

## Review Article

# Shaping Successful and Unsuccessful CD8 T Cell Responses Following Infection

**Maureen A. Cox and Allan J. Zajac**

*Department of Microbiology, University of Alabama at Birmingham, Birmingham, AL 35294, USA*

Correspondence should be addressed to Allan J. Zajac, azajac@uab.edu

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CD8 T cells play a vital role in the immunological protection against intracellular pathogens. Ideally, robust effector responses are induced, which eradicate the pathogen, and durable memory CD8 T cells are also established, which help confer protection against subsequent reinfection. The quality and magnitude of these responses is dictated by multiple factors, including their initial interactions with professional antigen-presenting cells, as well as the cytokine milieu and availability of CD4 T cell help. These factors set the transcriptional landscape of the responding T cells, which in turn influences their phenotypic and functional attributes as well as ultimate fate. Under certain conditions, such as during chronic infections, the development of these usually successful responses becomes subverted. Here we discuss advances in our understanding of the cellular and molecular determinants of T cell quality, and the formation of effector, memory, and exhausted CD8 T cells, during acute and chronic infections.

## 1. Introduction

The induction of CD4 and CD8 T cell responses, in conjunction with innate immunity and antibodies, helps protect against pathogens including viruses, bacteria, fungi, and eukaryotic parasites. CD8 T cells are of particular importance in mediating elimination of intracellular pathogens due to their ability to recognize and kill infected cells. Current vaccination strategies are aimed at eliciting B cell responses and conferring antibody-mediated protection; however interest has been steadily growing in the development of vaccines designed to prime durable memory T cells [1–5]. T cell-based protective approaches may be particularly advantageous in helping protect against or in controlling chronic infections such as hepatitis B virus (HBV), hepatitis C virus (HCV), and human immunodeficiency virus (HIV).

A better understanding of the factors that yield strong primary and memory T cell responses is likely to benefit the development of both T cell-based vaccines and new therapeutic options for chronic infections as well as tumors. Critical issues that are at best only partially understood include defining the variables which regulate the induction and maintenance of long-lived memory responses;

deciphering the distinguishing features of protective immune responses, including the molecular and cellular traits of “successful” T cell responses; and determining how these responses integrate with other immune system components and act in concert to confer immunological protection. Analyses of immune responses to infections in both animal model systems and in clinical studies using human samples are helping to unravel these complex issues.

## 2. Functional Quality of CD8 T Cell Responses during Acute and Chronic Infections

The functionality of CD8 T cells is shaped at each stage of the immune response by multiple factors, including the duration and strength of antigenic stimulation, interactions between T cells and antigen presenting cells (APCs), the inflammatory milieu, costimulatory requirements, cytokine and chemokine availability, and the presence of CD4 T cell help. These factors influence the expression of transcription factors that regulate the differentiation and phenotypic properties of CD8 T cells and collectively function to dictate

the development of effector T cells and the establishment of the memory pool.

Many acute infections elicit massive antigen-specific CD8 T cell responses which contribute to the successful clearance of the pathogen [6–15]. The overall pool of expanded effector cells, induced during the initial phase of the response, is heterogeneous and comprised of subsets which differ in their epitope-specificity, clonal abundance, anatomical location, effector potential, expression of surface markers, and fate. Typically only a fraction (5%–10%) of this expanded ensemble of antigen-specific CD8 T cells survives the subsequent downregulation of the response and proceeds to constitute the long-lived memory pool (Figure 1(a)). Thus, following the clearance of the infection, an increased number of antigen-specific T cells are now present which are tuned to rapidly respond if they reencounter infected cells [16–21]. Taken together, this highlights two points regarding the development of long-lived, protective, CD8 T cell memory. First, the ideal primary response not only eradicates the infection but also generates a set of “precursors” which can form the memory pool. Second, once the infection is controlled, it is critical to maintain both the physical presence as well as the functional potential of these memory cells in order to confer T cell-mediated immunological protection.

