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Cytokines and the Inception of CD8 T Cell Responses

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Abstract

The activation and differentiation of CD8 T cells is a necessary first step that endows these cells with the phenotypic and functional properties required for the control of intracellular pathogens. The induction of the CD8 T cell responses typically results in the development of a massive overall population of effector cells, comprised of both highly functional but short-lived terminally differentiated cells, as well as a smaller subset of precursors that are predisposed to survive and transition into the memory T cell pool. In this article we discuss how inflammatory cytokines and IL-2 bias the initial response towards short-lived effector generation and also highlight the potential counterbalancing role of IL-21.

The Pluripotency of Naïve CD8 T cells

The antigen-driven activation of naïve CD8 T cells is a critical first step in a differentiation process that generates heterogeneous subsets of cells which vary in their phenotypic attributes, functional capacity, anatomical location, and ability to persist over time. Single cell transfers [1] and DNA barcoding approaches [2,3] have shown that the developmental fate of an individual naïve CD8 T cell is not preset. Instead each naïve cell is immunologically pluripotent and possesses the capacity to give-rise to multiple distinct subsets. This permits the formation of short-lived, but highly functional, effector populations that operate to clear infections, as well as a set of memory precursor effector cells that are more likely to survive overtime, transition into memory T cells, and contribute to long-lived immunological protection (Figure 1) [4]. Because the developmental pathway taken by a naïve CD8 T cell is not predetermined, other factors such as the degree of antigenic activation, contact time with antigen-presenting cells, asymmetric division, and environmental cues, including cytokine availability and signaling, are vital forces that shape the overall outcome to the response (reviewed in 5,6).

The importance of cytokine signaling in dictating the differentiation of the responding cells is best described for CD4 T cells. The emergence of CD4 Th1, Th2, and Th17 populations as well as additional subsets such as Th9, Tr1, and T follicular helper cells, is principally governed by the composition of the cytokine milieu [7]. CD8 T cells can also attain analogous Tc2 [8,9] and Tc17 [10–12] associated phenotypes; however, in the case of CD8 T cells, early exposure to distinct cytokines arguably most commonly influences the balance

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between the development of short-lived terminally differentiated effector cells and memory precursors. Beyond the induction phase, cytokines also contribute to the regulation of the contraction of the response, as well as the long-term maintenance of memory CD8 T cells. Given that cytokines can impact the differentiation, efficacy, and durability of the T cell response they are attractive targets for immunotherapy and for potential use as adjuvants, as their levels can be manipulated with relative ease by exogenous administration, receptor blockade, or neutralization. This review will examine the effect of a select number of cytokines which serve as differentiation factors during the early phases of the CD8 T cell response, and influence the development of short-lived effector and memory precursor cells.

Inflammation and the initiation of the immune response

As naïve CD8 T cells become activated they require cognate antigenic signals through their T cell receptor (TCR), costimulatory signals provided by CD28-B7 interactions, and a third signal provided by inflammatory cytokines in order to fully elicit an immune response [13]. Partial activation of CD8 T cells, either in vitro or in vivo, with antigen and costimulation alone induces a brief abortive response, with poor induction of effector functions and potential deletion of the cells [14–16]. During the course of infections, different pathogen-associated molecular patterns and danger signals promote the production of inflammatory cytokines by innate immune cells such as dendritic cells and macrophages, prior to the activation of the adaptive immune response. The presence of certain inflammatory cytokines such as IL-12 or type I interferons allows the accumulation of activated CD8 T cells and drives their differentiation into fully functional effectors [14–20]. Adequate priming of CD8 T cells therefore requires antigen-specific recognition by the CD8 T cells as well as exposure to early alarm signals received in the form of cytokines.

