Published in final edited form as:

Semin Immunol. 2009 April ; 21(2): 84–91. doi:10.1016/j.smim.2009.02.003.

Biochemical Signaling Pathways for Memory T cell Recall

Donna L. Farber

Department of Surgery, University of Maryland School of Medicine Baltimore, MD 21201

Abstract

Memory T cells exhibit low activation thresholds and rapid effector responses following antigen stimulation, contrasting naive T cells with high activation thresholds and no effector responses. Signaling mechanisms for the distinct properties of naive and memory T cells remain poorly understood. Here, I will discuss new results on signal transduction in naive and memory T cells that suggest proximal control of activation threshold and a distinct biochemical pathway to rapid recall. The signaling and transcriptional pathways controlling immediate effector function in memory T cells closely resemble pathways for rapid effector cytokine production in innate immune cells, suggesting memory T cells use innate pathways for efficacious responses.

Keywords

T Lymphocytes; T Cell Receptors; Signal Transduction; transcription factors; innate immunity

Introduction

The development of memory T cells following antigen activation of naive T cells involves profound cellular and molecular changes. The memory differentiation process begins when naive CD4 T cells become activated by coordinate engagement of the T-cell receptor (TCR) and co-stimulatory receptor CD28 on the T cell surface by MHC-peptide antigen complexes and co-stimulatory ligands (B7-1/B7-2), respectively, on the surface of antigen-presenting cells (APC). Through integration of these signals, naive T cells are stimulated to proliferate and differentiate into different types of effector T cells producing effector cytokines. Most of these activated effector T cells die after a brief lifespan *in vivo*, yet a subset of primed, antigen-specific T cells persists as memory T cells via mechanisms that are not clearly defined. The resultant memory T cells can be maintained up to the lifetime of an individual via perpetual turnover and homeostasis, and when reactivated, coordinate the faster, stronger, and more prolonged memory immune response [1,2]. The ability of memory T cells to mediate efficacious responses is the cornerstone of the anamnestic immune response, although the pathways and mechanisms for the enhanced responses of memory T cells remain undefined.

Memory T cells acquire distinct phenotypic and functional properties that enable them to mediate enhanced secondary responses. Phenotypically, both naive and memory T cells are small resting cells with low level expression of the IL-2R α chain (CD25) and expression of

Corresponding Author: Donna L. Farber, Ph.D., Department of Surgery, University of Maryland School of Medicine, MSTF Building, Room 400, 685 W. Baltimore ST. Baltimore, MD 21201 Phone: 410-706-7458; FAX: 410-706-0311; e-mail: E-mail: dfarber@smail.umaryland.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

the IL-7R α chain (CD127) [3,4]. However, memory T cells express elevated levels of the adhesion markers CD44 [5] and CD11a [6], compared to naive T cells, which may facilitate their interactions with APC. Functionally, memory T cells exhibit rapid production of multiple effector molecules such as IFN- γ and TNF- α [7] or IL-4 and IL-5 within hours of stimulation whereas naive T cells require days of sustained activation to differentiate into effector cytokine producers [8,9]. The immediate response kinetics of memory T cells is similar to the cytokine response of innate immune cells, such as NK cells. In the case of memory CD8 T cells, increased expression of the cytotoxic molecules perforin and granzyme B also occurs at rapid times [10,11]. In addition, memory T cells have less stringent activation requirements compared to naive T cells, including a reduced activation threshold for low antigen doses [12], and an ability to be fully activated by diverse APC such as resting B cells, macrophages and endothelial cells [13,14] and DC [15] which are the primary APC for naive T cell activation. This rapid recall response and reduced activation threshold are the defining functional attributes of memory CD4 and CD8 T cells, although the cell-intrinsic mechanisms controlling these processes are undefined.

The study of TCR-coupled intracellular signaling pathways has revealed important insights into biochemical mechanisms controlling T cell activation, regulation and anergy. Analysis of signal transduction pathways in memory T cells has posed challenges due to the difficulty of obtaining sufficient numbers of memory T cells for biochemical analysis, and the fact that genetic deletion of key TCR-coupled signaling intermediates *in vivo* tends to disrupt T cell development and preclude any analysis of differentiated T cell populations. However, recent advances in single-cell analysis of signaling pathways and new results on signaling in diverse systems have provided new perspectives on biochemical control of memory T cell recall. Here, I will discuss new findings on memory T cell signal transduction that suggest proximal signaling control of activation threshold, as well as the acquisition of a separate biochemical pathway to rapid recall. In addition I will discuss aspects of memory T cell function, signaling and activation properties that bear striking similarities to innate cells such as NK and NKT cells, and suggest a common pathway to rapid cytokine responses in memory and innate immune cells. The embodiment of innate and adaptive properties in memory T cells through signaling pathways has important implications for understanding the evolution and regulation of immunological memory.

Signaling Analysis of memory T cells

The kinetics and magnitude of cytokine production by memory T cells following antigen stimulation could be due to enhanced interactions with APC, altered intracellular signaling, or a combination of both. There is now substantial evidence that rapid memory responses are due to cell intrinsic properties of memory T cells, independent of TCR specificity or APC-mediated effects. Notably, naive and memory CD4 or CD8 T cells derived from TCR-transgenic mice bearing the identical TCR exhibit the same functional and kinetics differences as polyclonal counterparts-- with TCR-transgenic memory T cells producing effector cytokines within 3-6 hours of TCR engagement by antigen, and naive T cells not producing measurable cytokines at these early timepoints [11,16]. It has also been suggested that the enhanced expression of adhesion markers and integrins on the surface of memory T cells may facilitate contact with APC and lead to more efficient activation [17,18]. However, rapid recall of memory T cells can result from TCR stimulation in the absence of APC, using anti-CD3 crosslinking, or PMA/ionomycin [16,19]. Moreover, *in vivo* image analysis using confocal laser scanning microscopy revealed no differences in the ability of naive or memory T cells to form conjugates with DC, and these contacts did not depend on expression of the integrin LFA-1 [20]. When taken together, the functional and imaging results indicate that memory T cells possess an intrinsic TCR-coupled pathway to rapid recall. Thus, distinct memory cell functions could derive from altered intracellular signaling coupled to the TCR, altered mobilization of transcription factors

in the nucleus, epigenetic changes in the loci of cytokine genes, and/or a combination of all three factors, as discussed below.

