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ORIGINS OF CD4+ EFFECTOR AND CENTRAL MEMORY T CELLS

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Abstract

Lineage-committed effector CD4+ T cells are generated at the peak of the primary response and are followed by heterogeneous populations of central and effector memory cells. Here we review the evidence that Th1 effector cells survive the contraction phase of the primary response and become effector memory cells. We discuss the applicability of this concept to the Th2, Th17, follicular helper cell, and induced regulatory T cell lineages. We also discuss how central memory cells are formed with an emphasis on the role that B cells play in this process.

The process of CD4+ memory T cell formation begins when the T cell antigen receptors (TCR) on naïve clones bind to major histocompatibility complex II-foreign peptide complexes (pMHCII) on antigen-presenting cells (APC)¹ in secondary lymphoid organs. Signals through the TCR and APC-derived costimulatory molecules such as CD28 cause the naïve cells to divide and become **effector cell** lymphoblasts^{2, 3}. Depending on the nature of cytokines produced by the innate immune system, these effector cells undergo a differentiation process that involves expression of specific transcription factors that control the capacity to produce certain lymphokines⁴. For, example effector cell differentiation in the presence of IL-12 promotes expression of T-bet, which commits the cells to the Th1 program of IFN- γ , but not IL-4 or IL-17 production; whereas differentiation in the presence of IL-4 promotes expression of GATA-3, which commits the cells to the Th2 program of IL-4, but not IFN- γ or IL-17 production. This differentiation also involves expression of homing receptors that facilitate the migration of effector cells to non-lymphoid sites of inflammation⁵ where these cells produce their cytokines to aid in antigen clearance. The number of effector cells peaks about a week into the response, at least in the case of antigens that are rapidly cleared from the body.

About 90% of the effector cells then die during the 1-2 week long contraction phase, leaving a residual population of long-lived cells. These cells, which are called **memory cells**, are predominantly quiescent but capable of intermittent self-renewal and long-term survival in the absence of the inducing pMHCII ligand⁶. Memory cells are heterogenous, however, and proposed to exist in at least two classes⁷. **Effector memory cells (Tem)** express homing receptors that facilitate migration to non-lymphoid sites of inflammation⁵ and produce a variety of microbicidal cytokines including IFN- γ , IL-4, and IL-5 within several hours of

TCR stimulation. **Central memory cells (T_{cm})** do not produce any of the prototypic effector cell lineage cytokines immediately after stimulation through the TCR, although they secrete IL-2 and proliferate extensively and acquire effector lymphokine production later. These cells express CD62L and CCR7, which are involved in migration through lymph nodes and mucosal lymphoid organs and positioning in the T cell areas of these organs⁸. It was therefore postulated that T_{cm} circulate through these locations, and would likely undergo secondary responses there⁷. This prediction was confirmed by studies in mice in which IL-2-producing CD4⁺ memory T cells were found primarily in the lymph nodes while IFN- γ -producing cells were located in the non-lymphoid organs⁵.

Here we will focus on two questions raised by these elegant models – what is the relationship between lineage-committed effector cells present at the peak of the response and the T_{em} that survive the contraction phase, and how are T_{em} and T_{cm} formed? We will review the evidence that some Th1, Th2, and Th17 effector cells become T_{em} and discuss B cells as drivers of the T_{em}/T_{cm} decision.

Evidence that lineage-committed effector cells become memory cells?

A key question in the immune memory field is how do the effector lymphoblasts present at the peak of the primary response relate to the quiescent memory cells that survive the contraction phase? A strong case can be made that some Th1 effector cells simply return to a quiescent state and become Th1 effector memory cells. Lohning and colleagues showed that highly purified *in vitro* differentiated Th1 cells derived from TCR transgenic naïve cells survived with a half-life of about 70 days after transfer into non-lymphophenic naïve recipients⁹. In addition, these investigators used cytokine capture flow cytometry to isolate IFN- γ -producing lymphocytic choriomeningitis virus (LCMV) pMHCII-specific TCR transgenic effector cells from adoptive hosts at the peak of acute infection, and showed that these cells survived with the same 25 day half-life after transfer in new recipients as did non-IFN- γ -producing effector cells. The reason that the *in vivo*-generated effector cells yielded memory cells with shorter lifespans than *in vitro*-generated cells was not clear, although excessive interclonal competition is a possibility. The memory cells derived from the IFN- γ -producing effector cells expressed the IL-7R, lacked CD62L expression, and rapidly produced IFN- γ but not IL-4 during a secondary response. Similarly, Harrington *et al.*¹⁰ used adoptive transfer of TCR transgenic LCMV pMHCII-specific CD4⁺ T cells from IFN- γ reporter mice to show that IFN- γ -producing effector cells from LCMV-infected mice gave rise to long-lived memory T cells. The memory cell population contained CD62L^{low} and CD62L^{high} subsets suggesting that both T_{em} and T_{cm} could be derived from IFN- γ -producing precursors in this case.