The inflammatory cytokine IL-12 promotes the differentiation of effector CD8 T cells (Figure 2) [14–17,21]. If inflammatory signals are abrogated during primary CD8 T cell response to *Listeria monocytogenes* (Lm) either by antibiotic treatment [21] or by infection of IL-12-deficient mice [17], then the effector response is diminished. Although the absence of inflammation curtails the development of terminally differentiated short-lived effector CD8 T cells, these conditions favor memory formation [18,21,22]. Conversely, increasing the amount or duration of IL-12 results in elevated expression of the transcription factor T-bet (*tbx21*) in responding CD8 T cells, which further enforces an effector (CD127^{lo}KLRG-1^{hi}) phenotype [21]. The presence of IL-12, therefore, drives maximal activation of the CD8 T cell response, leading to a strong terminally differentiated effector response. Thus, if the levels are too high then this can preclude the development of long-lived memory cells.

The induction of *tbx21* expression by IL-12 is dependant upon the mammalian target of rapamycin (mTOR) [23]. Inhibition of mTOR with rapamycin abrogates the effect of IL-12 on *tbx21*, but elevates expression of the closely related transcription factor Eomesodermin (*eomes*) [23] that is associated with the development of memory T cells. *Eomes* is more highly expressed in memory cells than in effector cells [24], and *eomes*-deficient CD8 T cells are defective in their maintenance and secondary proliferative potential [25]. Rapamycin treatment following infection increases memory cell formation, suggesting that the mTOR signaling pathway, which is regulated by inflammatory cytokines, is critically important in the effector versus memory fate decision of CD8 T cells [26,27]. In addition to mTOR and *tbx21* regulation, IL-12 signaling induces expression of CD25 [20], the high affinity IL-2R α chain, thereby potentiating IL-2 signals which further skew effector differentiation.

Although infections such as *Lm*, *Toxoplasma gondii* [28], *Leishmania* [29], and *Candida albicans* [30] stimulate the production of IL-12, other pathogens, especially viruses such as lymphocytic choriomeningitis virus (LCMV), vesicular stomatitis virus (VSV), vaccinia virus, and herpes simplex virus preferentially induce production of type I interferons (IFN) [31–33]. The types and amount of pro-inflammatory cytokines induced by these different infections also vary. This in turn likely contributes to the heterogeneity in the size and phenotype of the CD8 T cell responses observed following different infections. IFN- α is critically important following LCMV infection, as CD8 T cells deficient in the IFN- α receptor do not accumulate, and *ifna*^{-/-} mice fail to control the virus [34,35]. In a parallel way to IL-12, IFN- α also promotes the differentiation of effector CD8 T cells (Figure 2). These common outcomes are in part because both IL-12 and IFN- α cause similar patterns of chromatin remodeling, resulting in the acetylation of several genes, including those encoding IFN- γ , granzyme B, and CD25, which prolongs expression of these effector associated genes [36]. In addition to these effects on T cells after antigenic activation, signaling by IFN- α and IFN- β also pre-sensitizes naïve CD8 T cells for rapid activation and recruitment should they subsequently encounter antigen [37].

γ_c -cytokines: expanding effectors and maintaining memory potential

As primed CD8 T cells enter into the expansion phase a set of primarily T cell-derived cytokines also operate to support the continued expansion of the response and the acquisition of effector and memory traits. Two such cytokines, IL-2 and IL-21, belong to a larger family of cytokines, which all utilize the common gamma chain (γ_c) cytokine receptor in combination with other unique receptor chains to transduce signals [38]. Deficiency in the γ_c halts the marked proliferation of pathogen-specific effector CD8 T cells which occurs during the later stages of the expansion phase. Although effector generation is impaired by the absence of the γ_c , a greater fraction of the responding cells attain a memory precursor phenotype [39]. Thus, during the early phases of the response ablation of γ_c cytokine signaling appears to promote memory formation at the expense of effector generation. Despite this initial favoring of memory development, the subsequent maintenance of this population is compromised, highlighting the importance of γ_c cytokines throughout the course of the response [39].