TCR-coupled signaling pathways

The procession of TCR-coupled signaling events leading to IL-2 gene transcription in primary naive T cells and T cell lines has been elucidated (see [21] for a detailed review on the subject), revealing crucial processes necessary for transducing TCR engagement to nuclear events. TCR-coupled signal transduction can be divided into proximal, linker-adapter and distal processes. Events proximal to TCR engagement begin with phosphorylation of TCR-coupled CD3 subunits, CD3 ζ and CD3 ϵ , by the Src-family tyrosine kinases, Lck and Fyn [22], leading to the mobilization, phosphorylation and subsequent activation of the T cell-specific tyrosine kinase ZAP-70 (zeta-associated protein of 70kDa) [23,24] [25]. The ZAP-70 kinase subsequently phosphorylates the key linker/adapter molecules SLP-76 (SH2-containing protein of 76kDa) [26] and LAT (linker for activated T-cells) [27], which molecularly couple early phosphorylation signals to downstream signaling events [28,29]. Phosphorylated linker-adapter molecules form molecular complexes that trigger activation of the ras pathway through interactions with vav and promote release of calcium from the intracellular stores by interactions with PLC γ 1 [30,31]. Subsequent distal signaling events ensue, including protein kinase C activation, and activation of the MAP kinases Erk1/2 and p38 via the GDP-binding proteins (for reviews, see [32,33]). Together, activated MAP-kinases and Calcium flux promote nuclear translocation and mobilization of the nuclear factor for activated T cells (NFAT) for IL-2 gene transcription [34,35]. TCR-signaling also leads to activation of the transcription factor NF κ B, which is required for transcription of multiple genes involved in T cell activation, including CD25 and IL-2 [36-38].

Whether a similar orderly procession of signaling events as described above occurs in memory T cells has not been fully elucidated. Several laboratories, including my own, have applied conventional biochemical analysis to determine whether certain critical TCR-coupled signaling events occur in memory T cells. Analysis of TCR-coupled phosphorylation of signaling intermediates in phenotypic subsets of naive and memory T cells revealed decreased phosphorylation of proximal and distal molecules in mouse and human memory compared to naive CD4 T cells [39-43]. For CD8 T cells, there are no measurable phosphorylation differences between naive and memory subsets [44]. Interestingly, mouse memory CD4 T cells also exhibited decreased signaling at the linker/adapter level, with decreased phosphorylation of SLP-76 and association to downstream molecules [41]. These findings of reduced TCR-coupled phosphorylation and linker/adapter signaling in memory CD4 T cells were paradoxical considering their robust and rapid functional responses. Based on these results, we hypothesized that for memory CD4 T cells, fewer signaling events transduced from TCR engagement to functional output may represent a signaling “short-circuit” and a mechanism to promote rapid recall by memory T cells [9]. However, biochemical analysis of signaling pathways in lysates of naive and memory T cells did not indicate how specific signaling events were coupled to functional output, and new approaches were needed.

In recent years, the availability of antibodies that recognize signaling intermediates in the native form, has enabled visualization of signaling intermediates or their phosphorylated forms on the single cell level [45-47]. We applied this flow cytometry technique to analyze the expression and phosphorylation of signaling intermediates in resting and antigen-activated naive and memory CD4 T cells, and also used this approach for coordinate analysis of signaling events in conjunction with cytokine production. This combined method enabled direct assessment of the functional coupling of specific signaling events and whether effector cytokine production was associated with, or preceded by, a particular signaling event.

Single cell analysis of signaling intermediates enabled a thorough evaluation of the resting state signaling configuration in resting memory T cells. One hypothesis to account for the ability of memory T cells to signal for rapid recall is that signaling molecules are in a response-ready configuration in resting memory T cells. This potential could be achieved by the constitutive phosphorylation of key signaling intermediates, and/or pre-formed associations of specific signaling complexes. Biochemical analysis of lysates of resting naive and memory T cells indicated comparable low phosphorylation levels in both subsets [41,43,48,49]. Similarly, analysis of signaling intermediate expression by intracellular flow cytometry, confirmed low basal phosphorylation of key intermediates including ZAP-70, PLC- γ , Erk1/2, and p38 [16]. These results indicate that the ability of memory T cell to mediate rapid responses is not due to the presence of constitutively phosphorylated signaling intermediates. Another way in which signaling molecules could be poised for efficient biochemical responses could be via their association to membrane microdomains, called lipid rafts, which are cholesterol-rich membrane regions where signaling intermediates congregate [50,51]. Indeed, an increase in the association of signaling intermediates with membrane raft microdomains has been identified in antigen-specific memory CD4 and CD8 T cells [44,52]. Whether these observed differences in raft-associated signaling molecules had a functional consequence has not been demonstrated, and the role of lipid rafts in controlling T cell activation remains controversial [53].

Increased ZAP-70 expression in memory CD4 T cells

Although we did not detect differences in basal tyrosine phosphorylation in naive and memory T cells, we found that protein expression of certain signaling intermediates was remarkably different between these subsets. In particular, protein expression of the proximal ZAP-70 kinase was consistently 3-5-fold higher in antigen-specific memory compared to naive CD4 T cells, while expression of other signaling molecules such as CD3 ϵ and Erk was comparable ([16] and Fig. 1A). This augmentation in protein expression of a signaling intermediate in memory compared to naive CD4 T cells was apparent in antigen-specific and polyclonal populations of mouse memory CD4 T cells [16,54], and in human memory versus naive CD4 T cells (Fig. 1B). Interestingly, we found that new naive CD4 T cells from umbilical cord blood expressed the lowest level of ZAP-70; CD45RA⁺ CD4 T cells from adult peripheral blood which contain naive and non-naive cells [55] expressed higher levels of ZAP-70, and adult CD45RO⁺ memory CD4 T cells expressed the highest level of ZAP-70 (Fig. 1B). These results suggest developmentally-dependent increases in ZAP-70 expression in peripheral CD4 T cells.

In addition to resting state differences in ZAP-70 expression in CD4 T cell subsets, we also found that ZAP-70 expression increased progressively during antigen-stimulation and effector cell differentiation, and was comparably high in effector and memory CD4 T cells (Fig. 1C and [16]). Moreover, ZAP-70 co-localized to CD3 ϵ in effector and memory CD4 T cells (Fig. 1C), suggesting that an association of ZAP-70 with the TCR/CD3 complex marked cells with effector function. Consistent with this idea, we found that IFN- γ production from differentiated effector cells occurred only from the ZAP-70^{hi} population, and conversely, downmodulation of ZAP-70 expression by siRNA in memory CD4 T cells reduced rapid IFN- γ production [16]. These results suggested that a high basal level of ZAP-70 kinase expression and its association to CD3 ϵ in memory CD4 T cells facilitated propagation of downstream signals leading to rapid recall.

Our findings of elevated ZAP-70 expression in memory compared to naive CD4 T cells and its requirements for effector function by memory CD4 T cells, suggested a new mechanism for controlling T cell functional capacity by varying protein expression of a signaling intermediate. A recent study also reported a link between protein expression levels of signaling molecules and T cell functional capacity using a combination of computer modeling and single

cell experimental analysis [56]. In the case of memory T cells, increased ZAP-70 itself is probably not sufficient for propagating downstream signals leading to rapid effector function, for two reasons. First, our preliminary experiments in human T cells indicate that overexpression of ZAP-70 in newly activated naive T cells was not sufficient to promote rapid IFN- γ production (F.O. Okoye and D.L. Farber, unpublished data). Second, the increased level of ZAP-70 protein does not appear to promote enhanced levels of downstream signaling ([16,41] and see below), suggesting that either a different pathway is operative or additional downstream alterations are necessary to transduce rapid recall function in memory T cells. We propose that increased expression of ZAP-70 and its association to the TCR/CD3 complex may act to lower the activation threshold in memory T cells [16] and promote functional responses with reduced TCR triggering. In this way, increased expression of a proximal signaling initiator provides a biochemical basis for enhanced memory responses at the level of TCR engagement with its ligand.