Thus, both studies lead to the conclusion that some Th1-like effector cells can give rise to Th1 memory cells with the properties ascribed by Sallusto and Lanzavecchia¹¹ to T_{em}. It is not clear how many of the cells in the effector Th1 population became memory cells and whether this conversion was stochastic or determined by a small subset of dedicated precursors. In addition, the results of these studies must be viewed with some caution since they relied on adoptive transfer of TCR transgenic T cells^{12, 13}, which has been shown to influence memory cell generation and survival due excessive clonal competition¹⁴⁻¹⁸.

To avoid the issues related to adoptive transfer of TCR transgenic T cells, our laboratory studied effector to memory cell conversion by tracking endogenous polyclonal CD4⁺ T cells specific for a pMHCII derived from *Listeria monocytogenes* bacteria using a pMHCII tetramer-based cell enrichment method¹⁹. Intravenous infection with an attenuated strain of *L. monocytogenes*, which was cleared within several days, induced two effector cell populations of roughly equal size in the spleen and lymph nodes; one T-bet⁺ and one T-bet^{low}²⁰. The T-bet⁺ cells lacked ROR γ t and CCR7 and rapidly produced IFN- γ but not canonical Th2 or Th17 cytokines when stimulated *in vivo* with pMHCII. These effector cells were therefore indistinguishable from Th1 cells. The T-bet^{low} effector cells expressed CCR7 and produced none of the canonical lineage-defining cytokines immediately (although they could produce IFN- γ later) and therefore resembled Tcm despite being present during the effector phase of a Th1-driven response. Following the contraction phase and loss of 90% of the effector cells, the resulting memory cell population again consisted of equal subsets of T-bet⁺ CCR7⁻ and T-bet^{low} CCR7⁺ cells. These results are consistent with a model in which 10% of the cells within Th1 and non-Th1 effector cell populations survived the contraction phase and became Th1 effector memory cells and some other type of memory cells, respectively. Other groups reported similar results in other infection models where naive CD4⁺ T cells from pMHCII-specific TCR transgenic mice were tracked after transfer into normal recipients and infection^{21, 22}.

Both T-bet⁺ CCR7⁻ and T-bet^{low} CCR7⁺ *L. monocytogenes* pMHCII-specific memory T cell populations declined slowly over time with a half-life of 50 days, while maintaining an approximately 50:50 ratio for almost a year²⁰. The stability of this ratio suggests that the Th1 effector memory cells did not convert into Tcm. Rather, both subsets behaved as meta-stable non-convertible populations. The relative stability of these populations may be related to slow IL-15-driven homeostatic proliferation²³, although this process must be exceeded by death since both memory cell populations slowly decayed. LCMV pMHCII-specific CD4⁺ memory T cells induced by acute infection also declined slowly over time²⁴. Although pMHCII-specific CD4⁺ T cells induced by chronic viral infection were numerically stable, it is difficult to argue that these were memory cells since the relevant pMHCII was always present²⁵. These results suggest that the type of stable pMHC-independent memory described for CD8⁺ T cells⁶ is difficult if not impossible to achieve for CD4⁺ T cells. This difference may be related to the efficiency of IL-15 sensing, with CD8⁺ memory T cells having the advantage due to expression of more IL-15 receptors²⁶, or more memory stem cells²⁷.

