Immunology
The Journal of cells, molecules, systems and technologies

British Society for immunology

IMMUNOLOGY REVIEW ARTICLE

Generation, persistence and plasticity of CD4 T-cell memories

Jason R. Lees and Donna L. Farber
Department of Surgery, University of Maryland School of Medicine, Baltimore, MD, USA

doi:10.1111/j.1365-2567.2010.03288.x
Received 10 February 2010; revised 11 March 2010; accepted 12 March 2010.
Correspondence: Dr D. L. Farber, Department of Surgery, University of Maryland School of Medicine, MSTF Building, Room 400, 685 W. Baltimore St., Baltimore, MD 21201, USA. Email: dfarber@smail.umaryland.edu
Senior author: Donna L. Farber

Summary

The development of immune memory mediated by T lymphocytes is central to durable, long-lasting protective immunity. A key issue in the field is how to direct the generation and persistence of memory T cells to elicit the appropriate secondary response to provide protection to a specific pathogen. Two prevailing views have emerged; that cellular and molecular regulators control the lineage fate and functional capacities of memory T cells early after priming, or alternatively, that populations of memory T cells are inherently plastic and subject to alterations in function and/or survival at many stages during their long-term maintenance. Here, we will review current findings in CD4 T-cell memory that suggest inherent plasticity in populations of memory CD4 T cells at all stages of their development – originating with their generation from multiple types of primed CD4 T cells, during their persistence and homeostatic turnover in response to T-cell receptor signals, and also following secondary challenge. These multiple aspects of memory CD4 T-cell flexibility contrast the more defined lineages and functions ascribed to memory CD8 T cells, suggesting a dynamic nature to memory CD4 T-cell populations and responses. The flexible attributes of CD4 T-cell memory suggest opportunities and mechanisms for therapeutic manipulation at all phases of immune memory development, maintenance and recall.

Keywords: cellular differentiation; effector functions; homeostasis; immune memory; T lymphocytes

Introduction

The generation and persistence of immunological memory after pathogen encounter provides the basis for an efficient immune response, as previous immune activations are recorded and stored on the cellular and molecular level. Memory T cells direct and co-ordinate efficacious secondary immune responses through their enhanced functional, activation and migration properties compared with naive T cells. These enhanced immune responses ensure that an individual is protected from succumbing to repeated pathogen infections over a lifetime. In mouse models, memory CD4 and CD8 T cells mediate protective immunity to bacterial and viral pathogens, although in humans the ability to stimulate memory T-cell development and modulate their function in vaccines and anti-pathogen immunity remains elusive. For this reason, much of the current research on immune memory is concentrated on understanding mechanisms for the generation and persistence of functional memory T cells.

The life cycle of memory T cells can be broken down into three phases. The first phase comprises the initial generation of memory T cells following priming of naive, antigen-specific T cells with antigen and the appropriate co-stimulatory signals, resulting in their expansion and differentiation to effector cells within the first 7–9 days of infection. Following this acute phase, a large proportion of effector cells die and a population of surviving memory T cells emerges, with a decreased activation threshold and high functional capacity. During the second phase, memory T cells persist via steady-state homeostatic turnover in response to T-cell receptor (TCR) and/or cytokine-mediated signals. The final phase consists of the reactivation of memory T cells, leading to efficacious secondary responses.

A key issue in the field is how to direct the generation and persistence of memory T cells to elicit the appropriate secondary response to provide protection to a specific pathogen. Currently two prevailing views have emerged; that cellular and molecular regulators control the lineage

fate and functional capacities of memory T cells early after priming, or alternatively, that populations of memory T cells are inherently plastic and subject to alterations in function and/or survival at many stages during their long-term maintenance.

The majority of studies on memory T cells have focused on CD8 T cells mainly in the lymphocytic choriomeningitis virus infection model. These studies have defined some key molecular regulators of CD8 effector and memory T-cell fate. By contrast, mechanisms for memory CD4 T-cell development and persistence appear to be distinct from CD8 T cells and with less defined pathways and mediators. Moreover, CD4 T cells are required for the generation of memory CD8 T cells and are central regulators of secondary immune responses. Here, we will review the state of current findings in CD4 T-cell memory that suggest inherent plasticity in populations of memory T cells at all stages of their life cycle – originating with their development from primed CD4 T cells, dynamically altering over time during their persistence and homeostasis, and also following secondary challenge. This flexibility in memory CD4 T-cell development and functional maintenance suggests opportunities for therapeutic manipulation at all phases of immune memory development, persistence and secondary responses.

Memory CD4 T-cell generation: multiple precursors

The key issue in dissecting mechanisms for the generation of memory T cells is to define the signals and properties that distinguish short-lived effector T cells from memory T cells, which survive long-term. In studies on anti-viral effector and memory CD8 T cells, it is possible to isolate precursors to relatively short-lived effector cells (SLEC) and memory T cells (memory precursor effector cell; MPEC) based on expression of effector markers (T-bet, CD27), receptors for survival cytokines (interleukin-7 receptor; IL-7R), and markers associated with apoptosis (KLRG-1).[1] A number of groups have recently shown that altering inflammation and effector markers,[2,3] IL-2 responses,[4,5] and cytokine signalling or transcriptional regulation[6–9] lead to variations in the proportion of SLEC compared with MPEC. These findings indicate that effector or memory CD8 T-cell fate is determined at priming, which ultimately sets the extent of memory CD8 T-cell persistence and the potency of secondary CD8 T-cell-mediated immune responses. In response to different infections, SLEC and MPEC can both exhibit long-lived persistence,[10] suggesting that these populations may also exhibit some flexibility during their maintenance *in vivo*.