Functional coupling of signaling in naive and memory CD4 T cells: Dual pathways to memory recall

The coupling of specific signaling events to functional output in naïve versus memory T cells has been dissected using several approaches examining distal signaling events, costimulatory requirements and transcription factor utilization. From these multiple approaches, a view is emerging of TCR-coupled signaling in memory T cells that suggests two distinct pathways controlling recall responses. The different results that support this model are discussed below.

Distal Signaling events and the kinetic progression of memory responses

To trace the signaling events associated with rapid recall in memory T cells from the proximal ZAP-70 kinase, we simultaneously analyzed the phosphorylation of downstream mediators in conjunction with IFN- γ production in naive versus memory CD4 T cells following antigen activation over a broad kinetic window. Directly downstream of ZAP-70, we found that SLP-76 signaling was required for memory CD4 T cell recall (manuscript submitted). In addition, we found that naive and memory CD4 T cells exhibited similar kinetics for phosphorylation of signaling intermediates distal of ZAP-70 and SLP-76 including PLC- γ , p38, and p42. While resting and short-term-stimulated naive and memory T cells did not exhibit distal phosphorylation, we found that prolonged antigen activation for >24hrs resulted in the accumulation of phospho-PLC- γ , -p38, and -p42 in both subsets, enabling us to analyze whether IFN- γ production was associated with accumulated downstream phosphorylation. In naive CD4 T cells, IFN- γ production only occurred only after 24-48hrs of antigen stimulation from a population with extensive distal phosphorylation (p-PLC- γ^{hi} , p-p42 $^{\text{hi}}$, p-p38 $^{\text{hi}}$) (Fig. 2 and [16]). By contrast, IFN- γ production from memory T cells at early and later times post-antigen stimulation occurred from cells with different signaling signatures; after 6hrs of stimulation, IFN- γ producing T cells exhibited low levels of distal phosphorylation; and at later times (>24hr), IFN- γ production occurred from cells with high levels of distal phosphorylation (Fig. 2). This multiparameter analysis provided a key insight – that early memory recall may be triggered by a signaling pathway distinct from that triggering late effector function in either naive and memory CD4 T cells.

CD28 costimulation in memory T cell recall

The existence of two pathways for memory recall is further supported by their differential requirements for CD28 signaling. Signaling through the CD28 costimulatory receptor was initially found to be dispensable for reactivation of memory T cells based on *in vitro* studies and results using CD28-deficient mice. These earlier studies found that memory CD4 T cells could be activated to proliferate and produce multiple cytokines *in vitro*, by antigen presented by B7-deficient APC [13,57]. Similarly, LCMV infection of CD28-deficient mice led to

wildtype levels of memory CD8 T cells with intact recall function [58]. The notion of memory T cells being independent of the CD28 pathway was further consistent with their lower activation threshold through the TCR compared to naive T cells. However, further examination of memory T cell activation *in vivo* using specific inhibitors of the CD28 pathway and CD28-deficient mice revealed that wildtype memory CD4 and CD8 T cells do require CD28 costimulation for optimal IL-2 production and proliferative expansion *in vivo* [58-61]. In our studies, we found that inhibiting CD28 costimulation using the fusion protein CTLA4Ig which binds the B7 ligands, impaired IL-2 production and *in vivo* proliferation of antigen-stimulated memory CD4 T cells, but did not appreciably affect rapid IFN- γ production [61]. These findings indicated that CD28 costimulation was required for certain memory T cell functions, but not others.

We investigated whether the differential use of CD28 costimulation for IL-2 versus IFN- γ production could be attributed to different subsets of memory CD4 T cells, such as central and effector memory subsets that differ in homing receptor expression and can exhibit functional differences [19,62]. However, fractionated central (CD62L^{hi}) and effector (CD62L^{lo}) memory CD4 T cells from mice both produced IFN- γ and IL-2 in response to antigenic stimulation, and IL-2 production from both memory subsets was inhibited by interfering with CD28 costimulation [61]. These findings therefore suggest two pathways operative in memory CD4 T cells: an early pathway to effector cytokine production that does not require CD28 costimulation, and a later pathway leading to proliferation and IL-2 production that is CD28 dependent.

Nuclear transcriptional mechanisms

The different signaling pathways in memory T cells are marked by distinct kinetics, distal signaling and CD28 requirements; however, it is not known whether these pathways culminate in specific transcriptional activities in the nucleus. Thus far, there is some evidence for differential activation of transcription factors in naive and memory CD4 T cells. Polyclonal mouse memory CD4 T cells exhibited increased accumulation of NFAT following 36hrs of stimulation [63]; although the role of NFAT in rapid cytokine production was not examined. Expression of the transcription factor T-bet is essential for IFN- γ production by CD4 T cells and differentiation of the T-helper 1 (Th1) effector lineage [64]; however, IFN- γ production by CD8 T cells can be mediated either by T-bet or by the transcription factor Eomesodermin (Eomes) [65]. Both T-bet and eomesodermin have been shown to regulate memory CD8 T cell survival [65,66], but it is not known whether they are required for rapid recall of memory CD8 or CD4 T cells. The differential engagement of transcription factors on the promoters of cytokine genes in antigen stimulated memory compared to naive CD4 T cells has not been addressed.

We have examined T-bet expression and upregulation in antigen-specific memory compared to naive CD4 T cells, based on the hypothesis that enhanced transcriptional activation of the IFN- γ gene may be driven by elevated or constitutive expression of T-bet. By flow cytometry, we observed that T-bet expression was comparably low in resting naive and memory CD4 T cells, and was upregulated after antigen stimulation, with peak expression after 24hrs stimulation of memory CD4 T cells, and 48hrs stimulation of naive CD4 T cells (W. Lai and D.L. Farber, unpublished data). However, we identified a differential association of IFN- γ production with T-bet expression in naive and memory CD4 T cells. In naive CD4 T cells, IFN- γ production occurred following sustained (24-48hrs) antigen activation only from the T-bet⁺ population, consistent with a T-bet requirement for IFN- γ production by primary CD4 T cells [64]. By contrast, in memory CD4 T cells, early (≤ 6 hrs) IFN- γ production after antigen stimulation occurred from a T-bet^{lo} population that did not exhibit substantial T-bet upregulation, whereas late IFN- γ production occurred from a T-bet⁺ populations (W. Lai and

D.L. Farber, unpublished results). These results are consistent with the existence of two distinct signaling pathways to IFN- γ production in memory CD4 T cells—a pathway for early IFN- γ production that is T-bet independent, and a pathway to late IFN- γ production that requires T-bet, similar to naive T cells.

