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Divergent Generation of Heterogeneous Memory CD4 T Cells

Vaishali R. Moulton,*† Nicholas D. Bushar,*† David B. Leeser,* Deepa S. Patke,* and Donna L. Farber2*

Mechanisms for the generation of memory CD4 T cells and their delineation into diverse subsets remain largely unknown. In this study, we demonstrate in two Ag systems, divergent generation of heterogeneous memory CD4 T cells from activated precursors in distinct differentiation stages. Specifically, we show that influenza hemagglutinin- and OVA-specific CD4 T cells activated for 1, 2, and 3 days, respectively, exhibit gradations of differentiation by cell surface phenotype, IFN-γ production, and proliferation, yet all serve as direct precursors for functional memory CD4 T cells when transferred in vivo into Ag-free mouse hosts. Using a conversion assay to track the immediate fate of activated precursors in vivo, we show that day 1- to 3-activated cells all rapidly convert from an activated phenotype (CD25highIL-7RlowCD44high) to a resting memory phenotype (IL-7RhighCD25lowCD44high) 1 day after antigenic withdrawal. Paradoxically, stable memory subset delineation from undifferentiated (day 1- to 2-activated) precursors was predominantly an effector memory (CD62Llow) profile, with an increased proportion of central memory (CD62Lhigh) T cells arising from more differentiated (day 3-activated) precursors. Our findings support a divergent model for generation of memory CD4 T cells directly from activated precursors in multiple differentiation states, with subset heterogeneity maximized by increased activation and differentiation during priming. The Journal of Immunology, 2006, 177: 869–876.

The cellular precursors to memory T cells are distinguished by their selective survival within an activated/effector T cell pool that is otherwise susceptible to apoptosis. It was recently shown that expression of the cytokine receptor IL-7R and/or the CD8αα homodimer marked precursors for lymphocytic choriomeningitis virus-specific memory CD8 T cell development (8, 9). However, this correlation has not been upheld for memory CD8 T cells in similar systems (10–12), suggesting that the expression of specific genes may not drive memory T cell generation, but may be a secondary effect of other factors (not yet defined) that directly promote memory T cell generation. The identification of genes or factors directly involved in memory CD4 T cell generation has not yet been accomplished, although IL-7R expression has been implicated in memory CD4 T cell survival (13, 14).

An additional complication in deciphering the cellular origin of memory T cells is the remarkable heterogeneity of memory CD4 and CD8 T cells in phenotype and tissue distribution (15, 16). Two memory T cell subsets have been defined based on expression of the lymph node homing receptor(s) CD62L and/or CCR7, and are designated central memory T cells (TCM; CD62LhighCCR7+), which primarily reside in lymphoid tissue, and effector memory T cells (TEM; CD62LlowCCR7−), which are the predominant subset in nonlymphoid tissue (17–19). We and others have also identified additional variations in tissue-resident memory T cells (20, 21), suggesting that multiple memory subsets exist. Mechanisms for the generation of these heterogeneous memory subsets have not been elucidated, including whether distinct type of memory subsets are derived from specific precursors, or via distinct pathways.

Thus, the heterogeneity of memory T cells, the difficulty of associating specific gene expression with memory precursors, and the lack of information on factors affecting the fate of an activated/effector T cell, all present formidable challenges toward elucidating mechanisms for memory T cell generation. It has not been possible to establish a precursor-product relationship between activated/effector cells and the resultant memory T cells with the current in vivo models (largely viral infection (22)) for three reasons. First, the primary effector response in vivo is heterogeneous in terms of extent of activation and differentiation (23), and one cannot determine from which activated/effector population(s) memory T cell develops. Second, the fate of individual activated T cell clones in vivo cannot be tracked, and even T cells derived from TCR-transgenic mice expressing a fixed TCR develop into heterogeneous effector and memory populations (2, 19, 21, 23, 24). Finally, primary responses are typically examined at the peak effector response (after 1 wk) and memory responses months thereafter (8, 25, 26), with no analysis during the intervening period,
such that the immediate fate of activated T cells and their potential for memory development are not known.