Signaling by the γ_c cytokines, IL-2, IL-7, and IL-15, activates signal transducer and activator of transcription 5 (STAT5), resulting in prosurvival signals and upregulation of the anti-apoptotic molecule, Bcl-2. When this pathway is interrupted by deletion of STAT5 in T cells, the pathogen-specific CD8 T cells accumulate poorly due to enhanced apoptosis [40]. In contrast, constitutive activation of STAT5 greatly increases the abundance of virus-specific effector cells, which are CD127^{lo}KLRG-1^{hi} [41]. In addition to affecting proliferation and survival of CD8 T cells γ_c cytokines, such as IL-2 and IL-21, can impact the differentiation status, and functional quality of the responding CD8 T cells.

IL-2-driven effector generation

Upon initial activation CD8 T cells produce a burst of IL-2, but then enter into a transient refractory phase during which they maintain some effector functions but lose the capacity to produce IL-2 [42]. During this period the CD8 T cells are dependent upon extrinsic IL-2, presumably provided by CD4 T cells, for their continued proliferation and restoration of full functional capacity [43]. IL-2 signals during the primary immune response have been shown to be critical for programming the generation of highly functional memory CD8 T cells [44,45]; however, recent data have emphasized a contrasting role for IL-2 signaling in promoting the development of short lived effector cells (Figure 2).

IL-2 signals through a trimeric receptor comprised of CD25 (IL-2R α), CD122 (IL-2R β) and the γ_c . CD25 is not constitutively expressed but is instead transiently upregulated upon activation, following exposure to certain inflammatory cytokines such as IL-12, and also by IL-2 itself [20,46–48]. As the CD8 T cell response is launched these factors integrate to regulate the amount and duration of CD25 expression. This in turn controls the ability of the responding cells to receive IL-2 dependant signals, which has a profound influence on the formation of effector and memory pools.

CD4 T cells are principle producers of IL-2 and cooperate with CD8 T cells to promote the initial expansion of the response as well as the formation of durable memory cells that can elicit protective secondary responses. The secretion of IL-2 by CD4 T cells both upregulates CD25 expression by the responding CD8 T cells and also provides a source of IL-2, which increases the proliferation of the CD25⁺ CD8 T cells. Depletion of CD4 T cells prior to infection with vaccinia virus, Lm, or VSV, decreases the fraction of CD25⁺ CD8 T cells as well as reduces the relative amount of CD25 expression [46]. Consequently, the absence of CD4 T cells or CD25 expression on CD8 T cells restricts the expansion phase of the pathogen-specific response [45,46]. Nevertheless, the CD25-deficient (*il2ra*^{-/-}) CD8 T cells that are maintained appear to attain a more memory-like phenotype than their wild-type counterparts [45,46,49], which is consistent with the roles of IL-2 in supporting short-lived effector generation. Despite the ability of *il2ra*^{-/-} CD8 T cells to persist over time and resemble memory cells, their secondary proliferative capacity has been shown to be defective [44,45]. Collectively, these results demonstrate the importance of IL-2 signaling during the inception of the CD8 T cell response. This can have both short-term effects, pushing the generation of an overwhelming pool of highly potent effector cells, and long-term consequences, compromising memory populations.

The magnitude and duration of CD25 expression on CD8 T cells following activation tunes their ability to perceive IL-2 and likely dictates their effector or memory fates (Figure 3). The expression of CD25 on virus-specific CD8 T cells is initially high after LCMV infection, but becomes biphasic between days 3.5 and 5 post-infection. At this biphasic timepoint, cells that express lower levels of CD25 have a gene expression pattern similar to memory T cells: lower for *prf1* (perforin) and *prdm1* (Blimp-1), higher for *sell* (CD62L). In contrast, the transcriptional profile of the CD25^{hi} CD8 T cells more closely matches that of a prototypic effector cell [48]. Upon transfer into infection matched recipient mice, the CD25^{hi} cells undergo greater apoptosis and fail to accumulate, hallmarks of a terminally differentiated effector state. CD25^{hi} donor cells also express high levels of KLRG-1, lower levels of CD62L, and are defective in secondary expansion, further indicating that these cells are preferentially recruited to the effector cell lineage and are inefficient at forming an effective memory compartment [48]. By contrast the CD25^{lo} CD8 T cells were better able to survive overtime and mount vigorous recall responses, indicating that during the initial priming phase these cells are predisposed to form the memory compartment.