In contrast to CD8 T cells, there are as yet no defined precursors to effector and memory CD4 T cells, and the SLEC and MPEC designations have no direct correlates for CD4 T cells. Importantly, the production of effector cytokines does not seem to be a controlling factor for the development of memory CD4 T cells as shown in a number of studies. Mouse models in which interferon-γ (IFN-γ) -producing cells were genetically marked *in vivo* or isolated before adoptive transfer revealed memory CD4 T-cell generation from both IFN-γ^+ and IFN-γ^- populations.[11,12] Similarly, antigen-specific CD4 T cells that were primed for only short periods of time and consequently did not acquire effector function, were still able to develop into memory CD4 T cells *in vivo*.[13] These data suggest that the precursors to CD4 T-cell memory consist of a heterogeneous population of cells present within effector cytokine-producing and non-producing populations. The type of effector cells generated during priming may affect subsequent mechanisms for survival as memory T cells. Recently CD44 expression was shown to be required for memory CD4 T-cell generation from T helper type 1 (Th1) effector cells, but not Th2 or Th17 cells, because of its regulation of Fas-mediated apoptosis specifically in the Th1 subset.[14] These findings suggest that while multiple activated CD4 T-cell precursors may survive to become long-lived memory CD4 T cells, the specific mechanisms for their survival may differ.

Cytokines of the γc family can also affect memory T-cell generation and survival. Interleukin-7 is a known survival cytokine for T lymphocytes,[15,16] and precursors to memory CD8 T cells have been isolated based on up-regulation of IL-7R expression.[17] While memory CD4 T cells have been shown to require IL-7 for long-term persistence,[18] studies so far do not implicate IL-7R as a marker for memory CD4 T-cell precursors. While IL-7R is down-regulated after CD4 T-cell activation, it also can be rapidly up-regulated from IL-7R-negative activated T cells,[13] indicating that IL-7R down-regulation does not limit the potential to respond to IL-7 survival signals as memory T cells. Moreover, memory CD4 T-cell generation is not appreciably altered *in vivo* by fixed expression of the IL-7R or exogenous IL-7,[19,20] suggesting that IL-7 is not a predominant controlling factor for memory CD4 T-cell development.

Interleukin-2 has been shown to differentially drive effector or memory CD8 T-cell development,[21] with high IL-2 responses driving differentiation of terminal effector CD8 T cells.[4,5] By contrast, for memory CD4 T cells, increased responses to IL-2 quantitatively enhance the generation of both effector and memory CD4 T cells. Increased IL-2 production during priming correlated with increased frequency of memory CD4 T cells[22] and inhibited the apoptosis of effector CD4 T cells.[23] In addition, precursors to memory CD4 T cells all up-regulate the IL-2R, CD25,[13,24] in contrast to CD8 T cells where CD25 expression marks different effector and memory T-cell fates.[4] Hence, although IL-2 has quantitative effects on overall memory CD4 T-cell yield, it does not serve as a controller of cell fate.

Other factors that affect priming and development of memory CD4 T cells are the duration/dose of antigenic priming. Unlike CD8 T cells, which require only limited contact with antigen for initiating effector and memory development,[25,26] CD4 T cells require more sustained activation with antigen to promote full activation.[27] Prolonged antigen exposure during priming has been shown in several studies to increase the proportion of memory CD4 T cells generated,[13,28] although short-term activated T cells can also develop into memory populations.[13] When taken together, the flexible requirements for priming conditions and differentiation suggest multiple precursors to memory CD4 T cells (Fig. 1) as we have also previously suggested.[13,29] While primed/effector CD4 T cells can undergo activation-induced cell death in response to repeated or robust stimulation, below this threshold and above a minimum activation requirement, there appears to be a broad range of cellular activation states that can give rise to memory CD4 T cells (Fig. 1). Memory CD4 T-cell differentiation can therefore be viewed as multiple branched pathways from activated precursors to memory T cells surviving via homeostasis (Fig. 1 and[13]). The signals for branched memory T-cell development may be set during the initial cell division, leading to asymmetric apportioning of effector molecules to daughter cells,[30] or alternately, via the cessation of TCR signals that occurs following removal from antigen, as 'rested' effector CD4 T cells were shown to have similar gene expression profiles as *in vivo*-generated memory CD4 T cells.[31] These broadly defined requirements for precursors to memory CD4 T-cell generation can predispose the population to functional diversity.

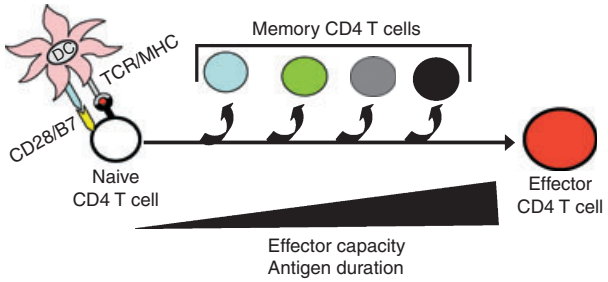

Figure 1. Multiple precursors generate memory CD4 T cells. The model depicts CD4 T-cell differentiation following antigen activation of naive CD4 T cells with increased effector capacity of the population following increased antigen stimulation. The end stage of T-cell differentiation is maximal differentiation to effector cells which ultimately die. At multiple stages before reaching the activation threshold for effector cell death, activated CD4 T cells at distinct differentiation states can branch off to develop and persist as memory CD4 T cells. DC, dendritic cell; MHC, major histocompatibility complex; TCR, T-cell receptor.

