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some built-in investigator bias toward robust responsiveness in the model systems. In the case of CD8+ T cells specific for pathogens, most bind well to tetrameric reagents, but for class II MHC responses, clearly some do not (Novak et al., 1999). Thus, the numbers obtained here are likely to be underestimates, but by how much is not clear. It is also important to point out that the study discussed here did not investigate the numerous αβ T cells (~10^7) found in the gut, and it would be interesting to know whether the αβ cells found in that compartment exhibit a similar or distinct repertoire.

This work also speaks to the size of the overall αβ T cell repertoire in that the authors note a diversity of V_βs in their ligand-specific populations. This indicates that there is considerable T cell receptor (TCR) diversity in these pools of T cells, consistent with calculations of the potential αβ TCR diversity of ~10^{15} (Davis and Bjorkman 1988) and with the work of Bousso et al. (1998) showing that individual mice of the same strain nonetheless expressed different TCR sequences. It would be interesting to know whether humans, with their much larger number of T cells, have more cells devoted to a given specificity or perhaps have a larger number of specificities. It is also intriguing to wonder why the ligand-specific T cell repertoire for αβ T cells in mice (10^6–10^7 seems like a reasonable compromise figure without trying to account for crossreactivity) is so nearly identical to that found for the B cell repertoire for mice in the pioneering studies of Klinman and colleagues (Cancro et al., 1978).

The beauty of the approach developed by Moon et al. lies not only in the technically superiority of this method for counting T cells of a given specificity, but also in the fact that one could have the T cells of interest "in hand," allowing all sorts of manipulations and analyses (of TCR diversity, for example) that will be invaluable for future studies. It will also move our discussions of T cell repertoires from the realm of speculation and philosophy to much more solid terrain.

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CD8+ T Cell Differentiation: Choosing a Path through T-bet

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Activated CD8+ T cells can differentiate into transient effector cells or long-lived memory cells. In this issue of *Immunity*, Joshi et al. (2007) suggest that inflammatory cytokines dictate this balance by regulating the expression of the transcription factor T-bet.

The adaptive immune response against pathogens has twin goals: to provide functional effector cells that augment and extend innate immune protection, and to establish immune memory, capable of mounting a quicker and more robust response to the same pathogen when encountered long into the future. The achievement of these distinct objectives requires the differential maintenance of stimulated lymphocytes: Effector cells are needed in vast numbers but for a short time, whereas the raison d'être of the antigen-reactive memory pool is that it should be long-lived yet low frequency (to maintain immune diversity). How and when is this balancing act regulated?

Previous work showed that precursors of the memory pool were evident as a rare population of IL-7Rα+ cells in the effector population (Kaech et al., 2003). The current report (Joshi et al., 2007) extends these earlier studies to show that the expression of the natural killer (NK) cell receptor KLRG1 can be used even earlier in the immune

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response (at stages when IL-7Rα is uniformly low) to identify cells that are destined to become short-lived effector cells (SLECs). KLRG1 (and, at later time points, IL-7Rα) expression therefore offered a way to separate activated cells into those destined to be SLECs versus a pool containing memory precursor effector cells (MPECs).

Although useful, these markers did not provide a direct insight into the mechanism of SLEC versus MPEC decision. Reactivity to interleukin-7 (IL-7) (requiring IL-7Rα) is necessary for the generation and maintenance of CD8$^+$ memory T cells (Kaech et al., 2003; Schluns et al., 2000), but recent work suggests IL-7Rα re-expression is not sufficient to drive effector cell differentiation into the memory pool (Hand et al., 2007). The role of KLRG1 is even more obscure: This molecule has been proposed to mark senescent cells, yet the authors find little impact of KLRG1 knockdown on SLEC production or longevity (Joshi et al., 2007). Nevertheless, these markers permitted the assessment of how other stimuli alter early effector cell commitment into SLEC versus MPEC fates—and to begin identifying the transcription factors controlling the decision making process.

