Cutting Edge Commentary: Differential TCR Signaling and the Generation of Memory T Cells

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There are currently two models for the generation of memory T cells: 1) memory T cells arise directly from activated effector T cells that have reverted to the resting state via an unknown mechanism; and 2) memory T cells are generated directly from naive T cells, bypassing an effector stage. I discuss here how recent results on the activation and signaling requirements of naive vs memory CD4 T cells favor the second model and how differential signaling of naive T cells may direct their developmental outcome. The Journal of Immunology, 1998, 160: 535–539.

One of the most familiar features of the adaptive immune system is its ability to confer lifelong protection against a pathogen previously encountered via illness or vaccination. These memory responses to previously encountered Ags surpass primary responses to newly encountered Ags both quantitatively and qualitatively in terms of their magnitude, expediency, and duration. Thus, an understanding of the mechanisms underlying immunologic memory is central to the development of more effective vaccines and immunologic therapies.

Although it is generally agreed that the memory immune response is mediated by memory T and B lymphocytes, basic questions regarding the generation, maintenance, and regulation of memory remain unresolved. This commentary will focus on the question of how immunologic memory is generated, with an emphasis on memory T lymphocytes. I will present a differential signaling model for the generation of Ag-specific memory T cells, based on recent findings identifying signaling and activation differences in naive, effector, and memory T cells and on advances in our understanding of TCR-mediated signal transduction. It will be argued that commitment to an effector T cell or memory T cell lineage will follow separate pathways, due to differential activation of the naive T cell, and that memory T cells may represent partially activated cells.

Immune responses commence when naive CD4 T cells recognize an Ag on the surface of an APC via the highly specific, cell surface TCR. This in turn triggers a cascade of intracellular events resulting in the differentiation of naive T cells into several types of effector T cells, which proliferate and produce myriad soluble factors to either recruit and activate additional immune cells such as macrophages, stimulate Ab production by B lymphocytes, and/or help cytotoxic T cells kill virally infected cells or tumor cells. It has been shown that most of these activated effector T cells subsequently die via activation-induced cell death (1, 2), yet a subset of Ag-specific T cells will persist in an individual as memory T cells (3, 4). When reactivated, memory T cells mediate and coordinate the faster, stronger, and more prolonged memory immune response.

There are currently two models to explain how memory T cells are generated, and these are illustrated in Figure 1A. Model I states that a subset of activated effector cells survives and “reverts” to the resting state to become memory T cells via an undefined mechanism. Model II states that memory T cells arise directly from activation of naive T cells and represent a developmental pathway distinct from that of effector T cells. The currently favored model (I) is often presented in textbooks and review articles on immunologic memory (4) and is used as a basis for the design of experiments investigating T cell memory (5). This model maintains that memory CD4 T cells arise directly from activated effector T cells, primarily because there are currently no phenotypic markers that reliably distinguish effector from memory T cells (6, 7). By contrast, generation of long-lived memory cells according to model II is believed to occur in B cells. B cell activation through the surface Ag receptor (or Ig) results in differentiation either to an activated effector B cell, specifically an Ab-secreting plasma cell, or to a memory B cell (8, 9). Plasma cells have a defined life span (measured between several weeks to several months (8)) and also lose surface expression of both Ig and MHC class II (10), whereas memory B cells can be identified by surface Ig expression and by the presence of class-switched and somatically mutated Ag receptors (11).

Because effector T cells do not permanently lose TCR expression and most TCR do not somatically mutate (with some exceptions (12)), B cell criteria cannot be applied to T cells. Thus, in determining the most appropriate model for the generation of memory T cells, it is necessary to consider at least two main criteria: first, does production of memory T cells depend on the generation of activated effectors; and second, although effector and memory T cells share certain phenotypic characteristics, do memory T cells resemble effector T cells mechanistically in terms of intracellular signaling and activation parameters?