In this study, we used an adoptive transfer system where we could follow the fate of activated memory precursors in vivo, to investigate mechanisms for development of heterogeneous memory CD4 T cells. We modeled heterogeneous activation during a primary response by altering the duration of Ag exposure during priming, and hypothesized that these differently activated cells could serve as specific precursors for development of distinct memory T cell subsets. We found that CD4 T cells specific either for influenza hemagglutinin (HA) or chicken OVA activated for 1, 2, or 3 days in vitro exhibited gradations of differentiation by cell surface phenotype, IFN-γ production, and entry into the cell cycle; however, they all developed into memory CD4 T cells in vivo in Ag-free intact mouse hosts. Using a conversion assay to track the immediate fate of differentially activated precursors in vivo, we found that acquisition of memory T cell markers such as IL-7R expression occurs as early as 1 day following Ag withdrawal. Paradoxically, effector memory (CD62L<sup>low</sup>) CD4 T cells were the predominant subset arising from short-term activated, undifferentiated CD4 T cells, with increased proportions of central memory CD4 T cells developing from differentiated precursors activated for longer times. Our results support a new divergent model for memory CD4 T cell generation directly from activated precursors independent of differentiation state, with subset heterogeneity maximized by increased activation and differentiation during priming.

Materials and Methods

Mice
BALB/c mice (8–16 wk of age) were obtained from Charles River Laboratories. HA-TCR transgenic mice (27) and DO11.10 OVA-TCR mice (28) bred as heterozygotes onto BALB/c (Thy1.2) hosts, were maintained in the animal facility at the University of Maryland (Baltimore, MD) under specific pathogen-free conditions, and all animal procedures were approved by the Institutional Animal Care and Use Committee at the University of Maryland School of Medicine.

Abs and peptides
The following Abs were purified from bulk culture supernatants and purchased from BioExpress; anti-CD8 (TIB 105), anti-CD4 (GK1.5), anti-I-A<sup>d</sup> (212.11), and anti-Thy-1 (TIB 238). The 6.5 anti-clonotype Ab directed against the HA-TCR (27), was purified and conjugated to biotin (Pierce). Generation of effector and memory CD4 T cells

CD4 T cells were purified from spleens of HA-TCR and OVA-TCR mice as described previously (1) and sorted for CD4 expression by magnetic sorting using AutoMACS (Miltenyi Biotec) resulting in 99–100% CD4<sup>high</sup> CD4 T cells, and cultured with 5.0 μg/ml HA peptide or 1.0 μg/ml OVA peptide, respectively, and mitomycin C-treated APCs prepared from BALB/c splenocytes as described previously (1) in complete Clicks me medium (Irvine Scientific) for 1, 2, or 3 days at 37°C. Differentially activated cells were purified by Ficoll centrifugation (LSM; ICN/Cappel), and sorted for CD25 expression using AutoMACS (Miltenyi Biotec), resulting in 90–95% pure CD25<sup>+</sup> CD4 T cells. Activated CD25<sup>+</sup> cells or naive CD4 T cells (1.5 × 10<sup>7</sup>/mouse) were injected i.v. into BALB/c (Thy1.1) adoptive hosts as described (24, 29), and persisting memory CD4 T cells were harvested 1–9 mo posttransfer.

Proliferation assays

Naive HA- or OVA-specific CD4 T cells were labeled with 2.5 mM CFSE (Molecular Probes), and 1 × 10<sup>6</sup> T cells were cultured with 3 × 10<sup>6</sup> splenic APCs plus HA peptide (5 μg/ml) or OVA peptide (1 μg/ml) in 24-well plates at 37°C, harvested at time points described in the text, and analyzed by flow cytometry. Analysis of cell division was conducted as described previously (30), with the number of undivided cohorts obtained by dividing the number of events (s) at each cell division n, by 2<sup>n</sup>. The percentage of dividing cells was obtained by the equation: percent dividing cells = (total no. of cohorts undergoing 1–9 divisions/total no. of cohorts undergoing 0–9 divisions) × 100.

Assays for cytokine production

Cytokine production from memory CD4 T cells was assessed by 18-h intracellular cytokine staining (ICS) analysis, as previously described (21, 31). Briefly, HA- or OVA-specific CD4 T cells (1 × 10<sup>6</sup>) were cultured with syngeneic APC (3 × 10<sup>6</sup>) with or without HA peptide (5 μg/ml) or OVA peptide (1 μg/ml) for 18 h. Monensin (Golgistop; BD Pharmingen) was added to cultures and cells were harvested after 6 h, surface stained with fluorescent mAbs to CD4, transgenic TCR and Thy1.2, fixed (Cytofix buffer; BD Pharmingen), permeabilized, and stained intracellularly with anti-IFN-γ Ab or its isotype control as described previously (21, 31), and analyzed using the FACS Calibur and CellQuest software (BD Biosciences). Cytokine production from day 1-, 2-, and 3-activated cells was assessed similarly by adding monensin in the last 6 h of each culture.