Memory T cell populations are remarkably heterogeneous and differ in many ways including their capacity to execute effector functions, their ability to proliferate upon secondary challenge, and also their anatomical location. Based on these traits, memory T cell populations have been broadly segregated into “effector-memory” and “central-memory” subsets. Effector-memory T cells express low levels of CD62L and CCR7 and generally exhibit a high degree of effector activity, low proliferative capacity, and preferentially reside in tissues where they can serve as sentinels to protect against localized infections. By contrast, central-memory T cells generally express high levels of CCR7 and CD62L, have a higher proliferative capacity, and reside in secondary lymphoid organs [22–24]. The lineage relationships between these two subsets, and what factors endow specific functions upon these populations, are an area of debate within the field [24–26].

Although CD8 T cells can be highly effective at controlling acute infections and contribute to protective secondary responses, protracted and chronic infections do arise and are often associated with the development of phenotypically and functionally inferior responses [14, 27–46]. These types of infections include many pathogens which are of significant global public health importance, such as HIV and HCV. A common feature of more protracted and chronic infections is that antiviral CD8 T cells are initially triggered, but qualitative and quantitative defects become apparent in the generation of robust sets of effector cells as well as the progression of memory T cell development. T cell responses generated under these conditions are susceptible to exhaustion, which is characterized by the stepwise and progressive loss of effector functions. Consequently, these responses are incapable of clearing the pathogen. The ability to produce the cytokine IL-2 and undergo robust proliferation appears to be highly sensitive to exhaustion

and are the first functional qualities lost by CD8 T cells during protracted and chronic infections [29, 39, 41]. TNF $\alpha$  production and IFN $\gamma$  production are more robust functions; however, even these abilities are lost at more severe stages of exhaustion, and T cells specific for certain epitopes may even be deleted [29, 39, 41, 47, 48] (Figure 1). Ex vivo killing activities are difficult to detect by exhausted cells; however, in vivo killing has been documented [29, 49]. Thus, these cells may retain some residual cytotoxic activity within the persistently infected host, although the biological significance of this killing activity is unclear given the failure to contain the infection.

Despite the functional ineptness of exhausted CD8 T cells, they express patterns of surface receptors which are commonly associated with activated effector T cells. They express high levels of CD69 and CD43 (1B11) but low levels of CD62L and CD127 [29, 30, 42, 45, 48, 50]. Exhausted CD8 T cells also express constellations of inhibitory receptors, most notably PD-1 [43, 51–53], but also LAG-3 and high levels of CD244 [54]. Varying levels of exhausted and defective CD8 T cell responses have been observed following many infections, including lymphocytic choriomeningitis virus (LCMV) [28, 29, 31, 47, 48, 55], polyoma virus [56], adenovirus [57], Friend leukemia virus [58–60], mouse hepatitis virus [61], HIV [34, 51, 62–64], HBV [65, 66], and HCV [33, 36, 52, 66, 67], and have also been observed in patients with malignancies [68–71]. As further discussed below, the development of the exhausted state is generally more severe if CD4 T cell help is inadequate.

### 3. Priming CD8 T Cell Responses by Professional Antigen-Presenting Cells

Naïve CD8 T cells become activated when they encounter professional antigen-presenting cells (APC) displaying cognate peptide antigen complexed with an appropriate MHC class I molecule. The ability of CD8 T cells to survey these MHC-peptide complexes allows these cells to fulfill their immunological surveillance functions and is the essential first step for launching the CD8 T cell response. The interaction between the naïve CD8 T cell and the APC allows the antigen-specific cells to receive TCR signals (signal 1) as well as costimulation (signal 2) [72] (Figure 2). These signals are conveyed as the T cell and APC form an immunological synapse (IS) consisting of TCR/MHC-peptide complexes and CD28/CD80-CD86 interactions, which provide signals 1 and 2, respectively, to the responding T cell. As the cells couple, these signaling molecules colocalize within the plasma membrane forming a central supramolecular activation cluster (cSMAC), while adhesion molecules such as lymphocyte function-associated antigen- (LFA-) 1 and intracellular adhesion molecule- (ICAM-) 1 surround the cSMAC, forming the peripheral supramolecular activation cluster (pSMAC) [73, 74]. LFA-1/ICAM-1 interactions mediate long duration contact between the T cell and the APC [75, 76], and it has been shown that ICAM-1 deficiency on the APC impedes long duration contact formation [76].