For the first criterion, generation of an activated effector CD4 T cell requires both a proliferative step, which is initiated following...
A. Current Models

Model I:

1. Proliferation
2. Differentiation
Naive CD4 T cell

Effect T cell

Death

Reversion to resting state

Effect T cell

Memory CD4 T cell

Model II:

1. Proliferation
2. Differentiation
Naive CD4 T cell

Effect T cell

Death

Differentiation

Memory CD4 T cell

B. Differential Signaling Model

1. CD3/ZAP-70 phosphorylation
2. B7-1/87-2 costimulation
3. Proliferation
4. Differentiation
Naive CD4 T cell

Effect T cell

1. CD3 phosphorylation
   no ZAP-70 phosphorylation
2. HSA or B7-1/87-2 costimulation
3. Differentiation
Naive CD4 T cell

Effect T cell

Memory CD4 T cell

FIGURE 1. Models for the generation of memory T cells. For explanation, see text.

costimulatory ligands on the APC (13). Using mice with targeted disruption of costimulatory receptor genes, it was shown that either HSA or CD28 could costimulate for production of memory CD8 T cells in both CD28 and HSA-deficient mice, whereas CD28 costimulation was required for effector T cell generation, as demonstrated by the lack of effector T cells in CD28-deficient mice (14). Because HSA delivers a weaker costimulatory signal than CD28 (14), these results suggest that generation of memory T cells can occur due to a lower level stimulus.

A second set of results generated in vitro using human and mouse CD4 T cells has likewise demonstrated the presence of “memory” T cells in the absence of effector T cells. These studies showed that in vitro recall responses are maximized when proliferation is inhibited during the priming step, either by treatment with cyclosporine or anti-IL-2R for mouse T cells (15) or by priming with chemically fixed APC, which do not costimulate naive T cells for proliferation and differentiation into effector T cells for human T cells (16). In the mouse system, addition of IL-2 to the priming culture inhibited the ability of the T cells to respond in a secondary stimulation (15). Although performed in vitro, these studies indicate that the ability of a CD4 T cell to elicit a secondary response following an initial priming step does not require proliferation and formation of effector T cells, supporting model II for the generation of memory CD4 T cells. Thus, both in vitro and in vivo evidence strongly support a lineage disparity between effector and memory T cells.

The second criterion for distinguishing between model I and II for generation of memory T cells states that if memory T cells arise from activated effectors that have reverted to a quiescent state, they should resemble effectors when reactivated. Based on cell surface phenotype and production of effector cytokines, effector T cells parallel memory T cells. Phenotypically, while mouse naive and memory CD4 T cells differ in their expression of the homing receptor MEL-14 (L-selectin) (17), CD44 (18) and CD45RB (19) and CD45RB isoforms of the CD45 glycoprotein, activated effector CD4 T cells exhibit similar CD44, CD45RB, and MEL-14 phenotypes as memory CD4 T cells (6). Functionally, both effector T cells and activated memory T cells produce effector cytokines in addition to IL-2, while naive CD4 T cells produce primarily IL-2 (7, 19, 20). Despite these similarities, when examined on a mechanistic level, as described below, memory CD4 T cells exhibit novel TCR-mediated biochemical signaling and activation properties distinct from effector counterparts.

The precise biochemical signals that drive peripheral T cell differentiation or direct specific functions and activation modes are not known, yet a basic scheme of the proximal signaling events coupled to the TCR has been elucidated (for a review, see Ref. 21). Initial contact of the multisubunit TCR/CD3 complex with its activating ligand initiates a cascade of intracellular signaling events involving a series of kinase activations and coupling of newly phosphorylated substrates. Initially, two intracellular protein tyrosine kinases (PTKs), CD4-associated p56lck and p59fyn (a portion that associates with TCR/CD3), phosphorylate tyrosine residues within ITAMs (immunoreceptor tyrosine-based activation motifs) in the cytoplasmic tail of each CD3 subunit of the TCR/CD3 complex. Subsequently, the tyrosine kinases ZAP-70 and, to a lesser extent, p72syk bind the phosphorylated ITAMs, resulting in tyrosine phosphorylation and ZAP-70/syk kinase activation (22, 23). These proximal events subsequently lead to second messenger generation, calcium flux, activation of the ras pathway, and the ultimate mobilization of transcription factors in the cell nucleus (24).