In vivo conversion assays

CD4 T cells isolated from naive HA-TCR mice were labeled with 2.5 μM CFSE (Molecular Probes) as described previously (32), and 1 × 10<sup>6</sup> T cells were cultured with 3 × 10<sup>6</sup> BALB/c splenic APC plus HA peptide (5 μg/ml) in 24-well plates at 37°C, harvested at 1, 2, or 3 days, sorted for CD25 expression as above, and adoptively transferred into intact BALB/c hosts. Each successive day, spleens and/or peripheral blood were harvested from individual mice, and CD4 T cells were isolated, stained for cell surface markers, and analyzed by flow cytometry. For analysis of IFN-γ production, splenocytes were isolated on successive days from recipients of CD25<sup>+</sup> day 1- to 3-activated cells, directly cultured for 4 h in vitro with monensin with or without PMA/ionomycin as described previously (21), and harvested for analysis of IFN-γ production by ICS.

Results

We previously found that priming of influenza HA-specific CD4 T cells from HA-TCR transgenic mice with HA peptide and APC for 3–5 days in vitro provided the necessary signals for generation of a long-lived HA-specific memory CD4 T cell population when transferred into Ag-free BALB/c and RAG2<sup>−/−</sup> hosts, with all of the phenotypic and functional qualities of memory T cells—including rapid effector function, heterogeneity in CD62L expression, and diverse distribution in lymphoid and nonlymphoid tissues (21, 24, 29, 31, 33). To test our hypothesis that differentially activated T cells give rise to distinct subsets of memory T cells, we used a variation of this in vitro priming/adoptive transfer system to isolate sufficient quantities of activated cells for subsequent tracking and analysis of memory development in vivo (see Fig. 1). We generated differentially activated T cells by stimulating purified naive TCR-transgenic (CD4<sup>high</sup>) CD4 T cells with peptide Ag and APC in vitro for 1, 2, or 3 days, respectively. The resultant activated cells (Thy1.2<sup>+</sup>) were sorted based on expression of the activation marker, CD25, to isolate primed cells and remove residual APC, transferred in equal numbers into syngeneic BALB/c (Thy1.1) mouse hosts (with control transfers of naive CD4 T cells) and assayed for development of memory CD4 T cells after 1–9 mo in vivo (Fig. 1). To verify that our findings were not specific to one antigenic system, we used purified CD4 T cells from both HA-TCR and DO11.10 (OVA-specific) transgenic mice.
Generation of differentially activated Ag-specific CD4 T cells

We initially evaluated the differentiation state of naive/day 0 (CD44<sup>low</sup>) Ag-specific CD4 T cells activated in vitro for 1 (day 1), 2 (day 2), and 3 days (day 3) with APC plus antigenic peptide, by assessing their phenotype, proliferation, and capacity for effector cytokine production (Fig. 2). Phenotypically, there was a progressive up-regulation of the activation markers CD44 and CD25 (Fig. 2A, second and third rows), and a progressive increase in cell size (first row) with increased Ag exposure from 1 to 3 days of activation that was comparable in both HA- (Fig. 2A) and OVA- (data not shown) specific CD4 T cells. By contrast, IL-7R was extensively down-regulated on day 1- to 3-activated Ag-specific CD4 T cells, with only a small proportion (2–3%) of IL-7R<sup>+</sup> T cells remaining at each time point (Fig. 2A, fourth row). For proliferation, there was minimal division of the day 1-activated CD4 T cells, a low proportion of dividing cells by day 2, yet a substantial proportion of cells undergoing multiple divisions by day 3 for both HA- (Fig. 2B) and OVA-specific (data not shown) CD4 T cells. Functionally, day 1-activated CD4 T cells produced very low levels of IFN-γ, with increased levels of IFN-γ from day 2- and day 3-activated CD4 T cells (Fig. 2C). For HA-specific CD4 T cells, peak IFN-γ production was observed from day 2 cells (Fig. 2C, left), whereas for OVA-specific CD4 T cells, peak IFN-γ production occurred from day 3 cells (right). These results indicate that altering the duration of Ag exposure affects the differentiation state of Ag-specific CD4 T cells with respect to phenotype, effector function, and proliferation; day 1-activated cells are undifferentiated by all parameters, day 3-activated cells are maximally differentiated by all criteria, and day 2-activated cells are intermediate-differentiated in effector cytokine production (particularly for HA-specific T cells), but not yet proliferating. Our results are likewise consistent with earlier studies that found limiting Ag exposure did not enable effector CD4 T cell differentiation (34, 35).