Memory homeostasis and persistence

Once generated, memory CD4 T cells can persist for up to the lifetime of an individual via long-term homeostasis and turnover. In humans, anti-viral memory CD4 T cells have been shown to persist for longer periods relative to memory CD8 T cells, which decay more quickly.[32,33] The very nature of their long-term maintenance suggests the potential for dynamic alteration of memory T cells over time based on their perception of signals and subsequent responses. Whether memory T cells require antigen and/or non-cognate T-cell stimulation has been extensively investigated. Memory CD8 T cells have been shown to persist independent of TCR signalling and major histocompatibility complex (MHC) class I engagement,[34,35] and to be more dependent on homeostatic cytokines such as IL-15 and IL-7 for their survival and homeostasis. By contrast, memory CD4 T cells appear to have more stringent requirements for TCR engagement for their persistence/homeostasis. Previous studies showed functional deterioration of memory CD4 T cells in MHC class II-deficient hosts,[36,37] and a role for TCR signalling in optimal persistence of memory CD4 T cells.[38] We recently showed that ablation of TCR signals in memory CD4 T cells through conditional deletion of the gene encoding SLP-76, a key TCR-coupled linker-adapter signalling molecule, resulted in almost complete inhibition of memory CD4 T-cell homeostatic turnover both in steady-state and lymphopenic conditions, and a corresponding diminution in their persistence.[39] The addition of excess levels of IL-7 *in vivo* could not restore wild-type levels of homeostatic turnover in SLP-76-deficient memory CD4 T cells,[39] suggesting that TCR signals are the predominant regulators of memory CD4 T-cell homeostasis. Further studies will be needed to precisely define how cytokines such as IL-7 and IL-15, which are also implicated in memory CD4 T-cell survival and/or homeostasis,[18,38,40] contribute to memory CD4 T-cell homeostatic turnover in conjunction with TCR-mediated signals.

The requirement for TCR signalling and MHC class II engagement for memory CD4 T-cell homeostasis has important implications. Continuous TCR engagement implies that signals are being dynamically perceived during memory CD4 T-cell maintenance with potential effects on functional and/or survival capacities. Memory CD4 T-cell turnover driven by TCR engagement can result in selective survival of specific clones as a result of their propensity to receive TCR signals, resulting in an overall narrowing of the TCR repertoire of memory CD4 T cells over time. Indeed, expanded clones of memory CD4 T cells have been detected in aged individuals, supporting this prediction.[41] This perception of T-cell signals during memory CD4 T-cell homeostasis can also result in functional alteration of these cells. As lymphopenia-driven homeostatic proliferation of naive T cells results in their

conversion to memory phenotypes and functions,[42,43] steady-state homeostasis of memory T cells, albeit at a slower rate, may also have functional consequences over time.

Memory CD4 T-cell heterogeneity

Populations of memory T cells persist in diverse subtypes in lymphoid and non-lymphoid tissues. Over 10 years ago, two major memory T-cell subsets were delineated based on expression of the lymph node homing receptors CD62 ligand (CD62L) and CCR7, which enable entry into lymph nodes through high endothelial venules.[44–46] The $CD62L^{lo}$ $CCR7^{lo}$ memory subtype is defined as effector-memory (T_{EM}) while the $CD62L^{hi}$ $CCR7^{hi}$-expressing subset is central-memory (T_{CM}). The initial finding in human peripheral blood CD4 T cells identified the T_{EM} subset as producing effector cytokines, with the T_{CM} subset predominantly producing IL-2.[45,47] However, in subsequent studies of antigen-specific subsets *in vivo*, both T_{EM} and T_{CM} populations were found to have effector function,[48–51] although the T_{CM} subset has a greater proliferative capacity compared with the T_{EM} subset in both humans and mice.[47]

Whether the T_{EM}/T_{CM} designations represent stable populations or populations that are dynamically altered during their persistence *in vivo* is not yet resolved. However, there is evidence that homeostatic proliferation of T_{CM} results in both maintenance of T_{CM} subsets and conversion of some progeny to T_{EM} phenotypes, mostly based on results with CD8 T cells.[52,53] Conversely, T_{EM} can convert to T_{CM} cells during persistence or following activation for CD8 or CD4 T cells,[50,54] indicating that T_{EM}/T_{CM} subset designations are not fixed. Moreover, the phenotypic markers that define T_{EM}/T_{CM} subsets do not encompass all of the multiple phenotypic variations seen in memory CD4 T cells (for a review, see ref. [51]). Notably, in humans, memory CD4 T cells were found to differ in a variety of chemokine receptors (distinct from CCR7), associated with different replicative histories as revealed by telomere length.[55] The complexities of phenotypic variation in memory T cells could arise either during their initial generation from multiple precursors or through changes and signals perceived during their homeostatic maintenance.

Memory T-cell diversity in homing/chemokine receptor expression reflects their diverse capacities for trafficking and residence in multiple lymphoid and non-lymphoid tissue sites, including spleen, lung, liver, gut and bone marrow.[56–58] Although expression of specific chemokine receptors can be associated with T-cell trafficking to certain tissue sites, such as intestine and skin,[59–61] mechanisms directing populations of memory T cells into non-lymphoid tissue sites remain largely undefined. In mice, dendritic cells from specific tissue sites can induce expression of tissue-specific homing molecules,[60,62–66] suggesting that priming conditions can direct memory T cells to specific tissues. Memory T cells resident in distinct tissues also have specific functional capacities with memory CD4 and CD8 T cells in gut and bone marrow having more effector-like properties,[67–71] and lung memory CD4 T cells having distinct homing properties.[48] The homing profile of memory cell subsets is also predictive of functional capacities, with specific chemokine receptors appearing coincident with Th1, Th2, Th17 and T follicular helper functional T-cell subsets.[57,72] These findings suggest that tissue-specific factors play a significant role in tuning the memory response in diverse anatomical sites.