A putative T-bet-independent pathway to IFN- γ production in memory CD4 T cells is certainly consistent with the known effects of T-bet in inducing stable epigenetic changes in the IFN- γ promoter during activation of naive T cells [67,68]. Reiner and colleagues showed that interaction of T-bet with a nuclear protein Hlx, during activation of naive T cells induced modifications in the IFN- γ locus resulting in increased promoter accessibility that were maintained during the effector stage [67,68]. These results suggested that T-bet was important for the induction but not maintenance of the capacity to produce IFN- γ [69]. For memory CD8 T cells, epigenetic changes involving increases in acetylated histones in the promoter regions of perforin, Eomes, and other effector molecule connote regions of active transcription, suggesting increased accessibility of promoter regions to transcription factor engagement in memory T cells [10,70]. These structural genetic changes may further obviate the need for certain transcription factors. A similar analysis of chromatin structure of memory CD4 T cells has not yet been reported.

If T-bet expression is not associated with early memory T cell IFN- γ production, then what is the critical transcription factor important for this rapid effector production? A likely candidate is NF κ B, as NF κ B has been shown to control IFN- γ transcription in Th1 effector populations [71,72], and controls many other genes important for T cell activation, including IL-2 and CD25, which are also more rapidly upregulated following TCR stimulation of memory T cells [48,73]. Activation of NF κ B requires phosphorylation and inactivation of the NF κ B inhibitor, I κ B, by the I κ B kinase. This pathway for TCR-mediated activation of NF κ B has been recently elucidated and involves the coupling of specific downstream mediators with PKC θ (for reviews, see [37,74]). Specifically, phosphorylation of Carma1 [75] and mobilization of the Carma1/Bcl10/MALT1 (CBM) complex leads to activation of the I κ B kinase (IKK) for phosphorylation of I κ B, and enables nuclear translocation of NF κ B, [36-38]. It is not known how the TCR-coupled signaling axis to NF κ B activation is oriented in memory T cells, and this pathway will be important to investigate. Another possibility is that early engagement of NF κ B or T-bet on the IFN- γ promoter may be driving the rapid recall and early and late pathways. Determining the temporal association of specific transcription factors on cytokine gene promoters following antigen activation will be crucial for dissecting mechanisms for rapid recall.

An Innate signaling pathway to rapid recall in memory T cells

The evidence discussed above implicates at least two distinct signaling pathways, designated “early” and “late” in memory CD4 T cells that are coupled to the production of the effector cytokine, IFN- γ (Fig. 3). Both of these pathways share certain properties in that they are marked by increased ZAP-70 expression and both require ZAP-70 and SLP-76 signaling (Fig. 3). Following this common proximal step, the two pathways appear to diverge. The late pathway for IFN- γ production in memory T cells is similar to that in naive T cells and arises following sustained TCR stimulation, leading to augmented distal phosphorylation and upregulation of T-bet expression which then translocates to the nucleus to initiate IFN- γ transcription (Fig. 3, right pathway). This late pathway for effector cytokine production requires CD28 signaling, and is coincident with IL-2 production and cell cycle entry. By contrast, the rapid pathway to early effector cytokine production is triggered within hours of TCR stimulation in memory but not naive T cells, and is not associated with downstream phosphorylation, T-bet upregulation or CD28 signaling (Fig. 3, left pathway indicated by a dotted line). These two pathways could

potentially be operative within the same cell, as individual memory T cells can be multifunctional and produce multiple cytokines and/or undergo proliferative turnover [7,76].

What is the nature of this rapid pathway for memory recall? We propose that the pathway coupled to TCR-mediated rapid effector function resembles an innate-like signaling pathway similar to NK cells and “innate-like” T cells, such as NKT cells, in terms of functional kinetics, activation requirements, signaling and transcriptional regulation. Importantly, memory T cells exhibit both the kinetics of effector cytokine production and the activation requirements of innate-type cells. Kinetically, IFN- γ production by antigen-specific memory CD4 and CD8 T cells occurs within hours of antigen stimulation [11,16,77], similar to the kinetics of IFN- γ production by NK cells in response to NK-activating receptors [78], and by innate-type NKT cells [79] in response to engagement of their invariant TCR by α -Gal-cer [80]. In addition, IFN- γ production by memory T cells can also occur via antigen-independent stimulation, similar to innate-type cells. For example, it has been shown by several groups that the cytokines IL-12 and IL-18 can stimulate memory CD8 T cells to produce rapid IFN- γ [81,82]. Recently, memory-phenotype CD4 T cells were also found to share this innate function, producing IFN- γ in response to IL-12 and IL-18 stimulation [83]. This ability of memory T cells to produce recall cytokines in response to inflammatory cytokines is shared by NKT cells, which can be stimulated to produce IFN- γ directly by exogenous IL-12 itself or as produced by TLR-stimulated dendritic cells (DC) [84,85]. When taken together, these findings suggest that memory T cells can be triggered early in an immune response by engagement of the TCR or cytokine receptors.

While the kinetic and functional data suggest innate-like functions to memory T cells, it is not known whether the signaling requirements and pathways for rapid IFN- γ production in memory T cells are similar to those in innate type cells. However, evidence from multiple studies suggest similar transcriptional profiles for rapid IFN- γ production in memory T cells and NK cells. Wilson and colleagues demonstrated the lack of T-bet expression did not impair IFN- γ production by NK cells *in vivo* following *Listeria* infection [86], consistent with our findings of lack of T-bet upregulation accompanying early recall of memory CD4 T cells. In addition, there is increasing evidence that NF κ B is integrally involved in signaling for IFN- γ production in NK cells, similar to its role in controlling IFN- γ production in previously activated effector CD4 T cells [71,72]. In NK cells, early IFN- γ requires NF κ B activation [87] and inhibition of NF κ B *in vivo* impairs IFN- γ production by both NK cell and T cells [88]. Importantly, two groups recently demonstrated that NK cells, like T cells, require the Carma1/Bcl10/MALT1 complex to signal for IKK activation and subsequent activation of NF κ B [89,90]. These results demonstrate that signaling intermediates upstream of NF κ B activation are common to both NK and T cells [89,90], and could be a shared mechanisms for rapid recall.

There is also evidence that non-cognate stimulation of memory T cells by IL-12 stimulation can mobilize NF κ B activity. IL-12 has been shown to promote nuclear translocation of NF κ B in human tumor-infiltrating memory CD4 T cells [91], and can promote IL-2 production and CD25 upregulation in CD8 T cells [92] which are both targets for NF κ B transcriptional activity. Given its central role as a transcriptional activator in both innate and adaptive pathways, NF κ B is a common link between early IFN- γ responses in memory T cells and innate cells. A thorough examination of NF κ B transcriptional activity and its engagement on the IFN- γ promoter in memory T cells, NKT cells and NK cells can establish whether NF κ B is the common denominator for rapid cytokine signaling in all of these cells types.

