the mixed bone marrow chimera altered the persistence of SLP-76–deficient AgSp cells, we assessed chimerism and quantitated CD45.2-derived cells. Longitudinal quantitation from serial samples of peripheral blood showed no change in chimerism of I-A\(^b\):gp61–specific CD4\(^+\) memory T cells throughout the course of the experiment (data not shown). Absolute numbers of I-A\(^b\):gp61–specific memory T cells were similar between WT, cHET, and cKO-derived cells in the spleen 18 wk p.i. (Fig. 5F). The competitive chimera results suggest that SLP-76–deficient AgSp memory T cells display a cell-intrinsic decrease in homeostatic turnover without a decrease in overall persistence.

Discussion
In this study, we combined timed genetic deletion of the SLP-76 adaptor with in vivo bacterial and viral infection to study the role of TCR signaling in the survival and turnover of AgSp CD4\(^+\) memory T cells. We found that SLP-76–mediated signals are essential for a normal rate of homeostatic turnover in both nonchimeric and competitive mixed bone marrow chimeric mice, but they are dispensable for maintenance of the polyclonal population. We also show for the first time, to our knowledge, that CD4\(^+\) AgSp memory T cells require SLP-76 to respond to antigenic TCR signaling, even in the context of the acute inflammatory environment produced by pathogenic infection.

A strength of this study is the evaluation of turnover and persistence in the context of an endogenous polyclonal response. TCR transgenics have been revealing but cannot recapitulate all of the influences of polyclonality on AgSp immune responses and homeostasis. The repertoire of memory cells with I-A\(^b\):gp61–80 specificity includes cells with varying TCR sequence and avidity. TCR transgenic T cells can vary in their capacity to generate memory depending on precursor frequency and infectious pathogen (26, 35–37). Using tetramer reagents to evaluate homeostasis of the polyclonal repertoire, we showed a reduction in BrdU incorporation in cKO mice following infection with either LCMV or Lm-gp61. As there are multiple clonal populations specific for I-A\(^b\):gp61–80, it remains possible that these will vary in homeostatic requirements. An additional strength of these studies is the investigation of I-A\(^b\):gp61–80–specific memory T cells generated by two different pathogens. SMARTA TCR transgenic T cells generate memory in response to LCMV but not Lm-gp61 (26). Similarly, whereas our data indicate that turnover is qualitatively similar, reduction in turnover seen in the absence of SLP-76 is quantitatively greater following L. monocytogenes infection (Fig. 3B versus 3C). This finding may be due to initial activation of clones with different avidities or differences in the inflammatory environment of the two pathogens. Thus, whereas TCR signals are required for optimal turnover in memory CD4\(^+\) T cells, clonal competition and the inflammatory environment at the time of
FIGURE 5. Decreased turnover in the absence of SLP-76 is a cell-intrinsic process. (A)and (D) Schematic depicting the generation of mixed bone marrow chimeras indicating the timing of infection, tamoxifen-induced deletion of SLP-76, and BrdU administration. WT, C57BL/6. (B)Spleen and lymph nodes depleted of CD8+ and MHC II+ cells gated on CD45.2+CD44hiI-Ab:gp61+ cells. Genotypes are shown above each contour plot. BrdU gating is determined using CD4+CD44hi cells from a WT mouse not administered BrdU. Inset numbers are relative percentage of cells within the gated area. Data are representative of five WT:WT, three WT:cHET, and four WT:cKO mice. (C)Compilation of BrdU incorporation from five WT:WT, three WT:cHET, and four WT:cKO mice previously infected with Lm-gp61. (E)Compilation of BrdU incorporation from five WT:WT, five WT:cHET, and five WT:cKO mice previously infected with LCMV. Compiled data in (C) and (E) were calculated by gating on CD45.2+CD44hiI-Ab:gp61+ cells followed by CD45.1+ or CD45.1- and determining the percentage BrdU+. Each point represents an individual mouse. (F)Fourteen weeks after deletion splenocytes were isolated and numbers of CD45.2+CD44hiI-Ab:gp61+ cells were calculated per spleen. Each point in (E) and (F) represents data from an individual mouse; n = 5 WT:WT, 5 WT:cHET, and 5 WT:cKO.

acute infection may dictate differences in homeostatic requirements.