Whether tissue-homing memory CD4 T cells further differentiate or can migrate back to the lymphoid compartment has not been resolved; however, evidence points to peripheral tissue-resident memory CD4 T cells as end-stage memory subsets. In general, non-lymphoid resident memory T cells have more effector-like properties,[68,73] and T_{CM} cells with primary residence in lymphoid tissue convert to T_{EM} phenotype cells in non-lymphoid sites,[53] supporting a role for lymphoid memory in seeding non-lymphoid sites through further differentiation. Furthermore, repeated boosting of antigen-specific memory populations results in their preferential residence in non-lymphoid sites,[74] indicating that increased differentiation is associated with peripheral resident memory T cells. Given the large fraction of persisting memory CD4 T cells residing in tissue sites, dissecting the mechanisms and specific responses of tissue-resident memory CD4 T cells will be an important focus for future research to target the generation or specific homing of memory T cells to the site of pathogen entry.

Environmental regulation during secondary activation

The idea of T-cell effector function as an indication of irreversible cellular differentiation to defined subsets has been re-evaluated in the context of a large body of evidence demonstrating significant plasticity in previously committed effector T-cell trafficking, cytokine production and transcriptional regulation. Indeed, effector T cells previously thought to represent fully differentiated states have now been clearly shown to be capable of co-expression of alternative effector molecules, or even of full conversion to wholly new phenotypes. Although expression of transcriptional regulators, such as T-bet or GATA-3, clearly directs cells towards Th1 or Th2 functional capacities, respectively,[75–77] they do not necessarily result in terminal differentiation as previously thought.[78–80]

Memory CD4 T-cell responses have been shown to maintain the effector functions from the primary response, based on studies showing conserved Th1 or Th2

cytokine profiles from populations of polarized Th1 or Th2 effector cells, respectively.[81,82] For CD8 T cells, expression of T-bet and effector cytokine production is conserved in effector and memory responses.[2] For memory CD4 T cells however, this functional capacity does not appear to be irreversibly committed. We initially demonstrated functional plasticity with a population of memory CD4 T cells that produced predominantly IFN-γ in the primary response, but could be differentially activated in the secondary response by altering the nature of the TCR stimulus to produce predominantly IL-4.[83] Human memory Th1 and Th2 cells were similarly found to have flexible functional profiles upon recall.[84] Inflammation and cytokine environment can likewise alter the outcome of memory CD4 T-cell responses with pro-inflammatory environments promoting Th1-like cytokines from populations of Th2 or uncommitted memory CD4 T cells,[12,85] and Th1-like memory CD4 T cells producing Th2 cytokines in a Th2 cytokine environment.[86] Continued environmental input was demonstrated to be necessary for induction of Th1 effector functions even following memory induction from a strongly Th1 primary response.[85] Together these studies suggest that during activation of secondary CD4 T-cell responses, environmental cues can adjust the functional outcome, and ultimately determine its efficacy.

Memory CD4 T-cell functional plasticity allows for continuous adjustment of immune responses to multiple and sequential pathogens and sites of infection. In Fig. 2, we present a schematic diagram of possible mechanisms for plasticity in secondary responses from a population of memory CD4 T cells either during antigenic recall and/or their persistence by homeostatic mechanisms. Upon antigenic recall, there are three possible outcomes for memory CD4 T-cell-mediated responses (Fig. 2, middle): (i) a conserved response in which antigenic recall of the memory CD4 T-cell cohort would elicit effector responses similar to those observed in the primary response; (ii) an altered response in which the memory CD4 T-cell cohort responds to a change in TCR stimulus or cytokine/inflammatory environment, and generates an effector response distinct from that observed in the primary response; (iii) a recall antigen could elicit a response from only a subset of pre-existing memory CD4 T cells with a subset of the functions. In these ways, a population of memory CD4 T cells could be uniquely poised to respond in a similar way to previous activations or to vary their response in altered environmental conditions.

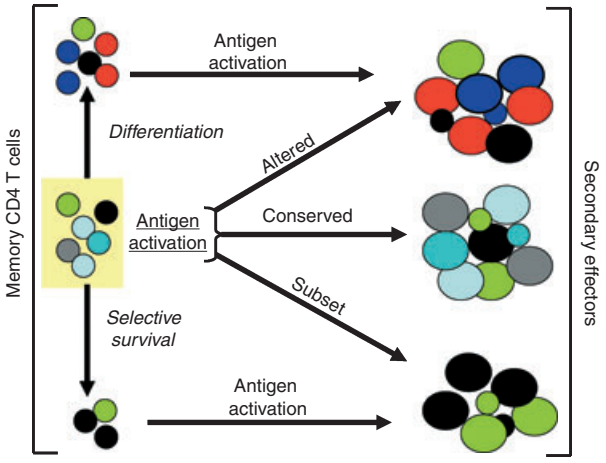

Figure 2. Model for memory CD4 T-cell plasticity in secondary responses. Schematic diagram of possible mechanisms for flexibility in recall responses by a population of antigen-specific memory CD4 T cells, because of direct antigenic recall (centre arrows), or via altered persistence because of homeostatic mechanisms (top and bottom arrows). For explanation, see text.

In addition to plasticity in antigenic recall responses, memory CD4 T cells can also undergo changes during their maintenance and homeostasis. Selective survival of a particular memory population as a result of TCR signals encountered during homeostasis can result in a narrowing of the cohort of surviving memory CD4 T cells responding to recall antigen (Fig. 2, bottom). In addition, homeostatic mechanisms with TCR or cytokine signals can result in further differentiation of a memory population leading to new functions elicited upon antigenic recall (Fig. 2, top arrow). With these mechanisms, a given population is not irreversibly fixed to mediate a specific recall function, but remains receptive to cues in the environment and during persistence to fine-tune responses. The effect of these altered secondary responses on long-term changes in specific memory populations is not known, although successive boosting of memory CD8 T-cell responses resulted in altered phenotypes and tissue distribution,[74] suggesting that each antigen exposure may imprint the persisting memory population in distinct ways.