While NF κ B may represent the signal transducer common to rapid function in memory and innate cells, the Itk tyrosine kinase appears to be the signaling intermediate that distinguishes between innate (or early) and adaptive (or late) type signaling. Itk is an IL-2 inducible Tec-family tyrosine kinase that regulates TCR signaling [93]. Several groups showed that mice

deficient in the Itk kinase develop peripheral CD8 and CD4 T cells with a predominant memory (CD44hi) phenotype [94-96]. CD8⁺CD44hi cells from Itk-deficient mice have been the most extensively studied from these mice, and were found to possess innate function, with rapid production of IFN- γ as a result of TCR engagement or IL-12 stimulation [94-96]. These cells have been referred to as “innate T cells” [97], although their expression of a conventional $\alpha\beta$ TCR, memory phenotype and function suggest that lack of Itk during development triggers T cells to convert directly to memory T cells, rather than generating naive T cell signaling configurations. Thus, the biochemical coupling of signaling pathways through the TCR in memory T cells may derive from the more primordial innate signaling, and that later in evolution, the TCR signaling configuration in naive T cells developed to uncouple rapid and innate function from TCR engagement.

In addition to similar kinetics, signaling and activation requirements for rapid IFN- γ production by memory T cells and innate cells, both memory T and NK cells also exhibit commonalities in the epigenetic changes in IFN- γ locus, that may also be important for promoting rapid cytokine responses. Methylation of histones is known to regulate promoter accessibility, with an “open” chromatin configuration characterized by hypomethylation at a specific promoter locus (for reviews, see [98]). Epigenetic modification defined by hypomethylation have been identified in the IFN- γ locus in polyclonal CD44hi memory CD8 T cells [99,100], and also in Th1 effector cells [101], and have been shown to regulate the efficiency of IFN- γ expression on the level of gene accessibility [98]. Interestingly, in NK cells, the IFN- γ promoter was found to be constitutively hypomethylated at regions similar to those found in Th1 cells [102,103]. These results, together with commonalities in signaling and activation pathways, suggest that a combination of intracellular signaling and promoter accessibility contribute to the rapidity of IFN- γ production following receptor engagement in both memory T cells and innate NK cells. Memory T cells must undergo prior activation with antigen to acquire the functional, signaling and epigenetic changes already present within NK cells, further suggesting that memory T cells use a primordial pathway for their recall responses.

Concluding Remarks

The analysis of intracellular signaling and its functional coupling in memory T cells has provided new insights into the mechanisms for rapid recall that endows memory T cells with their ability to respond efficaciously and coordinate a rapid anamnestic response. The emerging knowledge of the participants and intermediates in this memory recall pathway bear striking similarities to pathways in innate immune cells, including NK cells and NKT cells. Such similarities suggest a common innate pathway to rapid recall function in memory T cells that has important implications for the development and evolution of immune memory. Dissecting the intricacies of this pathway in future studies will reveal crucial insights into how the immune system uncouples rapid innate from delayed function, for proper immune regulation and homeostasis.

Acknowledgements

This work was supported by NIH AI42092 awarded to D.L.F. The author wishes to thank Martin Flajnik for critical reading of this manuscript.

References

1. Sprent J, Surh CD. T cell memory. *Annu Rev Immunol* 2002;20:551–79. [PubMed: 11861612]
2. Surh CD, Sprent J. Regulation of naive and memory T-cell homeostasis. *Microbes Infect* 2002;4:51–6. [PubMed: 11825775]

3. 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. [PubMed: 14625547]
4. Tan JT, Dudl E, LeRoy E, et al. IL-7 is critical for homeostatic proliferation and survival of naive T cells. *Proc Natl Acad Sci U S A* 2001;98:8732–7. [PubMed: 11447288]
5. Budd RC, Cerottini JC, Horvath C, et al. Distinction of virgin and memory T lymphocytes. Stable acquisition of the Pgp-1 glycoprotein concomitant with antigenic stimulation. *J Immunol* 1987;138:3120–9. [PubMed: 3106474]
6. Wallace DL, Beverley PCL. Phenotypic changes associated with activation of CD45RA⁺ and CD45RO⁺ T cells. *Immunol* 1990;69:460–7.
7. Slifka MK, Whitton JL. Activated and memory CD8⁺ T cells can be distinguished by their cytokine profiles and phenotypic markers. *J Immunol* 2000;164:208–16. [PubMed: 10605013]
8. Kaech SM, Wherry EJ, Ahmed R. Effector and memory T-cell differentiation: implications for vaccine development. *Nat Rev Immunol* 2002;2:251–62. [PubMed: 12001996]
9. Chandok MR, Farber DL. Signaling control of memory T cell generation and function. *Semin Immunol* 2004;16:285–93. [PubMed: 15528073]
10. Araki Y, Fann M, Wersto R, Weng NP. Histone acetylation facilitates rapid and robust memory CD8 T cell response through differential expression of effector molecules (eomesodermin and its targets: perforin and granzyme B). *J Immunol* 2008;180:8102–8. [PubMed: 18523274]
11. Kaech SM, Hemby S, Kersh E, Ahmed R. Molecular and functional profiling of memory CD8 T cell differentiation. *Cell* 2002;111:837–51. [PubMed: 12526810]
12. Rogers PR, Dubey C, Swain SL. Qualitative changes accompany memory T cell generation: faster, more effective responses at lower doses of antigen. *J Immunol* 2000;164:2338–46. [PubMed: 10679068]
13. Croft M, Bradley LM, Swain SL. Naive versus memory CD4 T cell response to antigen. Memory cells are less dependent on accessory cell costimulation and can respond to many antigen-presenting cell types including resting B cells. *J Immunol* 1994;152:2675–85. [PubMed: 7908301]
14. Dengler TJ, Pober JS. Human vascular endothelial cells stimulate memory but not naive CD8⁺ T cells to differentiate into CTL retaining an early activation phenotype. *J Immunol* 2000;164:5146–55. [PubMed: 10799873]
15. Zammit DJ, Cauley LS, Pham QM, Lefrancois L. Dendritic cells maximize the memory CD8 T cell response to infection. *Immunity* 2005;22:561–70. [PubMed: 15894274]
16. Chandok MR, Okoye FI, Ndejemi MP, Farber DL. A biochemical signature for rapid recall of memory CD4 T cells. *J Immunol* 2007;179:3689–98. [PubMed: 17785805]
17. Damle NK, Klussman K, Linsley PS, Aruffo A. Differential costimulatory effects of adhesion molecules B7, ICAM-1, LFA-3, and VCAM-1 on resting and antigen-primed CD4⁺ T lymphocytes. *J Immunol* 1992;148:1985–92. [PubMed: 1372018]
18. Dutton RW, Bradley LM, Swain SL. T cell memory. *Annu Rev Immunol* 1998;16:201–23. [PubMed: 9597129]
19. Sallusto F, Lenig D, Forster R, Lipp M, Lanzavecchia A. Two subsets of memory T lymphocytes with distinct homing potentials and effector functions. *Nature* 1999;401:708–12. [PubMed: 10537110]see comments
20. Westermann J, Bode U, Sahle A, et al. Naive, effector, and memory T lymphocytes efficiently scan dendritic cells in vivo: contact frequency in T cell zones of secondary lymphoid organs does not depend on LFA-1 expression and facilitates survival of effector T cells. *J Immunol* 2005;174:2517–24. [PubMed: 15728457]
21. Abraham RT, Weiss A. Jurkat T cells and development of the T-cell receptor signalling paradigm. *Nat Rev Immunol* 2004;4:301–8. [PubMed: 15057788]
22. Iwashima M, Irving BA, van Oers NS, Chan AC, Weiss A. Sequential interactions of the TCR with two distinct cytoplasmic tyrosine kinases. *Science* 1994;263:1136–9. [PubMed: 7509083]
23. Chan AC, Irving BA, Fraser JD, Weiss A. The ζ chain is associated with a tyrosine kinase and upon T-cell antigen receptor stimulation associates with ZAP-70, a 70-kDa tyrosine phosphoprotein. *PNAS* 1991;88:9166–70. [PubMed: 1717999]

