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Generation and Maintenance of Memory CD4+ T Cells

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Summary

In the course of an immune response to an infectious microbe, pathogen-specific naïve $CD4^+$ T cells proliferate extensively and differentiate into effector cells. Most of these cells die rapidly but a small fraction of effector cells persist as memory cells to confer enhanced protection against the same pathogen. Recent advances indicate that strong TCR stimulation during the primary response is essential for generation of long-lived memory $CD4^+$ T cells. Memory cells appear to be derived equally from all subsets of effector cells, and memory cells can also acquire additional functional capabilities during the secondary response. Resting memory $CD4^+$ cells are dependent on signals from contact with IL-7 and IL-15, but not MHC class II, for their survival and intermittent homeostatic proliferation.

Keywords

memory $CD4^+$ T cells; proliferation; differentiation; effector cells; cytokines

Introduction

$CD4^+$ T cells are essential players in adaptive immunity, providing help to other subsets of lymphocytes as well as mediating direct effector functions through cell-to-cell contact and via production of cytokines and chemokines [1]. Such multi-tasking capability is a reflection of the fact that functionally-distinct populations of effector $CD4^+$ T cells can emerge during an immune response, depending on the quality of the inflammatory conditions encountered. So far, Th1, Th2, T follicular helper (T_{FH}), and Th17 cells have been characterized as distinct subsets of effector $CD4^+$ T cells [1], but whether these effector cells maintain their polarized state upon differentiation into resting memory cells is only beginning to be understood. Although studies on $CD4^+$ T cell responses have been facilitated by the use of TCR transgenic (Tg) cells and MHC class II (MHC-II) tetramers, memory $CD4^+$ T cells are still less well understood than their $CD8^+$ counterparts. While the two subsets of T cells share some common characteristics, the mechanisms involved in the formation of $CD4^+$ memory T cells are different from $CD8^+$ memory T cells [2]. Here, we review recent advances in defining the mechanisms involved in the generation and maintenance of memory $CD4^+$ T cells.

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TCR signalling intensity and memory CD4+ T cell development

Unlike naïve $CD8^+$ T cells which can be easily triggered into a programmed course of differentiation with a relatively brief period of stimulation [3,4], naïve $CD4^+$ T cells require prolonged antigenic stimulation in order to differentiate into effector and memory cells [5,6]. Moreover, while past studies have suggested that weaker stimulation is better than strong stimulation for memory formation [7,8], more recent studies indicate that naive $CD4^+$ T cells require a high threshold of TCR signalling in order to fully differentiate into effector cells that can convert to memory cells. Much of the evidence supporting this notion emerged from the finding that the presence of an unphysiologically high precursor frequency of antigen (Ag)-specific naïve $CD4^+$ T cells during the primary response is detrimental for memory formation. Similar findings were reported previously for $CD8^+$ T cells [9,10], but it is now apparent that the influence of precursor frequency on memory cell generation is much greater for $CD4^+$ cells than $CD8^+$ cells. The negative effect of high precursor frequency on the quality of $CD4^+$ memory cells was initially reported for ovalbumin (ova)-specific OT-II Tg cells, and shown to impair their ability to undergo proliferation and secrete cytokines in response to *Listeria monocytogenes* (Lm) expressing ova [11]. A more thorough study with Eα-specific TEa Tg cells revealed that the response to vesicular stomatitis virus (VSV) expressing Eα at a high precursor frequency not only led to the generation of effector cells with impaired ability to secrete cytokines, but also that these cells were unable to survive as memory cells [12•]. Moreover, this defective immune response was mimicked when a low precursor frequency of naïve TEa Tg cells (which yields a normal immune response) was exposed to only limited amounts of Ag, i.e., by injecting a mAb that competes for the TCR ligand [12•].