Vaccine development and memory modulation

The plasticity in memory CD4 T-cell generation, persistence and recall function has important implications for vaccine strategies for protective immunity, and for memory modulation in disease. Flexibility in memory CD4 T-cell precursors suggests that the potential for generating memory CD4 T cells to a given antigen immunization is quite high. Whether the resultant memory populations mediate protection upon secondary challenge could be directly related to their migration or tissue-resident properties – therefore targeting memory T cells to the appropriate compartments may be more important than initial priming in vaccine design. In addition, long-term maintenance of specific memory CD4 T-cell populations may be favoured by intermittent boosting as memory CD4 T cells are optimally maintained with TCR signals. Hence, plasticity in memory

CD4 T-cell generation and homing suggests that targeting strategies following memory generation may be the best way to optimize memory CD4 T-cell responses.

Memory CD4 T cells can also mediate and perpetuate undesirable immune reactions in autoimmunity and also in rejection of transplanted organs.[87] Functional plasticity of memory CD4 T-cell populations suggests that they can be modulated by altering the *in vivo* environment or by immunotherapy.[88] Immunomodulators such as CD28 co-stimulation inhibition, previously thought to only affect naive CD4 T cells,[89] have recently been shown to affect memory CD4 and CD8 T-cell responses *in vivo*.[90,91] In addition, T-cell depleting strategies may differentially affect naive and memory CD4 T-cell homeostasis.[92] Elucidating mechanisms for modulating memory T-cell responses during the recall phase will have broad use in treating immunopathologies perpetuated by memory T cells.

Conclusions

We present here evidence for memory CD4 T-cell plasticity at each stage in memory T-cell development – beginning with memory CD4 T-cell generation from multiple types of activated precursors, during its persistence by homeostatic turnover in response to TCR-driven signals, and also in secondary responses to altered antigenic recall and cytokine environments. These multiple aspects of memory CD4 T-cell flexibility contrast the more defined lineages and functions ascribed to memory CD8 T cells, suggesting a dynamic nature to memory CD4 T-cell responses. It should be possible to exploit this plasticity for therapeutic use, to target the generation of specific types of memory responses long after initial priming, and for regulating memory responses that cause immunopathologies.

Acknowledgement

D.L.F. is supported by NIHAI083022 and a grant from the Juvenile Diabetes Research Foundation; J.R.L. is supported by a grant from the National Multiple Sclerosis Society.

Disclosures

The authors have no financial disclosures.

References

1 Kaech SM, Wherry EJ. Heterogeneity and cell-fate decisions in effector and memory $CD8^+$ T cell differentiation during viral infection. *Immunity* 2007; **27**:393–405.

2 Joshi NS, Cui W, Chandele A, Lee HK, Urso DR, Hagman J, Gapin L, Kaech SM. Inflammation directs memory precursor and short-lived effector $CD8^+$ T cell fates via the graded expression of T-bet transcription factor. *Immunity* 2007; **27**:281–95.

3 Sarkar S, Kalia V, Haining WN, Konieczny BT, Subramaniam S, Ahmed R. Functional and genomic profiling of effector CD8 T cell subsets with distinct memory fates. *J Exp Med* 2008; **205**:625–40.

4 Kalia V, Sarkar S, Subramaniam S, Haining WN, Smith KA, Ahmed R. Prolonged interleukin-2Rα expression on virus-specific $CD8^+$ T cells favors terminal-effector differentiation *in vivo*. *Immunity* 2010; **32**:91–103.

5 Pipkin ME, Sacks JA, Cruz-Guilloty F, Lichtenheld MG, Bevan MJ, Rao A. Interleukin-2 and inflammation induce distinct transcriptional programs that promote the differentiation of effector cytolytic T cells. *Immunity* 2010; **32**:79–90.

6 Araki K, Turner AP, Shaffer VO, Gangappa S, Keller SA, Bachmann MF, Larsen CP, Ahmed RmTOR regulates memory CD8 T-cell differentiation. *Nature* 2009; **460**:108–12.

7 Kallies A, Xin A, Belz GT, Nutt SL. Blimp-1 transcription factor is required for the differentiation of effector $CD8^+$ T cells and memory responses. *Immunity* 2009; **31**:283–95.

8 Rao RR, Li Q, Odunsi K, Shrikant PA. The mTOR kinase determines effector versus memory $CD8^+$ T cell fate by regulating the expression of transcription factors T-bet and Eomesodermin. *Immunity* 2010; **32**:67–78.

9 Rutishauser RL, Martins GA, Kalachikov S *et al.* Transcriptional repressor Blimp-1 promotes $CD8^+$ T cell terminal differentiation and represses the acquisition of central memory T cell properties. *Immunity* 2009; **31**:296–308.

10 Jameson SC, Masopust D. Diversity in T cell memory: an embarrassment of riches. *Immunity* 2009; **31**:859–71.

11 Harrington LE, Janowski KM, Oliver JR, Zajac AJ, Weaver CT. Memory CD4 T cells emerge from effector T-cell progenitors. *Nature* 2008; **452**:356–60.

12 Lohning M, Hegazy AN, Pinschewer DD *et al.* Long-lived virus-reactive memory T cells generated from purified cytokine-secreting T helper type 1 and type 2 effectors. *J Exp Med* 2008; **205**:53–61.

13 Moulton VR, Bushar ND, Leeser DB, Patke DS, Farber DL. Divergent generation of heterogeneous memory CD4 T cells. *J Immunol* 2006; **177**:869–76.

14 Baaten BJ, Li CR, Deiro MF, Lin MM, Linton PJ, Bradley LM. CD44 regulates survival and memory development in Th1 cells. *Immunity* 2010; **32**:104–15.