24. Chan AC, Iwashima M, Turck CW, Weiss A. ZAP-70: A 70 kd Protein-Tyrosine Kinase that associates with the TCR ζ chain. *Cell* 1992;71:649–62. [PubMed: 1423621]
25. Hatada MH, Lu X, Laird ER, et al. Molecular basis for interaction of the protein tyrosine kinase ZAP-70 with the T-cell receptor. *Nature* 1995;377:32–8. [PubMed: 7659156]
26. Jackman JK, Motto DG, Sun Q, et al. Molecular cloning of SLP-76, a 76-kDa tyrosine phosphoprotein associated with Grb2 in T cells. *J Biol Chem* 1995;270:7029–32. [PubMed: 7706237]
27. Zhang W, Sloan-Lancaster J, Kitchen J, Tribble RP, Samelson LE. LAT: the ZAP-70 tyrosine kinase substrate that links T cell receptor to cellular activation. *Cell* 1998;92:83–92. [PubMed: 9489702]
28. Myung PS, Boerthe NJ, Koretzky GA. Adapter proteins in lymphocyte antigen-receptor signaling. *Curr Opin Immunol* 2000;12:256–66. [PubMed: 10781404]
29. Jordan MS, Singer AL, Koretzky GA. Adaptors as central mediators of signal transduction in immune cells. *Nat Immunol* 2003;4:110–6. [PubMed: 12555096]
30. Finco TS, Kadlecsek T, Zhang W, Samelson LE, Weiss A. LAT is required for TCR-mediated activation of PLCgamma1 and the Ras pathway. *Immunity* 1998;9:617–26. [PubMed: 9846483]
31. Lewis RS. Calcium signaling mechanisms in T lymphocytes. *Annu Rev Immunol* 2001;19:497–521. [PubMed: 11244045]
32. Rincon M. MAP-kinase signaling pathways in T cells. *Curr Opin Immunol* 2001;13:339–45. [PubMed: 11406366]
33. Hardy K, Chaudhri G. Activation and signal transduction via mitogen-activated protein (MAP) kinases in T lymphocytes. *Immunol Cell Biol* 1997;75:528–45. [PubMed: 9492189]
34. Kuo CT, Leiden JM. Transcriptional regulation of T lymphocyte development and function. *Annu Rev Immunol* 1999;17:149–87. [PubMed: 10358756]
35. Northrop JP, Ho SN, Chen L, et al. NF-AT components define a family of transcription factors targeted in T- cell activation. *Nature* 1994;369:497–502. [PubMed: 8202141]
36. Rawlings DJ, Sommer K, Moreno-Garcia ME. The CARMA1 signalosome links the signalling machinery of adaptive and innate immunity in lymphocytes. *Nat Rev Immunol* 2006;6:799–812. [PubMed: 17063183]
37. Thome M. CARMA1, BCL-10 and MALT1 in lymphocyte development and activation. *Nat Rev Immunol* 2004;4:348–59. [PubMed: 15122200]
38. Weil R, Israel A. Deciphering the pathway from the TCR to NF-kappaB. *Cell Death Differ* 2006;13:826–33. [PubMed: 16439988]
39. Farber DL, Acuto O, Bottomly K. Differential T cell receptor-mediated signaling in naive and memory CD4 T cells. *Eur J Immunol* 1997;27:2094–101. [PubMed: 9295050]
40. Farber DL, Luqman M, Acuto O, Bottomly K. Control of Memory CD4 T cell activation: MHC class II molecules on antigen presenting cells and CD4 ligation inhibit memory but not naive CD4 T cells. *Immunity* 1995;2:249–59. [PubMed: 7535181]
41. Hussain SF, Anderson CF, Farber DL. Differential SLP-76 Expression and TCR-Mediated Signaling in Effector and Memory CD4 T Cells. *J Immunol* 2002;168:1557–65. [PubMed: 11823482]
42. Hall SR, Heffernan BM, Thompson NT, Rowan WC. CD4+ CD45RA+ and CD4+ CD45RO+ T cells differ in their TCR-associated signaling responses. *Eur J Immunol* 1999;29:2098–106. [PubMed: 10427972]
43. Krishnan S, Warke VG, Nambiar MP, Wong HK, Tsokos GC, Farber DL. Generation and biochemical analysis of human effector CD4 T cells: alterations in tyrosine phosphorylation and loss of CD3 ζ expression. *Blood* 2001;97:3851–9. [PubMed: 11389026]
44. Kersh EN, Kaech SM, Onami TM, et al. TCR Signal Transduction in Antigen-Specific Memory CD8 T Cells. *J Immunol* 2003;170:5455–63. [PubMed: 12759421]
45. Krutzik PO, Clutter MR, Nolan GP. Coordinate analysis of murine immune cell surface markers and intracellular phosphoproteins by flow cytometry. *J Immunol* 2005;175:2357–65. [PubMed: 16081806]
46. Krutzik PO, Hale MB, Nolan GP. Characterization of the murine immunological signaling network with phosphospecific flow cytometry. *J Immunol* 2005;175:2366–73. [PubMed: 16081807]
47. Perez OD, Nolan GP. Simultaneous measurement of multiple active kinase states using polychromatic flow cytometry. *Nat Biotechnol* 2002;20:155–62. [PubMed: 11821861]