Inflammatory cytokines could influence the relative frequency of effector versus memory cells. Harty and colleagues found that limiting inflammatory cues during CD8$^+$ T cell priming reduced the contraction phase of the response, resulting in more-efficient memory cell generation from the effector pool (Badovinac et al., 2004). Similarly, responses of IL-12-deficient animals to attenuated *Listeria monocytogenes* show the improved production of memory cells (Takemoto et al., 2006). Studies from Mescher and colleagues have shown that both IL-12 and type I interferon (IFN) can promote the differentiation of activated T cells into effector cells (Curtsinger et al., 2005). How do inflammatory cytokines impact the early appearance of SLECs and MPECs?

Joshi et al. also observe that in vivo inflammatory signals enhance the frequency of SLECs at the expense of MPECs, an effect that could be replicated by the administration of IL-12 in vitro. They go on to explore how these signals alter the expression of the transcription factor T-bet. T-bet and its close relative eomesodermin (Eomes) are important for both effector and memory CD8$^+$ T cell differentiation (Intlekofer et al., 2005; Pearce et al., 2003). T-bet is induced by T cell receptor (TCR) stimulation, but Joshi et al. show that T-bet expression is boosted by increasing doses of IL-12, in line with previous studies (Takemoto et al., 2006). Intriguingly, Joshi et al. show that T-bet deficiency leads to a profound absence of KLRG1^{hi} IL-7Rα^{lo} cells, suggesting a key requirement for T-bet in SLECs production. The link between T-bet expression and SLEC differentiation was reinforced by the use of T-bet heterozygous mice and an inducible T-bet expression system, with both approaches indicating that titrating up the amounts of T-bet promotes SLEC over MPEC commitment (Joshi et al., 2007).

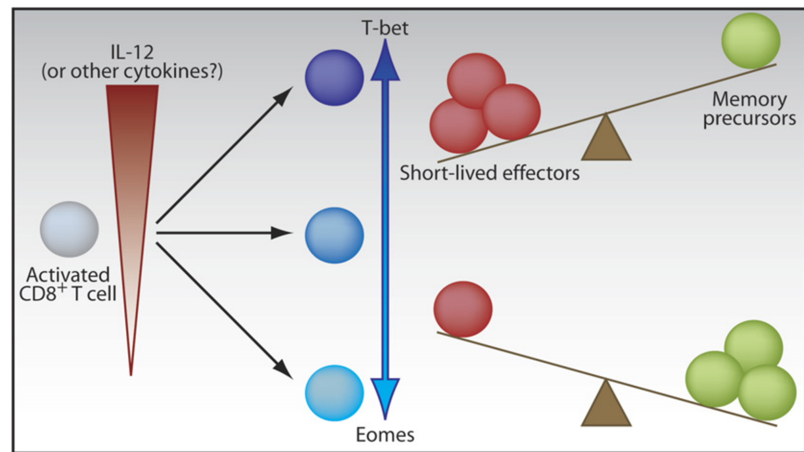

Figure 1. Model for the Role of Inflammatory Cytokines and T-bet in Directing Differentiation of Activated CD8$^+$ T Cells
IL-12 promotes the expression of T-bet (and can repress Eomes) in activated CD8$^+$ T cells. Increased T-bet levels (blue shading) lead to elevated frequencies of KLRG1^{hi} IL-7Rα^{lo} SLECs over KLRG1^{lo} IL-7Rα^{hi} MPECs (represented by the balance of red and green cells, respectively). Whether Eomes participates in regulating these pathways is unclear, as is the role of other inflammatory cytokines in dictating T-bet expression or SLEC-MPEC decision making.

So, do these data argue that T-bet expression, driven by IL-12, is a digital switch dictating SLEC versus MPEC fates? The system is likely to be more subtle than that (Figure 1). First, some minimal amount of T-bet appears to be required for normal memory T cell generation. Although Joshi et al. report that T-bet-deficient CD8$^+$ T cells are strongly skewed toward the MPEC pathway, the resulting T-bet-deficient memory CD8$^+$ T cells failed to upregulate CD122 (the β-chain of IL-2 and IL-15 receptors), a hallmark of normal CD8$^+$ memory T cells (Joshi et al., 2007). One might predict that T-bet-deficient memory cells will fail to undergo basal proliferation, an IL-15 driven process that maintains the normal CD8$^+$ memory T cell pool (Williams and Bevan, 2007). Such data suggest that memory CD8$^+$ T cell differentiation follows a "Goldilocks" model, in which amounts of T-bet need to be "just right" for memory pool production: Too much T-bet diverts cells to the SLEC pathway, whereas too little T-bet (perhaps reflecting a poor TCR signal) would shunt the cells into an alternative pool of memory-like cells that are impaired in sensing homeostatic signals from IL-15.