We sorted day 1- to 3-activated cells for CD25 up-regulation before adoptive transfer. Fig. 2D shows mean fluorescence intensities (MFIs) of CD44 and CD25 (left), IL-7R<sup>+</sup> and forward scatter (right) of the naive (day 0) and sorted CD25<sup>+</sup> day 1-, day 2-, and day 3-activated HA-specific CD4 T cells used for adoptive transfer. The progressive alterations in these phenotypic parameters are similar to those observed on unfractionated day 1- to 3-activated CD4 T cells.
cells (Fig. 2A and data not shown). To ensure that the adoptively transferred cells were not being further stimulated in vivo, we cultured the day 1, day 2 and day 3 CD25− cells with freshly isolated APCs, overnight. We found that CD25 expression remained unchanged or was slightly decreased after culture, and no IFN-γ production could be detected from these cultured cells (data not shown), suggesting that the level of Ag carryover was minute and not sufficient to further stimulate these cells in vivo.

**Memory T cell generation from differentially activated precursors**

We transferred sorted, CD25+ day 1- to 3-activated OVA and HA-specific CD4 T cells into BALB/c (Thy1.1) hosts, and found that they all gave rise to functional Ag-specific memory CD4 T cells based on three criteria: long-term persistence, a memory-specific phenotype, and rapid recall function. For OVA-specific CD4 T cells, we found significant numbers of persisting OVA-specific memory CD4 T cells from day 1- to 3-activated precursors in the spleen, lungs, and mesenteric lymph nodes of BALB/c hosts (Fig. 3A). By contrast, we observed complete attrition of OVA-specific naive CD4 T cells, which is known to occur in adoptive transfers into intact mice (36), and further establishes the persisting CD4 T cells in our system as memory. Although substantial proportions of memory CD4 T cells were generated from day 1- to 2-activated precursors, the frequency of persisting memory CD4 T cells from day 3 precursors averaged 5-fold more than that of day 1–2 in the spleen and lung and 10-fold more in the lymph node (Fig. 3A and data not shown). Spleen-derived OVA-specific memory T cells from day 1–3 precursors were small in size and exhibited a memory phenotype (CD25lowCD44highIL-7Rαhigh) (Fig. 3B), which was likewise observed in lymph node and lung-derived memory cells (data not shown). Functionally, day 1- to 3-derived memory T cells from spleen and lung all exhibited rapid IFN-γ production following in vitro recall with OVA peptide and APC, with an increased proportion of IFN-γ producers from day 3-derived compared with day 1- to 2-derived memory CD4 T cells (Fig. 3C). These results show that memory CD4 T cells can be generated from CD4 T cells exposed to Ag for only 1 day; however, memory T cell frequency and rapid recall capacity were greatest when derived from CD4 T cells activated with Ag for longer times.

We performed similar adoptive transfers of day 1- to 3-activated cells using HA-specific CD4 T cells into BALB/c (Thy1.1) hosts. Although overall yields were lower compared with OVA-specific CD4 T cells, we also observed memory generation from HA-specific day 1- to 3-activated precursors, with no persisting cells from transfers of naive HA-specific T cells (Fig. 4A, top row). We obtained the lowest frequency of HA-specific memory CD4 T cells from day 1 precursors, and comparable frequencies from day 2- and day 3-activated precursors (Fig. 4A and data not shown). Persisting day 1- to 3-derived HA-specific memory CD4 T cells in intact hosts likewise bore a memory phenotype (CD25lowCD44highIL-7Rαhigh) (data not shown), and exhibited rapid IFN-γ and IL-2 production upon restimulation with HA peptide in vitro, with a lower overall recall capacity from day 1-derived compared with day 2- and day 3-derived memory cells (Fig. 4B), similar to the OVA system. However, with HA-specific memory CD4 T cells, we typically find a lower proportion of IFN-γ producers after Ag stimulation compared with OVA-specific memory CD4 T cells (data not shown), due to the lower overall avidity of the 6.5 TCR compared with the KJ1-26 TCR (M. Ndejeimi and D. L. Farber, unpublished results). Furthermore, we obtained similar results for memory generation from HA-specific day 1- to 3-activated cells that were not sorted for CD25 up-regulation before transfer. These results establish that memory CD4 T cells can be generated from Ag-specific CD4 T cells at multiple stages of differentiation—from undifferentiated (day 1), to highly differentiated (day 3)—that is, generalizable to two diverse antigenic systems.