1 Abbreviations used in this paper: HSA, heat-stable antigen; ITAM, immunoreceptor tyrosine-based activation motif; APL, altered peptide ligand.
Although the nature of these TCR-coupled signaling pathways in ex vivo subsets of T cells is not defined, we and others have begun to examine proximal and downstream signaling events coupled to TCR/CD3 in mouse naive and memory CD4 (and to a lesser extent, CD8) T cells isolated on the basis of CD45 isoform expression. Thus far, four unique signaling properties of memory T cells have been identified: these include differences in the overall pattern of intracellular tyrosine phosphorylation, phosphorylation of the ZAP-70 kinase, phosphorylation of CD3ζ-associated proteins, and decreases in intracellular calcium flux (25–27). Following CD3 cross-linking, naive CD4 T cells exhibit the expected patterns of total tyrosine phosphorylation, ZAP-70 and CD3ζ phosphorylation and association, and calcium flux, comparable with that observed in T cell lines and clones (25–27). Memory CD4 T cells, by contrast, exhibit an altered and reduced pattern of total tyrosine phosphorylation, a virtual lack of ZAP-70 phosphorylation, and additional CD3ζ-associated phosphorylated proteins, although CD3ζ phosphorylation is comparable with naive CD4 T cells (25, 26). Both mouse CD4⁺ and CD8⁺ memory T cells have also been shown to exhibit substantially decreased calcium flux in response to stimulation by TCR cross-linkers, mitogens, and calcium ionophores, such as ionomycin (27, 28).

How do these TCR-coupled signaling processes in memory T cells compare with effector T cell counterparts? Although preliminary examination of TCR-mediated tyrosine phosphorylation in effector T cells generated from ex vivo-purified mouse naive CD4 T cells has revealed striking phosphorylation differences between naive, effector, and memory CD4 T cells (D. L. Farber, unpublished data), a thorough biochemical analysis of ex vivo effectors has not yet been performed. However, there are a wealth of data on signaling parameters in T cell clones that are maintained in vitro by continual stimulation with Ag and can be thought to approximate effector T cells. In responses to Ag or anti-CD3-mediated cross-linking, CD4 T cell clones exhibit a high level of total tyrosine phosphorylation, ZAP-70 phosphorylation, and calcium flux (29–33), contrasting these altered events in memory CD4 T cells.

Despite these differences in intracellular signaling, several important parallels can be drawn between signaling in memory T cells and in T cell clones treated with altered peptide ligands (APL), that may reveal a mechanism for the generation of memory T cells. Paul Allen and others have elegantly shown that changing the TCR contact sites of antigenic or agonist peptides, while not altering their ability to bind MHC class II, results in a ligand with lower affinity for the TCR, yielding a modified functional response in terms of proliferation, cytokine production, and signaling (34). Biochemically, APL alter proximal biochemical signaling coupled to the TCR, resulting in an altered pattern of CD3ζ phosphorylation, a virtual lack of ZAP-70 phosphorylation, and a greatly reduced calcium flux (29, 30, 33). These diminished proximal and downstream signaling events triggered by APL have led to the term “partial activation” (29). Thus, in terms of ZAP-70 phosphorylation and calcium flux, memory CD4 T cells exhibit biochemical properties of partially activated T cell clones.