Further evidence for the requirement for a high threshold of TCR stimulation for memory generation emerged from studies with Smarta1 TCR Tg $CD4^+$ T cells specific for lymphocytic choriomeningitis virus (LCMV) glycoprotein (gp) 61 presented by LCMV vs. Lm [13•]. Unexpectedly, although naïve Smarta1 cells underwent a comparable level of expansion to both LCMV and Lm expressing gp61 (Lm-gp61), memory $CD4^+$ T cells developed only with LCMV and not with Lm-gp61 infection. The failure to generate memory cells with Lm-gp61 occurred regardless of the T cell precursor frequency, indicating that clonal competition for Ag was not the issue [13•]. Rather, it appeared that Lm-gp61 infection led to a sub-threshold expression of Ag density, as suggested by the findings that Lm-gp61-induced effector cells displayed reduced effector function and a lower TCR avidity than control LCMV-induced effector cells [13•]. Even when the density of the Ag was not limiting, as in the case with LCMV infection, high precursor frequencies of Smarta1 cells led to generation of memory $CD4^+$ T cells with an abbreviated lifespan and impaired ability to mount a secondary response [14•]. Similar shortening in the lifespan of memory $CD4^+$ cells was also observed for two other lines of TCR Tg $CD4^+$ cells when they were stimulated with peptide and adjuvant at a high precursor frequency [15]. Interestingly, in the case with Smarta1 cells, competition for cytokines, in particular IFNγ [14•], appeared to be the main cause of aberrant memory cell development. Collectively, these reports indicate that strong TCR signalling, together with appropriate co-stimulatory signals, is required for optimal generation of long-lived memory $CD4^+$ T cells.

Recent intravital two-photon imaging studies provided a cellular mechanism for why clonal competition at a high precursor frequency of Ag-specific $CD4^+$ T cells is detrimental for a normal immune response. Here, an elegant system was devised to observe the initial interaction of T cells with Ag-loaded dendritic cells (DC). The approach involved reconstituting MHC-II^- mice with MHC-II^+ DC and TCR Tg $CD4^+$ T cells expressing different markers and then imaging the draining LN right after intravenous injection of the agonist peptide [16•]. In contrast to past reports of only transient interactions between T cells and DC during the first few hours of Ag recognition [17], prolonged T-DC interactions were observed immediately in this system and lasted for several hours [16•]. By terminating the T-DC interaction at various

time points with an injection of anti-MHC-II mAb, it was found that at least 6 hrs of continuous contact was required for naïve $CD4^+$ T cells to enter the cell cycle and more than 24 hrs for further cell proliferation and synthesis of cytokines [16•]. Importantly, prolonged T-DC interactions were readily observed with a low precursor frequency of Ag-specific $CD4^+$ T cells for the first 48 hrs, whereas the duration of T-DC interaction became much shorter, as previously reported, at a high precursor frequency of naïve $CD4^+$ T cells [18•]. Thus, clonal competition at a high precursor frequency appears to impair the ability of T cells to make prolonged meaningful contacts with Ag-loaded DC.

Linear versus non-linear differentiation of memory $CD4^+$ T cells

There are two competing models of memory T cell development: linear and divergent differentiation. In the first model, activated naïve T cells develop initially into cytokine-producing effector cells before a small fraction of these cells convert to memory cells. In the divergent model, a fraction of activated naïve T cells differentiate directly into memory cells, bypassing the effector phase. A report supporting the divergent model appeared several years ago with the finding that purified $IFN\gamma^+$ TCR Tg effector $CD4^+$ T cells generated with peptide immunization failed to survive upon adoptive transfer into naïve hosts, whereas $IFN\gamma^-$ Tg cells persisted as memory cells and produced IFNγ upon re-stimulation [19].

Two recent studies on the response to LCMV infection, however, are more consistent with the linear differentiation model. Using a similar approach to the previous report, one study found that generation of memory cells was equally efficient for $IFN\gamma^+$ and $IFN\gamma^-$ Smarta1 effector cells upon adoptive transfer into naïve hosts [20••]. The other study used two complementary genetic approaches to mark $IFN\gamma^+$ polyclonal effector T cells with CD90.1 (Thy-1.1) and then studied the fate of $CD90.1^+$ and $CD90.1^-$ cells upon adoptive transfer into naïve hosts. Again, $IFN\gamma^+$ effector $CD4^+$ (and $CD8^+$) T cells were found to differentiate into memory cells just as efficiently, or even more efficiently, than $IFN\gamma^-$ effector cells [21••]. Both reports found that memory cells derived from $IFN\gamma^+$ effectors were fully functional by all parameters analyzed and also confirmed that memory cells generated from $IFN\gamma^-$ effectors produced IFNγ upon re-stimulation. Therefore, these reports provide strong evidence that development of memory $CD4^+$ T cells, as with memory $CD8^+$ T cells, occurs through a linear pathway. Since $IFN\gamma^-$ effector cells gave arise to memory cells, it is also possible that the divergent pathway may contribute to memory $CD4^+$ T cell formation. One possible indication of the divergent pathway is that recently-activated naïve $CD8^+$ T cells initially undergo asymmetric cell division, the proximal daughter cell displaying both functional and phenotypic characteristics of effector cells and the distal daughter cell behaving more like a memory cell [22]. Asymmetric cell division was also observed for $CD4^+$ T cells, although whether distal daughter cells also exhibit memory function was not analyzed.