The presence of elevated concentrations of IL-2 following priming of naïve CD8 T cells also biases their development towards short-lived effector cells (Figure 3). IL-2 increases the expression of the effector proteins perforin, granzyme B, and IFN- γ , and enhances the cytolytic capacity of CD8 T cells [47]. Several transcription factors are known to regulate these genes as well as influence cell fate decisions in CD8 T cells. IL-2 signals directly upregulate *prdm1* [50] and *tbx21*, which are both transcription factors associated with differentiation and effector cell function [5,51]. This provides a molecular coupling between IL-2 and changes in the transcriptional landscape of the responding T cells, which enforces effector development. Bcl-6, in contrast, is a transcription factor associated with memory formation, and it is directly repressed by Blimp-1 [5,51]. At the highest doses of IL-2,

prdm1 expression is sustained while *bcl6* is repressed, further solidifying an effector phenotype within the cell [47].

Since Eomes is associated with memory formation, it is surprising that this transcription factor is dramatically upregulated in response to high concentrations of IL-2, although this is not the case when additional inflammatory stimuli are present [47]. Some evidence suggests that IL-12 signaling downregulates *eomes* expression [52], so it is plausible that during an infection, IL-2 signals drive the expansion and generation of effector cells, but the continued presence of inflammatory stimuli downregulates *eomes*. Accordingly, as the infection is controlled and inflammatory signals are curtailed, *eomes* may become derepressed allowing some of the cells to transition into the memory pool.

The hypothesis that high IL-2 levels do not completely ablate memory development is supported by experiments in which CD8 T cells were treated with high or low concentrations of IL-2 and then transferred into congenic recipient mice. Cells cultured with high IL-2 are phenotypically more effector like, express lower levels of CD62L, and are poorly maintained in the recipient mice. By 35 days after transfer, however, a subset of CD8 T cells that were initially exposed to high levels of IL-2 survived and expressed high levels of CD62L, and significantly, could rapidly proliferate in a secondary challenge [47]. This indicates that at least a fraction of the overall population of CD8 T cells primed in the presence of high levels of IL-2 could attain a bona fide memory phenotype. Consequently, the ultimate divergence between short-lived effector generation and the ability to form memory may reflect heterogeneity in the levels of CD25, even on CD8 T cells primed in the presence of elevated amounts of IL-2 (Figure 3). Thus, whether the responding CD8 T cells mature into effector or memory precursor cells is influenced by their ability to sustain expression of appropriate cytokine receptors, as well as the availability of γ_c cytokines, including IL-2, and the inflammatory milieu. Following activation this allows divergence within the CD8 T cell pool and regulates the balance between effector and memory-precursors.

The counterbalancing role of IL-21

Although CD4 T cell-derived IL-2 drives the continued expansion and terminal differentiation of CD8 T cells, CD4 T cells are also typically required for the development of a fully functional memory CD8 T cell pool. During the expansion phase of the immune response the production of IL-21 by CD4 T cells is an attractive contender for counterbalancing the effects of IL-2 on CD8 T cell differentiation (Figure 2).

Overexpression of IL-21 in mice results in gross exaggeration of the memory CD8 T cell population, implicating a role for this cytokine in the development and/or maintenance of memory cells [53].

During chronic LCMV infections, IL-21 acts directly on CD8 T cells to sustain the immune response, and a lack of IL-21 signaling within CD8 T cells results in the failure to contain the infection and causes severe exhaustion, leading to deletion of pathogen-specific CD8 T cells [54–56]. Exhaustion represents an extreme state of differentiation and arises as the short-lived effector cells lose their effector functions while maintaining expression of inhibitory receptors and activation markers, including PD-1 and CD43 [5,57,58]. This is driven by potent and sustained antigenic stimulation which the cells receive in the chronically infected host. Like short-lived effectors, certain exhausted cells are rapidly deleted. The more long-lived exhausted cells become addicted to their inducing antigen for maintenance and these altered homeostatic properties can result in the erosion of the population over time. Accordingly, IL-21 might slow the terminal differentiation of short-

lived CD8 T cells, possibly promoting the survival of a less developed progenitor of this population and curtailing the development of highly exhausted cells.