Second, as discussed above, T-bet has a close cousin, Eomes. The two factors exhibit distinct expression patterns during CD8$^+$ T cell differentiation and, relevant to the current study, IL-12 induces T-bet expression, whereas it inhibits Eomes transcription (Intlekofer et al., 2005; Takemoto et al., 2006). Furthermore, Eomes can also regulate CD122 expression, independent of T-bet (Intlekofer et al., 2005). Might Eomes play an overlapping (or opposing) role in regulating CD8$^+$ T

cell differentiation? Although the current report shows that manipulating the amount of T-bet can influence the SLEC versus MPEC decision, this outcome might involve an effect on Eomes activity (competition for shared target genes, for example). Joshi et al. state that Eomes expression is similar in KLRG1$^+$ and KLRG1$^-$ early effector cells, but given the mild changes in Eomes expression during CD8$^+$ T cell differentiation (Intlekofer et al., 2005; Takemoto et al., 2006), this might not be fully conclusive either. Further work will be needed to explore whether the balance between T-bet and Eomes dictates the fate of activated CD8 T cells.

Third, the impact of additional inflammatory cytokines other than IL-12 on SLEC versus MPEC fates is unclear. Data from Harty's group indicated that IFN-γ deficiency favored the generation of memory cells from early effectors in vivo (Badovinac et al., 2004). In seeming contrast, Joshi et al. report that IFN-γ was unable to directly promote SLEC differentiation of in vitro stimulated cells. However, Joshi et al. comment that they also observe fewer SLECs in immunized IFN-γ-deficient mice but correlate this with a failure to produce active IL-12. Such data suggest IFN-γ might, in certain situations, act through the induction of IL-12 (an interesting reversal of their normal roles). Furthermore, although some infections (such as *L. Monocytogenes*) induce robust amounts of IL-12, other pathogens (like the lymphocytic choriomeningitis virus, extensively used by Joshi et al.) preferentially provoke the production of type I IFN—a cytokine that can also promote CD8$^+$ T cell effector differentiation (Curtsinger et al., 2005). It will be interesting to see whether type I IFN (like IL-12) influences T-bet (or Eomes) expression, or whether the effects of this cytokine operate through a distinct mechanism.

The current work by Joshi et al. reinforces the widely held view that the "correct" amount of inflammation is critical for the construction of the memory pool and shows us a mechanism by which inflammatory cytokines mediate the formation of SLECs through expression of T-bet. Although one important criteria of a memory cell is its longevity, it will be of interest to examine whether the phenotypic and functional characteristics of MPECs (e.g., central versus effector memory subsets) and their ability to undergo protective recall responses is also influenced by amounts of inflammation and/or T-bet. Interestingly, recent studies by Reiner's group indicate that T-bet deficiency favors the generation of central rather than effector memory cells (Steve Reiner, personal communication), arguing for T-bet's influence beyond the SLEC stage.

Previous work has shown that limiting the contraction phase and thereby increasing the memory cell pool is possible if robust inflammation is prevented. In the context of the current work, it might therefore be possible to prevent the formation of SLECs and boost antigen-specific memory through immunization. However, as it is becoming clear that many types of phenotypically and functionally distinct memory populations exist (and presumably benefit the host), so too might multiple types of effector cells be advantageous. This raises the issue of whether SLEC and MPEC formation are mutually exclusive; for example, can effector cells be generated without impairing memory cell numbers in the same response? In their analysis of T-bet-deficient and heterozygous mice, Joshi et al. find similar MPEC numbers, whereas the size of the SLEC pool is drastically altered; yet their studies and earlier work (Badovinac et al., 2004) also suggest that inflammatory cues promote SLEC production at the expense of MPEC. The current report provides insight into effector cell commitment and might suggest vaccine approaches that temper T-bet expression to efficiently generate both SLEC and MPEC pathways—letting the immune system have its cake and eat it too.

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