IL-7 and selection of memory $CD4^+$ T cells

Signalling through the IL-7 receptor (IL-7R), a heterodimer of IL-7Rα and the common γ chain (γ_c), is essential for prolonged survival of naïve and memory T cells. IL-7R is normally expressed at high levels on resting T cells, and IL-7Rα is rapidly downregulated upon T cell activation and re-expressed by a small fraction of effector T cells destined to survive as memory cells [23]. Recent work suggests that contact with IL-2 during the early phase of activation promotes IL-7Rα re-expression on $CD4^+$ effector cells [24]. Nonetheless, for $CD8^+$ T cells it is now clear that IL-7R expression, while useful for detecting memory precursors, is not required for the effector-to-memory cell transition [25–27]. For $CD4^+$ T cells, however, IL-7R signalling has a much bigger role, and appears to be essential, though not sufficient, for development of memory $CD4^+$ cells. The requirement for contact with IL-7 is suggested by the finding that memory $CD4^+$ cells fail to arise when immune responses are generated in

IL-7^{-} hosts and that T cells expressing a mutant form of IL-7Rα (IL-7Rα^{449F}) cannot induce STAT5 activation [28,29]. Others examined the role of IL-7R signalling at different phases of the CD4^{+} T cell response. One study used polyclonal CD4^{+} T cells expressing chimeric GM-CSF/IL-7Rα chain to assess the effect of strong IL-7R signalling during the early phase of the immune response to LCMV, thereby eliciting intense IL-7R signalling delivered via the burst of GM-CSF produced during the first 6 d after infection. While this strategy increased the magnitude of the effector cell response, the contraction phase was reciprocally pronounced, thus resulting in no enhancement in the overall production of memory cells [26]. Nonetheless, another study found that provision of strong IL-7 signalling by administration of long-lived IL-7/mAb complexes at the peak of the response prevented the contraction of TCR Tg effector CD4^{+} cells by increasing their homeostatic proliferation and Bcl-2 upregulation [30•]. Although these findings indicate that signalling through IL-7 can augment the immune response, constitutive expression of Tg IL-7Rα on T cells failed to enhance (or impair) the production of effector and memory CD4^{+} (and CD8^{+}) cells [27,31•] and their ability to display normal secondary responses [31•]. Thus, continued expression of IL-7Rα alone is not sufficient for the development of memory CD4^{+} T cells.

Homeostasis of Memory CD4^{+} T cells

While most investigators agree that signalling from a combination of IL-7 and IL-15, but not MHC-I, regulates homeostasis of memory CD8^{+} T cells, the factors controlling homeostasis of the memory CD4^{+} T cell pool are more controversial [32]. Much of the confusion here seems to stem from the invalid assumption that memory-phenotype (MP) CD4^{+} T cells, which arise spontaneously without intentional immunization, and Ag-specific (AgSp) memory CD4^{+} T cells, which arise after intentional immunization with a specific Ag, display the same homeostatic requirements (see below). Recent studies with AgSp TCR Tg and polyclonal memory CD4^{+} cells demonstrate that these cells, like memory CD8^{+} T cells, rely on contact with both IL-15 and IL-7 but not MHC (MHC-II) for their homeostasis [28,33••,34•]. IL-7 appears to have a bigger role than IL-15 in supporting survival of memory CD4^{+} cells, but both cytokines seem equally essential for these cells to undergo basal homeostatic proliferation under normal conditions [33••,34•]. IL-15 has a less prominent role for memory CD4^{+} cells than for NK and memory CD8^{+} cells, these latter cells being much more reliant on IL-15 than IL-7 for their homeostasis [32]. This difference in IL-15 dependence correlates closely with the expression levels of the IL-15 receptor, CD122, which is displayed at much lower levels on memory CD4^{+} cells than on NK and memory CD8^{+} cells. For this reason, memory CD4^{+} cells compete less effectively for IL-15 than NK and memory CD8^{+} cells [33••], and this could be one of the main reasons why the lifespan of AgSp memory CD4^{+} cells tends to be shorter than that of memory CD8^{+} cells [35].