There is also evidence that Th2 effector cells can become Tem after the contraction phase of the immune response. *In vitro* differentiated Th2 cells derived from TCR transgenic naïve cells survived long term after transfer into non-lymphopenic naïve recipients⁹. Using an MHCII tetramer containing a peptide from a *Leishmania major* protein and an IL-4-GFP reporter mouse, Stetson and colleagues demonstrated that IL-4-competent cells are generated early after parasite infection²⁸. Some of the parasite pMHCII-specific IL-4 competent effector cells may have become memory cells because such cells remained long after the parasite was eliminated by antibiotic treatment²⁹. These long-lived Th2-like cells fit the definition of Tem based on low expression of CD62L and high expression of several gut homing molecules including α 4 β 7 integrin. These findings have been confirmed in

several other parasite infections including *Trichuris muris*³⁰. Although these results are consistent with Th2 effector cells surviving the contraction phase to become Th2 memory cells, a detailed phenotypic study of a polyclonal parasite pMHCII-specific population throughout the expansion, contraction, and memory cell phases after infection is needed to cement this conclusion.

The case for Th17 effector cells entering the memory cell pool is less clear. Although *L. monocytogenes* pMHCII-specific Th17 effector cells expressing ROR γ t⁺ but not T-bet were generated after intranasal infection, the number of ROR γ t⁺ cells declined after the contraction phase at a faster rate than Th1 effector memory cells induced by intravenous infection²⁰. The ROR γ t⁺ cells did not express CD27, which is associated with short lifespan³¹. It is therefore possible that the ROR γ t⁺ effector cells died²⁰.

It is also possible that the effector cells simply lost expression of ROR γ t. A recent study showed that T cells that produced IL-17 early during acute fungal infection lost the capacity to produce IL-17 over time³². In addition, there is mounting evidence for plasticity within the Th17 lineage. Numerous studies have demonstrated that *in vitro*-derived Th17 cells can turn into IFN- γ producers after transfer into mice³³⁻³⁶. Additionally, T-bet⁺ ROR γ t⁺ clones generated by injection of a peptide from myelin basic protein^{36, 37} reverted to Th1 cells *in vivo*. A population of IFN- γ and IL-17 double-producing T cells has also been identified in human peripheral blood³⁸⁻⁴⁰. The existence of these bipolar T cells suggests that certain priming conditions can generate effector cells that keep the Th1 or Th17 options open before committing to a final memory cell lineage. The fact that the Th1 lineage was the final choice may be explained by inhibition of ROR γ t by T-bet⁴¹. This idea is supported by the fact that conversion of Th1 cells to Th17 cells has not been reported to date.

The evidence that Treg cells can enter the memory pool is scarce. Although natural Treg cells comprised about 5% of the cells in a bacterial pMHCII-specific naïve T cell population, Foxp3⁺ cells were not present in the memory cell population formed after infection⁴². Foxp3⁺ Tbet⁺ cells can be generated *in vivo* under strong Th1 inflammatory conditions and after persistent infection with *Mycobacterium*⁴³. The cells may, however, lose Foxp3 before becoming memory cells. Evidence from a fate-mapping reporter mouse indicates that some Th1 effector cells or Tem expressed Foxp3 in the past⁴⁴.

Tfh cells marked by expression of CXCR5, ICOS, PD-1 and low levels of CCR7 can also been found at the peak of clonal expansion during immune responses in which germinal centers form^{45, 46}. Indeed, Tfh formation is dependent on pMHCII presentation by B cells⁴⁷ probably in germinal centers, and we find that Tfh cells persist only as long as germinal centers persist (unpublished data). Thus, it is possible that Tfh cells do not become memory cells. On the other hand, CXCR5⁺ CCR7⁺ cells lacking PD-1 and ICOS have been identified in humans and postulated to be resting Tfh memory cells⁴⁸⁻⁵⁰. As discussed below, however, it is also possible that these are Tcm that express CXCR5.

Tcm generation

As mentioned above, some Th1 effector cells generated in *L. monocytogenes*-infected mice appear to become Th1 effector memory cells. These infections, however, also induced