15 Tan JT, Dudl E, LeRoy E, Murray R, Sprent J, Weinberg KI, Surh CD. IL-7 is critical for homeostatic proliferation and survival of naive T cells. *Proc Natl Acad Sci U S A* 2001; **98**:8732–7.

16 Tan JT, Ernst B, Kieper WC, LeRoy E, Sprent J, Surh CD. Interleukin (IL)-15 and IL-7 jointly regulate homeostatic proliferation of memory phenotype $CD8^+$ cells but are not required for memory phenotype $CD4^+$ cells. *J Exp Med* 2002; **195**:1523–32.

17 Kaech SM, Tan JT, Wherry EJ, Konieczny BT, Surh CD, Ahmed R. Selective expression of the interleukin 7 receptor identifies effector CD8 T cells that give rise to long-lived memory cells. *Nat Immunol* 2003; **4**:1191–8.

18 Kondrack RM, Harbertson J, Tan JT, McBreen ME, Surh CD, Bradley LM. Interleukin 7 regulates the survival and generation of memory CD4 cells. *J Exp Med* 2003; **198**:1797–806.

19 Haring JS, Jing X, Bollenbacher-Reilley J, Xue HH, Leonard WJ, Harty JT. Constitutive expression of IL-7 receptor α does not support increased expansion or prevent contraction of antigen-specific CD4 or CD8 T cells following *Listeria monocytogenes* infection. *J Immunol* 2008; **180**:2855–62.

20 Tripathi P, Mitchell TC, Finkelman F, Hildeman DA. Cutting edge: limiting amounts of IL-7 do not control contraction of $CD4^+$ T cell responses. *J Immunol* 2007; **178**:4027–31.

21 Blattman JN, Grayson JM, Wherry EJ, Kaech SM, Smith KA, Ahmed R. Therapeutic use of IL-2 to enhance antiviral T-cell responses *in vivo*. *Nat Med* 2003; **9**:540–7.

22 Chandran SS, Verhoeven D, Teijaro JR, Fenton MJ, Farber DL. TLR2 engagement on dendritic cells promotes high frequency effector and memory CD4 T cell responses. *J Immunol* 2009; **183**:7832–41.

23 Zhang X, Giangreco L, Broome HE, Dargan CM, Swain SL. Control of CD4 effector fate: transforming growth factor beta 1 and interleukin 2 synergize to prevent apoptosis and promote effector expansion. *J Exp Med* 1995; **182**:699–709.

24 Tang AL, Bingaman AW, Kadavil EA, Leeser DB, Farber DL. Generation and functional capacity of polyclonal alloantigen-specific memory CD4 T cells. *Am J Transplant* 2006; **6**:1275–84.

25 van Stipdonk MJ, Hardenberg G, Bijker MS, Lemmens EE, Droin NM, Green DR, Schoenberger SP. Dynamic programming of $CD8^+$ T lymphocyte responses. *Nat Immunol* 2003; **4**:361–5.

26 Kaech SM, Ahmed R. Memory $CD8^+$ T cell differentiation: initial antigen encounter triggers a developmental program in naive cells. *Nat Immunol* 2001; **2**:415–22.

27 Obst R, van Santen HM, Mathis D, Benoist C. Antigen persistence is required throughout the expansion phase of a $CD4^+$ T cell response. *J Exp Med* 2005; **201**:1555–65.

28 Blair DA, Lefrancois L. Increased competition for antigen during priming negatively impacts the generation of memory CD4 T cells. *Proc Natl Acad Sci U S A* 2007; **104**:15045–50.

29 Moulton VR, Farber DL. Committed to memory: lineage choices for activated T cells. *Trends Immunol* 2006; **27**:261–7.

30 Chang JT, Palanivel VR, Kinjyo I *et al.* Asymmetric T lymphocyte division in the initiation of adaptive immune responses. *Science* 2007; **315**:1687–91.

31 McKinstry KK, Golech S, Lee WH, Huston G, Weng NP, Swain SL. Rapid default transition of CD4 T cell effectors to functional memory cells. *J Exp Med* 2007; **204**:2199–211.

32 Amara RR, Nigam P, Sharma S, Liu J, Bostik V. Long-lived poxvirus immunity, robust CD4 help, and better persistence of CD4 than CD8 T cells. *J Virol* 2004; **78**:3811–6.

33 Hammarlund E, Lewis MW, Hansen SG, Strelow LI, Nelson JA, Sexton GJ, Hanifin JM, Slifka MK. Duration of antiviral immunity after smallpox vaccination. *Nat Med* 2003; **9**:1131–7.

34 Murali-Krishna K, Lau LL, Sambhara S, Lemonnier F, Altman J, Ahmed R. Persistence of memory CD8 T cells in MHC class I-deficient mice. *Science* 1999; **286**:1377–81.

35 Polic B, Kunkel D, Scheffold A, Rajewsky K. How alpha beta T cells deal with induced TCRα ablation. *Proc Natl Acad Sci U S A* 2001; **98**:8744–9.

36 De Riva A, Bourgeois C, Kassiotis G, Stockinger B. Noncognate interaction with MHC class II molecules is essential for maintenance of T cell metabolism to establish optimal memory CD4 T cell function. *J Immunol* 2007; **178**:5488–95.

37 Kassiotis G, Garcia S, Simpson E, Stockinger B. Impairment of immunological memory in the absence of MHC despite survival of memory T cells. *Nat Immunol* 2002; **3**:244–50.

38 Seddon B, Tomlinson P, Zamoyska R. Interleukin 7 and T cell receptor signals regulate homeostasis of CD4 memory cells. *Nat Immunol* 2003; **4**:680–6.

39 Bushar ND, Corbo E, Schmidt M, Maltzman JS, Farber DL. Ablation of SLP-76 signaling after T cell priming generates memory CD4 T cells impaired in steady-state and cytokine-driven homeostasis. *Proc Natl Acad Sci U S A* 2010; **107**:827–31.