Maintenance of TCM and TEM populations was unaltered by deletion of SLP-76 throughout the 30-wk time course of these experiments, suggesting memory cell fate is determined prior to 30 d p.i. In addition to CD62L expression, we also examined cell surface levels of IL-7R and CD27. Unlike AgSp memory T cells maintained in the absence of MHC II (19), SLP-76–deficient CD4+ memory T cells retained similar levels of IL-7R compared with cHET controls. Binding of CD27 to its ligand CD70 is a costimulatory signal for activation and influences survival of the AgSp memory population (38–40). The retention of CD27hi cells from both cHET and cKO YFP+I-Ab:gp61+ populations suggest that this signaling pathway may still be intact and represent a mechanism for persistence. Retention of both IL-7Rs and CD27 supports a model where progressive differentiation of CD4+ AgSp memory T cells is unaltered by a lack of continuous differentiation of CD4+ AgSp memory T cells in the context of chronic Ag exposure. Experiments to evaluate the role of SLP-76–dependent signals in the setting of chronic infection are of great interest and ongoing.

The finding that impaired turnover did not influence overall persistence of SLP-76–deficient CD4+ AgSp memory T cells is unexpected and intriguing. Either increased responsiveness of SLP-76–deficient cells to cytokines or independent regulation and maintenance of the SLP-76–deficient pool could explain the uncoupling. One potential model postulates that SLP-76–deficient AgSp cells have an increased half-life on an individual cell basis through activation of alternative pathways, for example in response to homeostatic cytokines. At low doses, IL-7 supports the survival of resting AgSp memory cells through elevated levels of antiapoptotic Bcl2 and reduced levels of proapoptotic Bim (10, 43–45). However, we have previously shown that there is no alteration in STAT5 phosphorylation in response to IL-7 or IL-15 treatment of SLP-76–deficient CD4+ MP cells (20). In contrast to the decrease seen in AgSp cells maintained in the absence of MHC II (19), preliminary experiments showed no difference in Bcl2 protein levels among YFP+ cells (data not shown). Together with a lack of alteration in STAT5 phosphorylation, unaltered Bcl2 levels suggest that SLP-76 deficiency does not promote increased survival. Therefore, although possible, a model where SLP-76 loss increases responsiveness to homeostatic cytokines and individual cell survival is unlikely to account for the divergence seen between turnover and persistence.

An alternative model to explain the discrepancy between turnover and persistence is that there are two populations within the AgSp memory T cell compartment. We propose that a pool of AgSp CD4+ memory T cells is quiescent and maintained normally in the absence of SLP-76–dependent signals. A second pool of actively proliferating cells dependent on SLP-76/TCR signals is also generated. In this second pool, proliferation and cell death are TCR-dependent and matched; in the absence of SLP-76, this pool therefore has both decreased division and decreased death. In support of the existence of two separate pools of AgSp memory cells, we noted that the percentage of YFP+I-Ab:gp61+ cells remains constant over time in cKO animals, despite the continued proliferation of the YFP+ fraction (data not shown). This model draws parallels with hematopoietic stem cells (HSCs), where long-term dormant HSCs under homeostatic conditions
divide rarely (once every 145 d), but in times of stress/repair can switch to an active state to repopulate the pool and maintain cell numbers (46). Interestingly, self-renewal pathways in memory T cells are similar to those described in long-term dormant HSCs (47). AgSp memory cells undergo glucose-based metabolism, representing a shift from their effector predecessors to a less active and more quiescent state (48–50). Also supportive of this model, a population of long-lived, dormant AgSp memory T cells were recently described that remain quiescent for the life of the host unless stimulated through TCR plus IL-2 or by reinfec tion (51). Quiescence of the dormant AgSp memory cells could prevent exhaustion and depletion of the pool, prevent cancerous mutations caused by excessive division, and provide a stable source of AgSp memory T cells to respond to reinfec tion (52–55).

The experiments presented in this study show that deletion of SLP-76 does not affect the ability of AgSp CD4+ memory T cells to persist. The reduction in BrdU incorporation, yet equal maintenance in YFP+ cKO versus cHET cells, confirms that a normal rate of persist. The reduction in BrdU incorporation, yet equal maintenance in YFP+ cKO versus cHET cells, confirms that a normal rate of persistence of CD4+ memory T cells is not necessary for persistence of I-Aα-gp51+CD4+ memory T cells. Our results strongly support a model of memory stem cells. This model provides for long-lived dormant memory T cells whose limited division prevents deleterious genetic alterations and whose sole purpose is to respond rapidly upon reinfection.

Acknowledgments

We thank Phil Scott and Marc Jenkins for helpful discussions and Jenni Punt and Steve Emerson for critical reading of the manuscript. We thank Hao Shen and E. John Wherry for providing Lin-gp51 and LCMV-Armstrong and the National Health Institutes/Emory Tetramer Facility for the I-Aα-gp51 tetramer.

Disclosures

J.S.M.’s spouse has an equity interest in GlaxoSmithKline and is a current employee of Morphotek. The other authors have no financial conflicts of interest.

References