Mice deficient in the IL-21 receptor have intact responses to acute pathogens [54,55,59,60] and peptide immunization [59], although greater expansion of antigen-specific CD8 T cells has been reported in the liver of *il21*^{-/-} mice following peptide immunization [59]. This enhanced expansion might reflect the role of IL-21 in restricting effector cell generation, thereby limiting the size of T cell population that can traffic into tertiary sites such as liver. The ability of IL-21 to limit the effector response, while permitting memory generation, is further supported by the observation that providing IL-21 signals during the priming phase can cause the majority of the responding T cells to express a central memory phenotype [61]. Nevertheless, IL-21 is not required for the formation of central memory CD8 T cells, as these cells are detectable in *il21*^{-/-} and *il21r*^{-/-} mice [60,62]. Qualitative differences in the memory compartment do, however, manifest in the absence of IL-21 signals. *il21r*^{-/-} memory CD8 T cells are outcompeted by their *il21r*^{+/+} counterparts following secondary LCMV infection of mixed bone marrow chimeras [60]. Additionally, blunted CD8 T cell recall responses are also observed following adenovirus infection of *il21r*^{-/-} mice [62]. Collectively, these findings suggest that IL-21 may impact both the effector and memory compartments.

How IL-21 functions to shape effector and memory CD8 T cell responses is still not fully understood. IL-21 has been shown to induce expression of *tcf7* (TCF-1) and *lef1* (LEF-1) in CD8 T cells [63], which are components of the Wnt pathway and interact with β -catenin in the nucleus to regulate transcription of several genes, including *eomes*, which drive memory formation [64,65]. Nevertheless, in vitro studies indicate that CD8 T cells activated by IL-21 express lower amounts of *eomes*, despite sustaining CD62L expression and the ability to survive and protect against tumor challenge [63]. Thus, the roles of IL-21 in this process are not yet fully defined and warrant further investigation. IL-21 not only modulates *tcf7* and *lef1* but also induces *prdm1* (Blimp-1) expression [66], which is known to drive terminal differentiation in CD8 T cells. The transcription factor Bcl-6 can suppress Blimp-1, and in B cells, *bcl6* is induced by IL-21. So it is possible that IL-21 signaling in CD8 T cells induces both *prdm1* and *bcl6*, and the balance between terminal differentiation and memory cell formation is then determined by additional signals. If a cell receives higher IL-2 signals, this would further induce *prdm1*, which could then shut-down *bcl6* and drive terminal differentiation. If instead, the cell received strong IL-21 but weaker IL-2 signals, then *bcl6* expression might predominate and repress Blimp-1 induced differentiation. However, this is still speculative, as induction of *bcl6* by IL-21 in CD8 T cells has not yet been reported.

Looking ahead

Our knowledge of how individual cytokines shape the differentiation of CD8 T cells and determine short-lived effector versus memory fate decisions is continuing to advance. Nevertheless, our understanding of these processes is far from complete, and certain outstanding questions are outlined in Box 1. Overall, it remains necessary to better understand how the composition of the cytokine milieu is regulated during the induction of an immune response, and how this exerts molecular control over the differentiation of CD8 T cells as they respond to antigenic stimuli. Further dissecting these issues might reveal potential approaches for not only boosting T cell activities and memory to pathogens, but also dampening possibly immunopathogenic responses. For example, immunotherapy with IL-2 alone is only effective in a subpopulation of melanoma and renal cell carcinoma patients [67], and does not enhance immune protection in HIV infected individuals [68]. Although IL-2 therapy can enhance the number of virus-specific cells following acute infection [69], the ability of this cytokine to drive differentiation of the responding T cells might be

responsible for its clinical limitations. In contrast, IL-21 therapy has shown some efficacy in preclinical studies [70]. Thus combination or sequential IL-2, IL-21 therapies could be explored as these may be more effective at not only expanding the T cell response but also at sustaining greater immunological pluripotency and proliferative potential in these cells.