**Direct conversion of differentially activated precursor cells into Ag-specific memory CD4 T cells**

Our finding that even short-term activated yet undifferentiated Ag-specific CD4 T cells (day 1-activated) gave rise to long-lived memory T cells contrasted with the prevailing view that memory T cells derive from differentiated effector cells. We therefore asked...
whether memory generation from day 1- to 3-activated precursors in our system occurred via direct “resting down” to a memory phenotype, or whether differentiation to effector cells continued in vivo due to an initial activation program, as suggested for CD8 T cells (37). To distinguish these possibilities, we developed a conversion assay to track the acquisition of memory characteristics from day 1- to 3-activated CD4 T cells on successive days following their transfer into adoptive hosts in vivo. For this assay, we transferred CFSE-labeled day 1-3 CD25 active HA-specific CD4 T cells into BALB/c mice, and recovered T cells from peripheral blood and spleen each successive day up to 4 days post-transfer. From this analysis, we found that the CD25highIL-7RlowCD44 high phenotype of activated T cells rapidly converted to a resting memory (CD25lowIL-7RhighCD44high) phenotype after 1 day in vivo (Fig. 5 and data not shown). After only 24 h in vivo, CD25 expression was significantly down-regulated (Fig. 5A, first row), and IL-7R expression was substantially up-regulated (Fig. 5B, first row) from day 1- to 3-activated cells, and these phenotypic changes continued after 2–3 additional days in vivo (Fig. 5, A and B, second and third rows). Fig. 5C depicts the corresponding MFIs for CD25 (left panel) and IL-7Rα (right panel) expression of the cells in vitro (black symbols) and in vivo (red symbols). CD44 expression was also further up-regulated after an additional 1–3 days in vivo on day 1- to 3-activated CD4 T cells (data not shown).

To address whether the day 1- to 3-activated cells were undergoing further turnover and/or functional differentiation, we likewise monitored proliferation and IFN-γ production at successive days posttransfer into Ag-free hosts. As shown in Fig. 5D, day 1- to 3-activated precursors all continued to divide in vivo, with a similar extent of proliferation observed at day 4 for all precursors. By contrast, analysis of de novo IFN-γ production from day 1- to 3-activated cells recovered ex vivo did not reveal significant IFN-γ production (Table I; columns marked “in vivo”), indicating that these cells were not producing IFN-γ de novo, although they could be further stimulated in vitro with PMA/ionomycin to produce IFN-γ (Table I). These results indicate that day 1-activated cells
acquire memory T cell characteristics (i.e., CD25<sup>low</sup>IL-7R<sup>high</sup>CD44<sup>high</sup> phenotype and ability to produce IFN-γ upon re-stimulation) without eliciting substantial de novo effector function during priming. When taken together, our results demonstrate a rapid conversion of CD25<sup>+</sup> day 1- to 3-activated precursors into resting memory CD4 T cells in vivo upon removal of the antigenic stimulus, in the absence of further differentiation, yet accompanied by proliferative turnover.