In contrast to AgSp memory CD4^{+} cells, MP CD4^{+} cells comprise a heterogeneous population of cells with different homeostatic requirements. While the majority of MP cells appear to resemble AgSp memory CD4^{+} cells, a small fraction of MP CD4^{+} cells undergoes a very rapid rate of homeostatic proliferation under both normal and lymphopenic conditions. Significantly, such rapid proliferation is driven largely by TCR interaction with MHC-II and not by contact with IL-7 or IL-15 [33••,36]. The presence of a population of fast-dividing MP CD4^{+} cells has been interpreted to indicate a role for TCR-MHC-II signalling in homeostasis of memory CD4^{+} cells [37]. However, this conclusion is questionable because AgSp memory CD4^{+} cells typically divide quite slowly [28,33••,34•]. The function of the fast-dividing subset of MP CD4^{+} cells and the nature of the Ag that drives their rapid proliferation have yet to be defined.

Role of apoptotic proteins in memory T cell survival

A delicate balance of pro- and anti-apoptotic molecules dictates survival of T cells [38]. Both IL-7 and IL-15 have been shown to support T cell survival by increasing the expression of anti-apoptotic molecules, such as Bcl-2 and Mcl-1 [39]. On the other hand, the pro-apoptotic BH3-only molecule Bim has been implicated in effector cell death during the contraction phase [40]. This notion was recently confirmed for $CD4^+$ T cells with the finding that a larger pool of effector cells survived to differentiate into memory cells in $Bim^{-/-}$ mice than in normal mice; however, Bim^- memory $CD4^+$ cells did not have an overtly extended lifespan, implying that other homeostatic mechanisms beside Bim are involved in mediating death of memory cells [41]. In addition, high levels of Bim expression were found on effector $CD4^+$ T cells derived from sub-optimally activated naïve $CD4^+$ T cells, i.e., cells that failed to develop into memory cells [13].

The role of Bcl-2 in $CD4^+$ memory cell generation has been studied in $Bim^{+/-}$ $Bcl\text{-}2^{-/-}$ mice, thus circumventing the problem of early death of $Bcl\text{-}2^{-/-}$ mice [42•]. In contrast to the severe loss of naïve T cells in $Bim^{+/-}$ $Bcl\text{-}2^{-/-}$ mice, production of LCMV-specific memory $CD4^+$ cells was only slightly reduced. However, much of the memory $CD4^+$ cell production in these mice appeared to reflect enhanced lymphopenia-driven proliferation; thus, these memory $CD4^+$ cells rapidly disappeared upon adoptive transfer into normal T cells-sufficient hosts [42•]. The conclusion therefore is that Bcl-2 has a prominent role in controlling the survival of memory $CD4^+$ T cells.

In a study using human T cells, gene expression profiling and proteomic analysis suggested that survival of human memory $CD4^+$ T cells is mediated in part by inhibiting the transcription of Bim and Fas via inactivation of forkhead box O3a (FOXO3a) transcription factor combined with activation of STAT5a [43•]. Indeed, silencing the active form of FOXO3a by introducing small interfering RNA or a dominant negative form of FOXO3a was recently found to enhance survival of memory $CD4^+$ T cells in HIV patients [44•]. Another BH3-only pro-apoptotic molecule, Noxa, was also reported to play an intermediary role in controlling memory $CD4^+$ T cell generation [45]. Although the absence of Noxa itself did not affect development of naïve and memory T cells, significant defects were seen with loss of the polycomb group gene, Bmi1, which controls Noxa expression. Thus, $CD4^+$ T cells deficient in Bmi1 underwent normal proliferation and differentiation into effector cells but were severely impaired in their ability to further differentiate into memory cells [46]. Interestingly, the premature death of $Bmi1^-$ effector cells appeared to be mediated through increased Noxa expression, indicating that Bmi1 promotes memory $CD4^+$ cell generation through repression of the Noxa gene.