40 Purton JF, Tan JT, Rubinstein MP, Kim DM, Sprent J, Surh CD. Antiviral $CD4^+$ memory T cells are IL-15 dependent. *J Exp Med* 2007; **204**:951–61.

41 Schwab R, Szabo P, Manavalan JS, Weksler ME, Posnett DN, Pannetier C, Kourilsky P, Even J. Expanded $CD4^+$ and $CD8^+$ T cell clones in elderly humans. *J Immunol* 1997; **158**:4493–9.

42 Hamilton SE, Wolkers MC, Schoenberger SP, Jameson SC. The generation of protective memory-like $CD8^+$ T cells during homeostatic proliferation requires $CD4^+$ T cells. *Nat Immunol* 2006; **7**:475–81.

43 Surh CD, Sprent J. Homeostasis of naive and memory T cells. *Immunity* 2008; **29**:848–62.

44 Sallusto F, Langenkamp A, Geginat J, Lanzavecchia A. Functional subsets of memory T cells identified by CCR7 expression. *Curr Top Microbiol Immunol* 2000; **251**:167–71.

45 Sallusto F, Lenig D, Forster R, Lipp M, Lanzavecchia A. Two subsets of memory T lymphocytes with distinct homing potentials and effector functions [see comments]. *Nature* 1999; **401**:708–12.

46 Campbell JJ, Murphy KE, Kunkel EJ *et al.* CCR7 expression and memory T cell diversity in humans. *J Immunol* 2001; **166**:877–84.

47 Sallusto F, Geginat J, Lanzavecchia A. Central memory and effector memory T cell subsets: function, generation, and maintenance. *Annu Rev Immunol* 2004; **22**:745–63.

48 Bingaman AW, Patke DS, Mane VR, Ahmadzadeh M, Ndejembi M, Bartlett ST, Farber DL. Novel phenotypes and migratory properties distinguish memory CD4 T cell subsets in lymphoid and lung tissue. *Eur J Immunol* 2005; **35**:3173–86.

49 Champagne P, Ogg GS, King AS *et al.* Skewed maturation of memory HIV-specific CD8 T lymphocytes. *Nature* 2001; **410**:106–11.

50 Wherry EJ, Teichgraber V, Becker TC, Masopust D, Kaech SM, Antia R, von Andrian UH, Ahmed R. Lineage relationship and protective immunity of memory CD8 T cell subsets. *Nat Immunol* 2003; **4**:225–34.

51 Verhoeven D, Teijaro JR, Farber DL. Heterogeneous memory T cells in antiviral immunity and immunopathology. *Viral Immunol* 2008; **21**:99–113.

52 Geginat J, Lanzavecchia A, Sallusto F. Proliferation and differentiation potential of human $CD8^+$ memory T-cell subsets in response to antigen or homeostatic cytokines. *Blood* 2003; **101**:4260–6.

53 Marzo AL, Yagita H, Lefrancois L. Cutting edge: migration to nonlymphoid tissues results in functional conversion of central to effector memory CD8 T cells. *J Immunol* 2007; **179**:36–40.

54 Schwendemann J, Choi C, Schirrmacher V, Beckhove P. Dynamic differentiation of activated human peripheral blood $CD8^+$ and $CD4^+$ effector memory T cells. *J Immunol* 2005; **175**:1433–9.

55 Song K, Rabin RL, Hill BJ *et al.* Characterization of subsets of $CD4^+$ memory T cells reveals early branched pathways of T cell differentiation in humans. *Proc Natl Acad Sci U S A* 2005; **102**:7916–21.

56 Sigmundsdottir H, Butcher EC. Environmental cues, dendritic cells and the programming of tissue-selective lymphocyte trafficking. *Nat Immunol* 2008; **9**:981–7.

57 Rivino L, Messi M, Jarrossay D, Lanzavecchia A, Sallusto F, Geginat J. Chemokine receptor expression identifies Pre-T helper (Th)1, Pre-Th2, and nonpolarized cells among human $CD4^+$ central memory T cells. *J Exp Med* 2004; **200**:725–35.

58 Schaerli P, Ebert L, Willimann K, Blaser A, Roos RS, Loetscher P, Moser B. A skin-selective homing mechanism for human immune surveillance T cells. *J Exp Med* 2004; **199**:1265–75.

59 Connor SJ, Paraskevopoulos N, Newman R, Cuan N, Hampartzoumian T, Lloyd AR, Grimm MC. CCR2 expressing $CD4^+$ T lymphocytes are preferentially recruited to the ileum in Crohn's disease. *Gut* 2004; **53**:1287–94.

60 Mora JR, Bono MR, Manjunath N, Weninger W, Cavanagh LL, Rosemblatt M, von Andrian UH. Selective imprinting of gut-homing T cells by Peyer's patch dendritic cells. *Nature* 2003; **424**:88–93.

61 Reiss Y, Proudfoot AE, Power CA, Campbell JJ, Butcher EC. CC chemokine receptor (CCR)4 and the CCR10 ligand cutaneous T cell-attracting chemokine (CTACK) in lymphocyte trafficking to inflamed skin. *J Exp Med* 2001; **194**:1541–7.

62 Campbell DJ, Butcher EC. Rapid acquisition of tissue-specific homing phenotypes by $CD4^+$ T cells activated in cutaneous or mucosal lymphoid tissues. *J Exp Med* 2002; **195**:135–41.

63 Kassiotis G, Stockinger B. Anatomical heterogeneity of memory $CD4^+$ T cells due to reversible adaptation to the microenvironment. *J Immunol* 2004; **173**:7292–8.