Box 1

Unanswered Questions

- How do the complex signals which CD8 T cells initially receive as they differentiate integrate to control the short-lived effector versus memory precursor fate decision?
- Is memory formation the default setting of an activated CD8 T cell; do accessory signals primarily function skew this process to permit the generation of effector cells?
- Do factors that trigger CD25 expression independently of IL-2 mainly influence CD8 T cell differentiation because of CD25 induction, or do these factors have other distinct effects on the responding cell?
- Is the maintenance of CD25 on subsets of responding CD8 T cells principally a reflection of recent antigenic stimulation or is CD25 expression more prominently driven and maintained by inflammatory signals such as IL-12?
- What is the biological significance of IL-2 induced Eomes expression; is Eomes driving terminal differentiation and, if so, what are the requirements for Tbet under these conditions?
- How does IL-21 potentially limit CD8 T cell differentiation; is this primarily due to regulation of the Blimp-1-Bcl-6 axis?
- Do the factors which determine effector and memory precursor development during primary responses also similarly regulate the generation and abundance of secondary and tertiary effector cells, which arise as memory cells are re-exposed to their cognate antigen?

Regulation of cytokine receptor expression is also a key area that requires further study, as this is likely to be a rate-limiting step in effector cell generation. It has been proposed that initial asymmetric division by CD8 T cells influences the developmental fate of their daughter cells [71]. CD25 is one molecule that is asymmetrically inherited and it is tempting to speculate that higher expression of CD25 on the proximal daughter cell results in a feedback loop, allowing the cell to receive stronger IL-2 signals, thus maintaining CD25 expression and driving effector formation. Conversely, the development of the effector response may be more plastic, and dependant upon relative levels of inflammatory, IL-2, and TCR-derived pro-differentiation signals, as well as opposing signals from IL-21 and potentially other yet to be defined molecules. Thus, determining whether the cytokine driven fate of the response can be further enforced or reversed may foster the development of strategies for manipulating the response, as well as provide fundamental insights into how the phenotypic and functional diversity of CD8 T cell responses are controlled.

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Figure 1.

Naïve CD8 T cells have the potential to differentiate into both short-lived effectors and memory precursors following activation. Short-lived effector cells are commonly defined as CD127^{lo}, KLRG-1^{hi} and express a panel of transcription factors, which promote effector activities but limit their proliferative capacity and survival. By contrast, memory precursors are typically CD127^{hi}, KLRG-1^{lo}. These cells also have certain effector properties including the ability to produce IFN- γ , but unlike their short-lived counterparts they are more likely to survive the downregulation of the response and transition into memory populations, which persist over time and help confer long-lived immunological protection. Different priming strategies and infections can skew the developmental process in either direction, which is determined by various factors including duration of stimulation and the composition of the cytokine milieu. This likely results in a spectrum of differentiated states, ranging from terminally differentiated effector cells to memory precursors.

Figure 2.

Inflammatory cytokines, type I IFN, IL-2, and IL-21, dictate the balance between short-lived effector and memory precursor generation. Infections often cause increases in the levels of the pro-inflammatory cytokine IL-12 and/or type I IFN, which in conjunction with antigenic activation and costimulation, promote the differentiation of short-lived effector cells but restrict the formation of memory precursors. High levels of IL-2 similarly enforce short-lived effector formation, whereas the related cytokine IL-21 potentially plays a less well defined role in restricting the terminal differentiation of the T cell population.

Figure 3.

IL-2 levels and CD25 expression influence the short-lived effector versus memory precursor fate decision. IL-2 induces the expression of CD25, a component of cognate receptor for this cytokine. This allows the activated cells to receive longer and greater IL-2 signals which drive their differentiation towards a short-lived effector state. Lower amounts of IL-2, which may occur if CD4 T cells are defective or not available, as well as the loss of CD25 expression limits the terminal differentiation of the responding cells and favors the formation of memory precursors.