48. Ahmadzadeh M, Hussain SF, Farber DL. Effector CD4 T cells are biochemically distinct from the memory subset: evidence for long-term persistence of effectors in vivo. *J Immunol* 1999;163:3053–63. [PubMed: 10477569]
49. Farber DL. Differential TCR signaling and the generation of memory T cells. *J Immunol* 1998;160:535–9. [PubMed: 9551883]
50. Douglass AD, Vale RD. Single-molecule microscopy reveals plasma membrane microdomains created by protein-protein networks that exclude or trap signaling molecules in T cells. *Cell* 2005;121:937–50. [PubMed: 15960980]
51. Drevot P, Langlet C, Guo XJ, et al. TCR signal initiation machinery is pre-assembled and activated in a subset of membrane rafts. *Embo J* 2002;21:1899–908. [PubMed: 11953309]
52. Watson AR, Lee WT. Differences in signaling molecule organization between naive and memory CD4+ T lymphocytes. *J Immunol* 2004;173:33–41. [PubMed: 15210756]
53. Kenworthy AK. Have we become overly reliant on lipid rafts? Talking Point on the involvement of lipid rafts in T-cell activation. *EMBO Rep* 2008;9:531–5. [PubMed: 18516088]
54. Okoye FI, Krishnan S, Chandok MR, Tsokos GC, Farber DL. Proximal signaling control of human effector CD4 T cell function. *Clin Immunol* 2007;125:5–15. [PubMed: 17692570]
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. [PubMed: 15905333]
56. Feinerman O, Veiga J, Dorfman JR, Germain RN, Altan-Bonnet G. Variability and robustness in T cell activation from regulated heterogeneity in protein levels. *Science* 2008;321:1081–4. [PubMed: 18719282]
57. London CA, Lodge MP, Abbas AK. Functional responses and costimulator dependence of memory CD4+ T cells. *J Immunol* 2000;164:265–72. [PubMed: 10605020]
58. Suresh M, Whitmire JK, Harrington LE, et al. Role of CD28-B7 interactions in generation and maintenance of CD8 T cell memory. *J Immunol* 2001;167:5565–73. [PubMed: 11698427]
59. Borowski AB, Boesteanu AC, Mueller YM, et al. Memory CD8+ T cells require CD28 costimulation. *J Immunol* 2007;179:6494–503. [PubMed: 17982038]
60. 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. [PubMed: 18178855]
61. Ndejambi MP, Teijaro JR, Patke DS, et al. Control of Memory CD4 T Cell Recall by the CD28/B7 Costimulatory Pathway. *J Immunol* 2006;177:7698–706. [PubMed: 17114440]
62. 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. [PubMed: 15032595]
63. Dienz O, Eaton SM, Krah T, et al. Accumulation of NFAT mediates IL-2 expression in memory, but not naive, CD4+ T cells. *Proc Natl Acad Sci U S A* 2007;104:7175–80. [PubMed: 17438271]
64. 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. [PubMed: 10761931]
65. Intlekofer AM, Takemoto N, Wherry EJ, et al. Effector and memory CD8+ T cell fate coupled by T-bet and eomesodermin. *Nat Immunol* 2005;6:1236–44. [PubMed: 16273099]
66. Juedes AE, Rodrigo E, Togher L, Glimcher LH, von Herrath MG. T-bet controls autoaggressive CD8 lymphocyte responses in type 1 diabetes. *J Exp Med* 2004;199:1153–62. [PubMed: 15096540]
67. Mullen AC, High FA, Hutchins AS, et al. Role of T-bet in commitment of TH1 cells before IL-12-dependent selection. *Science* 2001;292:1907–10. [PubMed: 11397944]
68. Mullen AC, Hutchins AS, High FA, et al. Hlx is induced by and genetically interacts with T-bet to promote heritable T(H)1 gene induction. *Nat Immunol* 2002;3:652–8. [PubMed: 12055627]
69. Martins GA, Hutchins AS, Reiner SL. Transcriptional activators of helper T cell fate are required for establishment but not maintenance of signature cytokine expression. *J Immunol* 2005;175:5981–5. [PubMed: 16237092]
70. Fann M, Godlove JM, Catalfamo M, et al. Histone acetylation is associated with differential gene expression in the rapid and robust memory CD8(+) T-cell response. *Blood* 2006;108:3363–70. [PubMed: 16868257]

71. Corn RA, Aronica MA, Zhang F, et al. T cell-intrinsic requirement for NF-kappa B induction in postdifferentiation IFN-gamma production and clonal expansion in a Th1 response. *J Immunol* 2003;171:1816–24. [PubMed: 12902482]
72. Shin HM, Minter LM, Cho OH, et al. Notch1 augments NF-kappaB activity by facilitating its nuclear retention. *Embo J* 2006;25:129–38. [PubMed: 16319921]
73. Ahmadzadeh M, Hussain SF, Farber DL. Heterogeneity of the memory CD4 T cell response: persisting effectors and resting memory T cells. *J Immunol* 2001;166:926–35. [PubMed: 11145669]
74. Thome M, Tschopp J. TCR-induced NF-kappaB activation: a crucial role for Carma1, Bcl10 and MALT1. *Trends Immunol* 2003;24:419–24. [PubMed: 12909454]
75. Sommer K, Guo B, Pomerantz JL, et al. Phosphorylation of the CARMA1 linker controls NF-kappaB activation. *Immunity* 2005;23:561–74. [PubMed: 16356855]
76. Wille-Reece U, Flynn BJ, Lore K, et al. Toll-like receptor agonists influence the magnitude and quality of memory T cell responses after prime-boost immunization in nonhuman primates. *J Exp Med* 2006;203:1249–58. [PubMed: 16636134]
77. Wherry EJ, Teichgraber V, Becker TC, et al. Lineage relationship and protective immunity of memory CD8 T cell subsets. *Nat Immunol* 2003;4:225–34. [PubMed: 12563257]
78. Kirwan SE, Burshtyn DN. Regulation of natural killer cell activity. *Curr Opin Immunol* 2007;19:46–54. [PubMed: 17142026]
79. Lantz O, Bendelac A. An invariant T cell receptor alpha chain is used by a unique subset of major histocompatibility complex class I-specific CD4+ and CD4-8- T cells in mice and humans. *J Exp Med* 1994;180:1097–106. [PubMed: 7520467]
80. Bendelac A, Savage PB, Teyton L. The biology of NKT cells. *Annu Rev Immunol* 2007;25:297–336. [PubMed: 17150027]
81. Berg RE, Crossley E, Murray S, Forman J. Memory CD8+ T cells provide innate immune protection against *Listeria monocytogenes* in the absence of cognate antigen. *J Exp Med* 2003;198:1583–93. [PubMed: 14623912]
82. Kambayashi T, Assarsson E, Lukacher AE, Ljunggren HG, Jensen PE. Memory CD8+ T cells provide an early source of IFN-gamma. *J Immunol* 2003;170:2399–408. [PubMed: 12594263]
83. Hu J, August A. Naive and innate memory phenotype CD4+ T cells have different requirements for active Itk for their development. *J Immunol* 2008;180:6544–52. [PubMed: 18453573]
84. Nagarajan NA, Kronenberg M. Invariant NKT cells amplify the innate immune response to lipopolysaccharide. *J Immunol* 2007;178:2706–13. [PubMed: 17312112]
85. Tyznik AJ, Tupin E, Nagarajan NA, Her MJ, Benedict CA, Kronenberg M. Cutting edge: the mechanism of invariant NKT cell responses to viral danger signals. *J Immunol* 2008;181:4452–6. [PubMed: 18802047]
86. Way SS, Wilson CB. Cutting edge: immunity and IFN-gamma production during *Listeria monocytogenes* infection in the absence of T-bet. *J Immunol* 2004;173:5918–22. [PubMed: 15528324]
87. Tato CM, Mason N, Artis D, et al. Opposing roles of NF-kappaB family members in the regulation of NK cell proliferation and production of IFN-gamma. *Int Immunol* 2006;18:505–13. [PubMed: 16481345]
88. Tato CM, Villarino A, Caamano JH, Boothby M, Hunter CA. Inhibition of NF-kappa B activity in T and NK cells results in defective effector cell expansion and production of IFN-gamma required for resistance to *Toxoplasma gondii*. *J Immunol* 2003;170:3139–46. [PubMed: 12626571]
89. Gross O, Grupp C, Steinberg C, et al. Multiple ITAM-coupled NK-cell receptors engage the Bcl10/Malt1 complex via Carma1 for NF-kappaB and MAPK activation to selectively control cytokine production. *Blood* 2008;112:2421–8. [PubMed: 18192506]
90. Hara H, Ishihara C, Takeuchi A, et al. Cell type-specific regulation of ITAM-mediated NF-kappaB activation by the adaptors, CARMA1 and CARD9. *J Immunol* 2008;181:918–30. [PubMed: 18606643]
91. Broderick L, Brooks SP, Takita H, Baer AN, Bernstein JM, Bankert RB. IL-12 reverses anergy to T cell receptor triggering in human lung tumor-associated memory T cells. *Clin Immunol* 2006;118:159–69. [PubMed: 16271513]