In addition to signaling similarities, memory CD4 T cells also resemble partially activated T cell clones in certain activation requirements. While APL-activated T cell clones are rendered anergic, and thus unresponsive to subsequent activation by agonist or antigenic peptide (34), memory T cells are potently activated by Ag to differentiate into memory effector T cells and are clearly not anergized in the conventional sense (7). However, mouse memory CD4 T cells have been shown to be hypersensitive to certain noncognate TCR stimuli that activate naive CD4 T cells and T cell clones, such as the bacterial superantigen staphylococcal enterotoxin B (SEB) (35), mitogenic lectins such as Con A (36, 37), and APC-directed anti-CD3 cross-linking in the presence of CD4 ligation by Abs or MHC class II (25, 38). Negative signaling through CD4 via Ab-mediated CD4 cross-linking has also been shown to cause a partially activated phenotype in T cell clones treated with agonist peptide, as evidenced by the lack of ZAP-70 phosphorylation and associated functional alterations (39). These findings suggest that a common mechanism for APL-derived T cell anergy and the “partial anergy” observed in memory T cells is due to modifications in the configuration of CD4 relative to the TCR/CD3 complex. The increased ability to negatively regulate memory CD4 T cells via the CD4 molecule may be due to the realignment of CD4 during previous partial activation of a naive CD4 T cell. Indeed, it has been shown that the CD4 molecule on the surface of memory CD4 T cells is differentially associated with TCR and CD45 when compared with naive CD4 T cells (40).

Presented in Figure 1B is a differential signaling/activation model for memory T cell generation that represents a modified version of model II. Activation of a naive T cell by Ag/MHC class II presented on the surface of a professional APC expressing costimulatory ligands can result in two possible outcomes. If the Ag/MHC complex has a high affinity for the TCR and/or behaves like a peptide agonist, and the APC expresses B7-1/B7-2 costimulatory ligands, ZAP-70 phosphorylation/activation occurs; the naive cell will proliferate and subsequently differentiate into an effector T cell (Fig. 1B). The types of effector T cells that are generated may depend on the cytokine environment or on ligand density on APC during repeated stimulation (41). If the Ag/MHC complex has a lower affinity for the TCR and/or behaves like a peptide antagonist or partial agonist, and the APC expresses either B7-1/B7-2 or HSA, the naive T cell may be partially activated and will differentiate directly into a long-lived memory T cell, bypassing a proliferative state (Fig. 1B). The partial activation will change the configuration of the TCR and CD4 on the T cell surface and will alter the coupling of signaling intermediates to the TCR/CD3 complex. When reactivated with cognate Ag, the memory T cell will be rapidly activated to directly produce effector cytokines due to the previous partial activation/differentiation step. This model may apply to both CD4 and CD8 T cells, as partial activation in response to APL (with a concomitant absence of ZAP-70 phosphorylation) also occurs in CD8 T cell clones (42).

This model is analogous to the differential avidity model of thymocyte selection, supported by experimental systems demonstrating that peptides that interact with the TCR on thymocytes through a low affinity interaction promote positive selection, or survival, and peptide/MHC complexes that interact with a high affinity (or at high ligand density) promote negative selection, or death (43, 44). Thus, disparate activation/signaling of double-positive thymocytes determines their differentiative fate. By analogy, the model presented in Figure 1B predicts that partial activation of naive, peripheral T cells leads to long term survival via the production of memory T cells, and full activation of peripheral CD4 T cells generates effector T cells, which ultimately die.

The idea that low affinity ligands favor generation of memory T cells appears in sharp contrast to evidence that persisting memory responses have a greater affinity for their antigenic ligand (45). However, several changes that accompany differentiation into a memory T cell are likely to raise the effective affinity of the TCR for its cognate ligand. These changes include a significant increase in the expression of adhesion molecules, such as LFA-1, CD2, and CD44 (18, 46, 47), and a switch in CD45 isoform expression from the highly charged CD45RB-containing isoforms to the less charged, smaller CD45RO isoform (19). It is believed that adhesion molecules and abundant surface molecules such as CD45 can affect the apparent “affinity” of the TCR for its ligand, with stable
adhesive and cell-cell contact interactions compensating for a low affinity interaction of the TCR with Ag/MHC (48) (TCR solution affinities for so-called “high-affinity” ligands are also very low (49)). Thus, a memory T cell undergoes “affinity maturation,” not by somatic mutation and Ag selection for high affinity receptors as in memory B cells, but by the change in expression of accessory and adhesion molecules that facilitate contact and activation by Ag.