Recall response of memory $CD4^+$ T cells

Rapid recall immune responses by memory T cells are attributed largely to their increased precursor frequency of Ag-specific cells plus their heightened responsiveness to Ag. One of the major reasons for increased sensitivity to Ag is the reduced co-stimulatory requirements for activation of memory T cells compared to naïve T cells. The poised state of memory $CD4^+$ T cells also appears to reflect elevated expression of the TCR proximal tyrosine kinase, Zap70, in memory $CD4^+$ T cells [47]. With regard to co-stimulatory signals, recent work confirmed that these signals are not required for the initial activation of memory $CD4^+$ T cells to Ag, but nonetheless are important for activated memory cells to mount a sustained immune response. Thus, in vivo blocking of CD28/B7 interactions by injection of CTLA4-Ig considerably reduced the magnitude of memory cell expansion and impaired the capacity of the expanded cells to secrete IL-2 and IFNγ [48]. Moreover, another group found that while CD40/CD40L interactions were dispensable for the expansion of memory $CD4^+$ T cells, CD40L signals were nevertheless required for these cells to synthesize IFNγ [49].

With regard to the kinetics of memory cell proliferation, recent work on viral infections has confirmed the long-held belief that naïve and memory $CD4^+$ T cells commence cell proliferation at a similar time point after Ag exposure and undergo a similar rate of cell division [50]. Thus, LCMV-specific Smarta1 memory $CD4^+$ T cells, like naïve Smarta1 cells, did not initiate cell division until 3–4 days after LCMV infection, despite the fact that memory cells synthesized IFNγ within several hours after the infection. The reason for the lag before entry into cell division is not clear but seems to be dictated by the microenvironment of the infected host. Thus, transfer of Smarta1 cells into mice pre-infected with LCMV 2 days before entered cell division earlier [50]. In light of these findings, the higher magnitude of the secondary response compared to the primary response can be attributed largely to the increased precursor frequency of Ag-specific cells. Nonetheless, another study found that LCMV-specific polyclonal memory $CD4^+$ cells proliferated at a slower rate than naïve $CD4^+$ cells towards the end of the response [51]. The reduced proliferation of memory cells appeared to be due to their low production of IL-2 and increased synthesis of IFNγ, as provision of exogenous IL-2 or anti-IFNγ mAb increased the proliferation of the memory cells [51].

Differentiation of naïve $CD4^+$ T cells into Th1 or Th2 subsets of effector cells is known to involve heritable epigenetic changes that allow for expression of a restricted set of cytokines. For this reason, memory $CD4^+$ cells are widely believed to maintain the polarized cytokine profile of their predecessors. This notion, however, has recently been challenged by two studies demonstrating that Th1 and Th2 memory cells can be induced to synthesize the cytokines of the alternate lineage when stimulated under the opposing conditions. One group analyzed the memory cells generated from in vitro-polarized Th2 (and Th1) Smarta1 effector cells that were adoptively transferred into normal hosts. While Th2 Smarta1 memory cells synthesized Th2 cytokines when left unstimulated, infection with LCMV, a strong inducer of Th1 cell development, caused the bulk of Th2 memory cells to produce IFNγ in addition to Th2 cytokines [20••]. The other group used DC pulsed with an agonist peptide plus either a Th1-inducing bacterial extract or Th2-inducing worm egg Ag to generate Th1 or Th2 responses in normal mice. When the primed mice were boosted with Ag in Th1- or Th2-inducing conditions, a large fraction of memory cells in both situations produced both Th1 and Th2 cytokines [52•]. These findings indicate that memory $CD4^+$ cells derived from polarized effectors are much more amenable than previously thought and are reminiscent of a past study which found that polarized human Th1 and Th2 cells can synthesize cytokines of the alternate lineage [53].

Concluding remarks

Much progress has been made in recent years in understanding the generation and maintenance of memory $CD4^+$ T cells. It is now clear that the strength of TCR signalling during priming has a strong impact on memory formation and that memory cells can develop from both cytokine-producing and non-producing effector cells. Memory $CD4^+$ cells are maintained by signals from contact with both IL-7 and IL-15, but not from TCR interaction with MHC-II ligands. Memory cells generated from polarized Th1 and Th2 effector cells are more flexible than previously considered in terms of their cytokine production capability. This finding implies that pathogenic Th cells in chronic and autoimmune disease could be manipulated to express more benign traits of a different Th lineage. Despite these advances, many key issues still remain outstanding. Two areas are particularly noteworthy. First is the age-old question of the distinguishing feature of the small fraction of effector cells that is selected to persist as memory cells. Even with recent advances, this issue remains largely an enigma. The second important question is why the lifespan of memory $CD4^+$ cells is shorter than for memory $CD8^+$ cells. This question is also unresolved and highlights the increasing realization that many of mechanisms involved in the generation and maintenance of memory $CD4^+$ and $CD8^+$ cells are subtly, but distinctly different.

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