92. Valenzuela J, Schmidt C, Mescher M. The roles of IL-12 in providing a third signal for clonal expansion of naive CD8 T cells. *J Immunol* 2002;169:6842–9. [PubMed: 12471116]
93. Liao XC, Littman DR. Altered T cell receptor signaling and disrupted T cell development in mice lacking Itk. *Immunity* 1995;3:757–69. [PubMed: 8777721]
94. Atherly LO, Lucas JA, Felices M, Yin CC, Reiner SL, Berg LJ. The Tec family tyrosine kinases Itk and Rlk regulate the development of conventional CD8+ T cells. *Immunity* 2006;25:79–91. [PubMed: 16860759]
95. Hu J, Sahu N, Walsh E, August A. Memory phenotype CD8+ T cells with innate function selectively develop in the absence of active Itk. *Eur J Immunol* 2007;37:2892–9. [PubMed: 17724684]
96. Broussard C, Fleischacker C, Horai R, et al. Altered development of CD8+ T cell lineages in mice deficient for the Tec kinases Itk and Rlk. *Immunity* 2006;25:93–104. [PubMed: 16860760]
97. Schwartzberg PL, Finkelstein LD, Readinger JA. TEC-family kinases: regulators of T-helper-cell differentiation. *Nat Rev Immunol* 2005;5:284–95. [PubMed: 15803148]
98. Schoenborn JR, Wilson CB. Regulation of interferon-gamma during innate and adaptive immune responses. *Adv Immunol* 2007;96:41–101. [PubMed: 17981204]
99. Fitzpatrick DR, Shirley KM, Kelso A. Cutting edge: stable epigenetic inheritance of regional IFN-gamma promoter demethylation in CD44^{high}CD8+ T lymphocytes. *J Immunol* 1999;162:5053–7. [PubMed: 10227972]
100. Fitzpatrick DR, Shirley KM, McDonald LE, Bielefeldt-Ohmann H, Kay GF, Kelso A. Distinct methylation of the interferon gamma (IFN-gamma) and interleukin 3 (IL-3) genes in newly activated primary CD8+ T lymphocytes: regional IFN-gamma promoter demethylation and mRNA expression are heritable in CD44^(high)CD8+ T cells. *J Exp Med* 1998;188:103–17. [PubMed: 9653088]
101. Avni O, Lee D, Macian F, Szabo SJ, Glimcher LH, Rao A. T(H) cell differentiation is accompanied by dynamic changes in histone acetylation of cytokine genes. *Nat Immunol* 2002;3:643–51. [PubMed: 12055628]
102. Chang S, Aune TM. Histone hyperacetylated domains across the Ifng gene region in natural killer cells and T cells. *Proc Natl Acad Sci U S A* 2005;102:17095–100. [PubMed: 16286661]
103. Tato CM, Martins GA, High FA, DiCioccio CB, Reiner SL, Hunter CA. Cutting Edge: Innate production of IFN-gamma by NK cells is independent of epigenetic modification of the IFN-gamma promoter. *J Immunol* 2004;173:1514–7. [PubMed: 15265878]

Fig. 1. Increased ZAP-70 protein expression in resting memory compared to naive CD4 T cells
 (A) Expression of surface CD3 ϵ and intracellular total ZAP-70 and phospho-ZAP-70 in ovalbumin-specific naïve and memory CD4 T cells. Adapted from Ref. 16, with permission.
 (B). Intracellular ZAP-70 expression in human CD4 T cells obtained from umbilical cord blood, and naïve (CD45RA) and memory (CD45RO) subset of adult peripheral blood. Representative of 3 different donors.
 (C) Confocal analysis of CD3 ϵ and ZAP-70 expression separately and in a merged view of ovalbumin-specific naïve, effector and memory CD4 T cells. Effector CD4 T cells were obtained by activation of naïve CD4 T cells with ovalbumin peptide and APC from 2 days, and memory CD4 T cells were obtained from adoptive hosts

that received primed effector CD4 T cells 8-12 weeks previously. Adapted from Ref. 16 with permission.

Fig. 2. Functional coupling of distal phosphorylation following antigen activation of naive and memory CD4 T cells

Graph shows the percent of IFN- γ producing cells as a function of phosphorylated PLC- γ 1 (row 1) and phosphorylated p38 MAP kinase (Row 2), following antigen-stimulation of naive or memory CD4 T cells. The frequency of IFN- γ -producing cells that also exhibited phosphorylated PLC- γ 1 or p38 (P-PLC- γ 1⁺ or P-p38⁺) is indicated by a black line, and the frequency of IFN- γ -producing cells that did not express phosphorylated PLC- γ 1 or p38 (P-PLC- γ 1⁻ or P-p38⁻) is indicated by a red line at increasing times following antigen activation. Adapted from Ref. 16 with permission.

Fig. 3. A Two-Pathway model for TCR-coupled signaling leading to IFN- γ production in memory CD4 T cells

A schematic diagram of a memory CD4 T cell is shown with surface TCR and CD28. TCR engagement triggers sequential signals through CD3, ZAP-70 and SLP-76 as shown. Two pathways then diverge distal to SLP-76 that couple to IFN- γ gene transcription in the nucleus. The Late pathway (right side) requires both TCR and CD28 engagement, occurs following sustained activation and is associated with an accumulation of MAP kinase and distal phosphorylation and upregulation of T-bet expression. The Rapid pathway (left side) does not require CD28 costimulation, and is not associated with increased distal phosphorylation or T-bet upregulation. This rapid pathway requires NF[κ]B transcriptional activity.

Table I

Signaling events preceding effector cytokine production following antigen activation of memory and naive CD4 T cells.

	Memory (early)	Memory (late)	Naive (late)
Kinetics	1-6hrs	24-48hrs	24-48hrs
CD28 costimulation	Not required	Required	required
ZAP-70 expression	High	High	High
ZAP-70 signaling	Required	Required	Required
Distal MAPK Phosphorylation	Low phosphorylation	High phosphorylation	High phosphorylation
Transcription factor exp.	T-bet ^{lo}	T-bet ^{hi}	T-bet ^{hi}