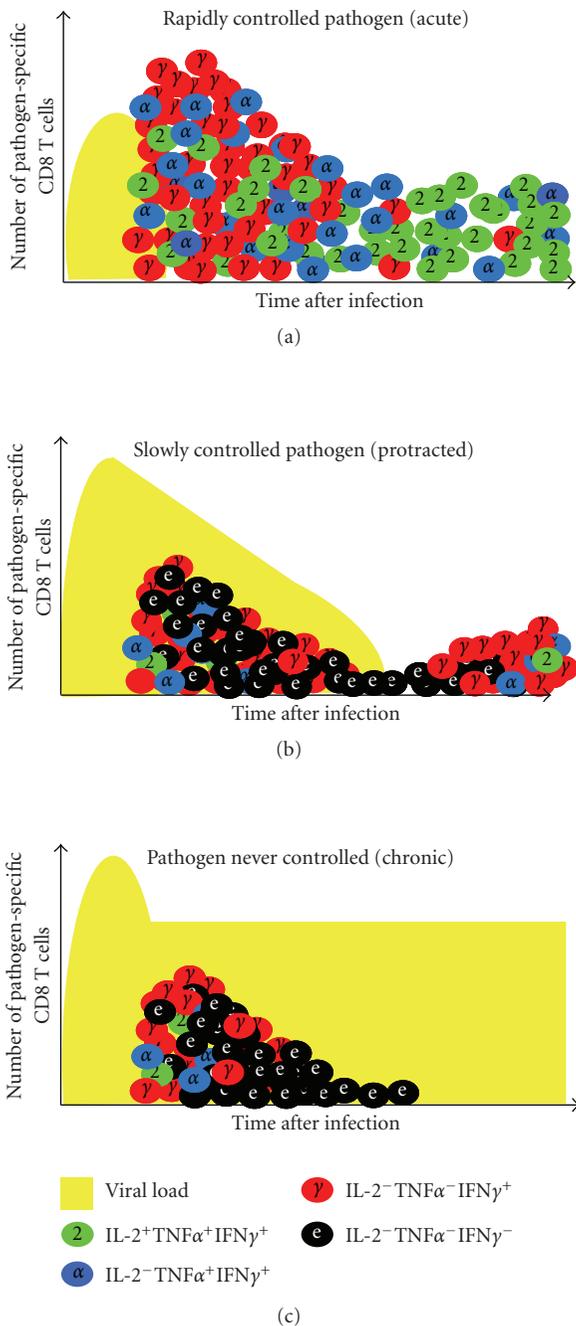


FIGURE 1: Pathogen-specific T cell responses to acute and chronic infections vary in magnitude and functional attributes. (a) Acute infections elicit a large CD8 T cell response, and after resolution of the infection the memory pool is typically composed of polyfunctional T cells capable of coproducing  $IFN\gamma$ ,  $TNF\alpha$ , and  $IL-2$  (green circles) or  $IFN\gamma$  and  $TNF\alpha$  (blue circles). (b) Protracted infections are associated with weaker responses and reductions in the functional quality of the responding T cells, such as the emergence of CD8 T cells which only produce  $IFN\gamma$  (red circles) as well as the development of exhausted cells that are functionally incapacitated (black circles). The overall quality of the response may improve as the infection is brought under control. (c) High-grade viral persistence during chronic infection results in severe functional exhaustion and the possible deletion of antigen-specific CD8 T cells.