64 Dudda JC, Lembo A, Bachtanian E *et al.* Dendritic cells govern induction and reprogramming of polarized tissue-selective homing receptor patterns of T cells: important roles for soluble factors and tissue microenvironments. *Eur J Immunol* 2005; **35**:1056–65.

65 Dudda JC, Martin SF. Tissue targeting of T cells by DCs and microenvironments. *Trends Immunol* 2004; **25**:417–21.

66 Mora JR, Cheng G, Picarella D, Briskin M, Buchanan N, von Andrian UH. Reciprocal and dynamic control of CD8 T cell homing by dendritic cells from skin- and gut-associated lymphoid tissues. *J Exp Med* 2005; **201**:303–16.

67 Di Rosa F, Pabst R. The bone marrow: a nest for migratory memory T cells. *Trends Immunol* 2005; **26**:360–6.

68 Masopust D, Vezys V, Marzo AL, Lefrancois L. Preferential localization of effector memory cells in nonlymphoid tissue. *Science* 2001; **291**:2413–7.

69 Masopust D, Vezys V, Wherry EJ, Barber DL, Ahmed R. Cutting edge: gut microenvironment promotes differentiation of a unique memory CD8 T cell population. *J Immunol* 2006; **176**:2079–83.

70 Tokoyoda K, Zehentmeier S, Hegazy AN, Albrecht I, Grun JR, Lohning M, Radbruch A. Professional memory $CD4^+$ T lymphocytes preferentially reside and rest in the bone marrow. *Immunity* 2009; **30**:721–30.

71 Zhang X, Dong H, Lin W *et al.* Human bone marrow: a reservoir for ''enhanced effector memory'' $CD8^+$ T cells with potent recall function. *J Immunol* 2006; **177**:6730–7.

72 Moser B, Schaerli P, Loetscher P. $CXCR5^+$ T cells: follicular homing takes center stage in T-helper-cell responses. *Trends Immunol* 2002; **23**:250–4.

73 Masopust D, Lefrancois L. CD8 T-cell memory: the other half of the story. *Microbes Infect* 2003; **5**:221–6.

74 Masopust D, Ha SJ, Vezys V, Ahmed R. Stimulation history dictates memory CD8 T cell phenotype: implications for prime-boost vaccination. *J Immunol* 2006; **177**:831–9.

75 Szabo SJ, Kim ST, Costa GL, Zhang X, Fathman CG, Glimcher LH. A novel transcription factor, T-bet, directs Th1 lineage commitment. *Cell* 2000; **100**:655–69.

76 Zhang DH, Cohn L, Ray P, Bottomly K, Ray A. Transcription factor GATA-3 is differentially expressed in murine Th1 and Th2 cells and controls Th2-specific expression of the interleukin-5 gene. *J Biol Chem* 1997; **272**:21597–603.

77 Zheng W, Flavell RA. The transcription factor GATA-3 is necessary and sufficient for Th2 cytokine gene expression in CD4 T cells. *Cell* 1997; **89**:587–96.

78 Zhou L, Chong MM, Littman DR. Plasticity of $CD4^+$ T cell lineage differentiation. *Immunity* 2009; **30**:646–55.

79 Zhu J, Paul WE. Heterogeneity and plasticity of T helper cells. *Cell Res* 2010; **20**:4–12.

80 Rowell E, Wilson CB. Programming perpetual T helper cell plasticity. *Immunity* 2009; **30**:7–9.

81 Swain SL. Regulation of the generation and maintenance of T-cell memory: a direct, default pathway from effectors to memory cells. *Microbes Infect* 2003; **5**:213–9.

82 Swain SL, Hu H, Huston G. Class II-independent generation of CD4 memory T cells from effectors. *Science* 1999; **286**:1381–3.

83 Ahmadzadeh M, Farber DL. Functional plasticity of an antigen-specific memory CD4 T cell population. *Proc Natl Acad Sci U S A* 2002; **99**:11802–7.

84 Messi M, Giacchetto I, Nagata K, Lanzavecchia A, Natoli G, Sallusto F. Memory and flexibility of cytokine gene expression as separable properties of human T(H)1 and T(H)2 lymphocytes. *Nat Immunol* 2003; **4**:78–86.

85 Pakpour N, Zaph C, Scott P. The central memory $CD4^+$ T cell population generated during *Leishmania major* infection requires IL-12 to produce IFN-gamma. *J Immunol* 2008; **180**:8299–305.

86 Krawczyk CM, Shen H, Pearce EJ. Functional plasticity in memory T helper cell responses. *J Immunol* 2007; **178**:4080–8.

87 Bingaman AW, Farber DL. Memory T cells in transplantation: generation, function, and potential role in rejection. *Am J Transplant* 2004; **4**:846–52.

88 Ndejembi MP, Tang AL, Farber DL. Reshaping the past: strategies for modulating T-cell memory immune responses. *Clin Immunol* 2007; **122**:1–12.
89 Bluestone JA, St Clair EW, Turka LA. CTLA4Ig: bridging the basic immunology with clinical application. *Immunity* 2006; **24**:233–8.
90 Ndejembi MP, Teijaro JR, Patke DS, Bingaman AW, Chandok MR, Azimzadeh A, Nadler SG, Farber DL. Control of memory CD4 T cell recall by the CD28/B7 costimulatory pathway. *J Immunol* 2006; **177**:7698–706.
91 Fuse S, Zhang W, Usherwood EJ. Control of memory $CD8^+$ T cell differentiation by CD80/CD86-CD28 costimulation and restoration by IL-2 during the recall response. *J Immunol* 2008; **180**:1148–57.
92 Sener A, Tang AL, Farber DL. Memory T-cell predominance following T-cell depletional therapy derives from homeostatic expansion of naive T cells. *Am J Transplant* 2009; **9**:2615–23.