CCR7⁺ effector cells, and subsequently memory cells that express low levels of T-bet and lack other lineage-defining transcription factors²⁰. Could the early cells be Tcm precursors and the later cells their Tcm progeny? The fact that the T-bet^{low} memory cells induced by this infection produce IL-2 (unpublished data) is consistent with this possibility. Another prediction would be that these cells should have the flexibility to give rise to diverse types of effector cells if they are Tcm. Along these lines, Mosmann and colleagues have described uncommitted Th primed precursor cells, which are generated in response to immunization with soluble protein antigens and produce IL-2 but not IFN- γ or IL-4, and have the subsequent capacity to produce these lymphokines when exposed to polarizing cytokines⁵¹. The finding that *L. monocytogenes* infection induces effector cells that give rise to both Tem and non-Tem (Tcm?) raises the question of how naïve T cells decide which path to follow. Several observations indicate that strong transient TCR signaling favors Tem formation. Activation of naïve TCR transgenic T cells under conditions where they are very abundant in adoptive recipients generates predominantly Tcm, perhaps due to reduced TCR signaling as a consequence of interclonal competition¹⁴⁻¹⁸. In addition, naïve T cells that enter a draining lymph node late in the primary response and are activated by dwindling pMHCII complexes primarily become Tcm⁵². Also, reduction of the metabolic activity of effector cells by inhibition of the mTOR pathway increased the number of CD8⁺ Tcm generated by acute viral infection^{53,54}, whereas increased activity of T-bet or Blimp-1 increased effector cell generation⁵⁵. Furthermore, intracellular fluorescent dye labeling experiments showed that the more effector cells divide, the more likely they are to lose CD62L⁵⁶. These results suggest that strong stimulation is needed for commitment to one of the Tem lineages, while weaker stimulation favors the generation of less committed Tcm. In a case of persistent pMHCII presentation, weaker TCR signaling is needed for Tcm and Tem formation, likely by preventing T cell exhaustion⁵⁷.

B cells may also play a role in the appearance of different memory T cell lineages during the primary response. Acute viral infection of B cell-deficient mice had no effect on effector cell generation but resulted in a dramatic reduction in the generation of CD4⁺ memory T cells by a mechanism that does not depend on secreted antibody⁵⁸. This result suggests that the generation of all memory T cells, Tem and Tcm, depends on B cells in this infection. In contrast, we found that T-bet⁺ (Th1) but not T-bet^{low} (Tcm?) memory cells formed in B cell-deficient mice after acute intravenous infection with attenuated strain of *L. monocytogenes* (unpublished data). These results are reminiscent of findings in mice or humans lacking ICOS. Memory T cell generation is altered in the absence of ICOS⁵⁹ or ICOSL such that Th1 formation is increased⁶⁰⁻⁶² while a population of CXCR5⁺ memory cells (78) is decreased^{48, 50, 63}. These ICOS-dependent CXCR5⁺ memory cells may be the descendants of Tfh, which also depend on ICOS⁶⁴. This link, however, has not been proven.

Model of Tcm generation

Based on these results we propose the following model of Tcm generation. Naïve T cells are first stimulated by pMHCII presentation by dendritic cells and then proliferate to form effector cells under the influence of the cytokines from the innate immune system (Fig. 1). Some of these early effector cells, perhaps those that do not interact with B cells, commit to a lineage (e.g. Th1) and some of these survive to become lineage-committed Tem (I). Treg

cells may also be induced but fail to survive or change into Th1 effector memory cells (II). Other early effector T cells interact with B cells and receive signals through ICOS (III). Some these cells become Tfh effector cells that survive as long as antigen and pMHCII-presenting germinal center B cells are present. After the germinal center reaction ends, some of the Tfh become quiescent memory cells while retaining some Tfh markers such as CXCR5 and losing others such as PD-1. In this case the nonlineage committed Tcm-like CXCR5⁺ memory cells observed in several systems are the descendants of Tfh²⁰⁻²². Alternatively, some early effector cells become CXCR5⁺ Tfh and others CXCR5⁺ Tcm precursors, both in response to ICOS signals from B cells. The Tfh effector cells then all die as the germinal center reaction ends while the Tcm survive. In this case, Tcm derive from different effector cells than Tfh. The two variants of this model can be tested in mice lacking the Bcl-6 transcription factor, which is essential for Tfh development. If the first version is correct, then Tcm-like cells will not form, if the second is correct, then they will.

In conclusion, the current evidence indicates that committed Th1, Th2, and perhaps Th17 effector cells survive the contraction phase to form Tem. Other evidence is pointing to B cells as important drivers of the Tem/Tcm decision. However, the big question of why only 10% of the effector cells become memory cells is still unresolved. The answer will likely come from new tools with the power to determine whether CD4⁺ memory T cells are chance survivors of a stochastic process or descendants of dedicated memory cell precursors.

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Figure 1. Model for simultaneous generation of Tem and Tcm cells
See text for description.