Nevertheless, the model presented in Figure 1B does not limit the types of interactions to low affinity ligands. High affinity Ag present in low ligand density on the surface of an APC, as occurs late in an infection, may have a similar effect in driving a naive T cell toward the memory lineage. Also, contributions by different APC in promoting or limiting contact of the TCR with its ligand may also affect effector vs memory T cell generation. Moreover, it appears that not all naive CD4 T cells have similar life spans or turn over at the same rate (50). Although we refer to T cells in the periphery that have not contacted their antigenic ligand as naive, in fact all of these T cells have been positively selected via contact of the TCR with peptide/MHC on thymic epithelia. Certain signals, the nature of which is unknown, have been transduced through the TCR/CD3 complex during the selective process and may affect the way a “naive” cell may respond once contacted in the periphery. Thus, certain naive T cells may be predestined toward an effector or memory outcome as a result of signaling variations during positive selection.

Another possibility to account for the partial activation phenotype of memory T cells derives from the idea that memory T cells may be maintained over a long period of time by continuous stimulation by either low levels of persisting Ag or by noncognate, cross-reactive environmental Ags (43). Although a full discussion of the maintenance of memory is beyond the scope of this commentary, several points should be noted. First, it is well documented that the long term maintenance of CD8 T cell memory can occur in the complete absence of cognate Ag (5, 51–53), although the maintenance of CD4 T cell memory is currently unresolved (54). Second, transfer experiments of previously activated TCR-transgenic CD8 T cells have revealed that these memory phenotype T cells can be maintained in the absence of the appropriate MHC class I protein, although MHC class I expression is required for long term maintenance (53). While these results suggest that an interaction between the TCR and MHC is necessary to maintain memory T cells, the nature of this interaction is unknown. Furthermore, the proliferation of memory T cells observed following transfer into MHC-positive hosts without Ag (53) may indicate either full, rather than partial activation of a fraction of memory T cells or the transfer of a population of effector T cells within the previously activated memory pool. Investigation of the activation and signaling parameters of transferred memory CD4 and CD8 T cells over a long period of time in a variety of adoptive transfer systems will determine whether constant activation is necessary for their maintenance.

I have presented evidence to suggest that generation of memory vs effector T cells follows different developmental pathways, analogous to B cells. Can the same mechanism of differential signaling be applied to generation of memory vs effector B lymphocytes? Although differential signaling in B cells triggered by Ag is not yet documented, one can consider the dichotomy between T-dependent Ags, which generate memory B cells, and T-independent Ags, which are poor and ineffective producers of memory B cells. Although it has been demonstrated that interactions with T cells, primarily through CD40, play a role in memory B cell generation (55), it is possible that the high affinity and high degree of cross-linking that are the hallmark of T-independent Ags always fully activate a naive B cell and drive it to the effector state. T-dependent Ags, by contrast, are of varying affinities and differentially cross-link the B cell receptor in the presence of T cell help, leading to the generation of both effector and memory B cells. Determination of the signaling pathways triggered by Ags that differentially cross-link the B cell receptor should help to resolve this issue.

In summary, activation, signaling, and costimulatory requirements of memory CD4 T cells point to a differential signaling model for memory T cell generation, in contrast to the currently favored linear model for generation of memory T cells directly from activated effectors. Such a mechanism for memory T cell generation may provide for the efficient functioning of the immune response. For example, high concentrations of Ag found early in an infection may promote full activation to effector cells, while later in an immune response when much of the Ag is cleared, low concentrations of Ag may promote generation of memory T cells. Further analysis of signaling and activation parameters in naive, effector, and memory T cells in a variety of in vivo systems will uncover the complex mechanisms underlying the generation of immunologic memory.

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