**Heterogeneity of differentially generated Ag-specific memory CD4 T cells**

Our results above show that Ag-activated CD4 T cells in distinct stages of differentiation can directly give rise to functional memory CD4 T cells. Although we observed heterogeneous tissue distribution in lymphoid and nonlymphoid compartments from day 1–3 precursors (see Fig. 3), we also asked whether the stable distribution of memory T<sub>CM</sub> and T<sub>EM</sub> subsets varied according to the extent of priming by examining CD62L expression in long-term memory T cells derived from day 1- to 3-activated precursors. We found that HA- and OVA-specific memory CD4 T cells derived from day 1 precursors were primarily CD62L<sub>low</sub>, consistent with a T<sub>EM</sub> phenotype, with an increased proportion of CD62L<sub>high</sub> T<sub>CM</sub> cells in day 2- and day 3-derived memory T cells (Fig. 6A). Expression of CD62L likewise cosegregated with CD45RB<sup>high</sup> expression (data not shown), which we previously found to also delineate mouse T<sub>CM</sub> (21). The proportion of HA- and OVA-specific T<sub>CM</sub> (CD62L<sup>high</sup>) memory CD4 T cells in individual BALB/c mice from multiple experiments is depicted in Fig. 6B, and reveals two consistent findings: first, there was a preponderance of CD62L<sup>low</sup> T<sub>BM</sub> memory CD4 T cells derived from day 1 and day 2 precursors in all experiments, and second, the proportion of CD62L<sup>high</sup> T<sub>CM</sub> cells from day 3 precursors was significantly higher than that of day 1 and day 2 precursors (<i>p < 0.001</i>) for both HA- and OVA-specific memory CD4 T cells (Fig. 6B). This trend of increased CD62L<sup>high</sup> memory T cells from day 3 vs day 1 and 2 precursors was also evident in lymph nodes from recipient mice, and was independent of whether activated precursors were sorted for CD25 before transfer, sorted for pure CD4<sup>+</sup> naive T cells before activation, or transferred into BALB/c or Rag2<sup>−/−</sup> hosts (Fig. 6B and data not shown). These results demonstrate that delineation of CD62L subsets of memory CD4 T cells can be affected by the differentiation state of the activated precursor.

**Discussion**

Mechanisms for the generation of memory CD4 T cells and delineation into subsets have proven difficult to establish. We reveal here that influenza HA- and OVA-specific CD4 T cells activated for 1, 2, and 3 days, respectively, exist in distinct differentiation states, yet all serve as direct precursors for functional memory CD4 T cells upon withdrawal from the antigenic stimulus. Paradoxically, CD62L<sup>low</sup> T<sub>EM</sub> cells represent the predominant subset generated from undifferentiated (day 1-activated) precursors, with more differentiated precursors giving rise to CD62L<sup>high</sup> T<sub>CM</sub> cells. Our results support a new model for generation of memory T cells from multiple precursors via branched diversions on the pathway of T cell activation, effector differentiation and death (Fig. 7). In this model, the potential for memory generation from activated cells occurs immediately upon withdrawal of Ag generating a “pre-memory” T cell with up-regulated IL-7R expression and down-regulated CD25 expression (Fig. 7). The less differentiated T cells skew toward development of T<sub>EM</sub> cells, whereas more differentiated precursors give rise to increased development of T<sub>CM</sub> cells and increased subset heterogeneity.

Our model for memory generation contrasts the prevailing linear model for memory generation from differentiated effector cells, which was supported by earlier adoptive transfer studies demonstrating memory T cell generation from populations of activated T cells producing effector cytokines (3, 4). In analogous transfer studies, activated T cells that did not produce effector cytokines were also shown to persist in vivo as memory T cells (6, 38).
of the TEM rather than the TCM subset (26), and day 1-activated lymphoid homing and less differentiated CD62LhighCD45RBhigh precursors to TCM cells. However, Ahmed and colleagues (19) have demonstrated a direct conversion of CD62Llow TEM to TCM memory CD8 T cells in spleen (26, 41), although it was not possible in those studies to identify the direct precursors to virus-specific memory CD8 T cells (8), this correlation has not been upheld for peptide-primed CD8 T cells (10), and its role in memory CD4 T cell generation is not defined. Our results showing that Ag withdrawal triggers up-regulation of IL-7R on the majority of activated T cells within 1 day in vivo suggest that acquisition of qualities that promote survival of memory T cells is controlled by extrinsic factors, rather than being an inherent property of the activated T cell. We propose that Ag withdrawal is a primary trigger for memory development during activation, although other host factors (e.g., cytokines) may likewise influence memory conversion and subset delineation. We previously found that CD62L expression on effector cells was not maintained in the resultant memory T cell population (21), and we found here that CD62L expression was not altered on day 1–3 precursors up to 1 wk in vivo (data not shown), contrasting the rapid phenotypic conversion of CD25 and IL-7R. These results suggest that stable delineation into CD62L subsets occurs after the initial priming stage, after an extended period in the host in vivo.

In conclusion, identifying pathways for memory T cell differentiation is critical for understanding long-term immunity, and has broad implications for the manipulation of memory heterogeneity in vaccines and autoimmune disease. We reveal here that memory CD4 T cells can derive from multiple cellular precursors upon Ag withdrawal, suggesting a novel divergent model of memory T cell development.

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Disclosures

The authors have no financial conflict of interest.

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MULTIPLE PRECURSORS FOR MEMORY CD4 T CELL DEVELOPMENT