Stable formation of the IS allows strong signaling to the responding CD8 T cell and also keeps the cell in close proximity to the priming APC. This allows the CD8 T cell to receive “signal 3” in the form of cytokines such as  $IL-12$  and other inflammatory mediators, which also influence the functional attributes of the developing response [77–83]. Signaling via  $IL-12$  supports the proliferation and development of cytolytic activity in CD8 T cells in vitro [77, 79, 84] and can act as an adjuvant during peptide immunization in vivo [78, 80–82]. This ability to promote effector CD8 T cell responses is further shown by findings that  $IL-12$  can drive the formation of effector CD8 T cells, which are distinguished by their expression of high levels of  $KLRG-1$  and low levels of  $CD127$  [82, 83]. The primary producers of  $IL-12$  are dendritic cells, macrophages, and monocytes, which produce the cytokine in response to inflammatory signals including  $IFN\gamma$ . These two cytokines act in a positive feedback loop in which  $IFN\gamma$  activates the APC to produce  $IL-12$ , and then  $IL-12$  induces more  $IFN\gamma$  production by the CD8 T cell [85, 86]. In addition to promoting  $IFN\gamma$  production,  $IL-12$  and  $IFN\alpha$  have both been shown to enhance induction of other effector molecules, such as granzyme B and Fas ligand, when combined with TCR ligation and costimulation [77, 84].

#### 4. CD4 T Cell Help for CD8 T Cells

CD4 T cells contribute to immunological protection against both intracellular and extracellular pathogens. These cells have multifaceted roles and function to help B and T cell responses by promoting the activation of professional APCs via interactions with costimulatory molecules and by the secretion of cytokines and chemokines. During typical viral infections, CD4 T cells are primarily polarized to the Th1 lineage and produce the cytokines  $IFN\gamma$ ,  $TNF\alpha$ , and  $IL-2$  [8, 87, 88]. The more recently described T follicular helper (Tfh) subset of CD4 T cells, which promote germinal center formation, are also generated following infection [89, 90].

The importance of CD4 T cells in helping CD8 T cells has become increasingly well described [29, 30, 39, 48, 91–105]. Although deceptively potent primary CD8 T cell responses can be induced in the absence of CD4 T cell help, the subsequent establishment of long-lived, functionally robust memory CD8 T cells is compromised [29, 30, 91–102, 106–109]. CD8 T cell responses elaborated in the absence of CD4 T cell help may remain in an effector-like state, associated with the constitutive expression of the transcription factor T-bet, but develop a spectrum of functional defects which increase in severity with time [29, 30, 48, 97, 101, 110]. Prototypic memory CD8 T cells primed and maintained in the presence of CD4 T cell help are typically  $CD62L^{lo/hi}$ ,  $CD122^{hi}$ , and  $CD127^{hi}$  whereas their helpless counterparts are predominantly  $CD62L^{lo}$ ,  $CD122^{lo}$ , and  $CD127^{lo}$  and may not be stably maintained [30, 91, 97]. Additionally, marked reductions in the capacity to produce cytokines, especially  $IL-2$ , as well as diminished secondary proliferative responses are hallmarks of inadequately helped memory CD8 T cells [30, 91, 92, 95–97, 99–101].

It has been proposed that CD4 T cells operate to “program” the development of robust, long-lived memory CD8 T cells [95, 96]. This model suggests that as naïve CD8 T cells respond to antigenic stimulation, CD4 T cells imprint the subsequent development of memory traits. Accordingly, if CD4 T cell help is not applied, the responding CD8 T cells are misprogrammed and are incapable of fully differentiating into authentic memory T cells. An alternative maintenance model has also been put forward to account for the helper dependency of memory CD8 T cell responses [93, 101]. This model suggests that CD4 T cells are not required for the initial programming of memory precursors during the priming phase but are instead required for their subsequent survival and the maintenance of functional potential. Which of these models best accounts for defective CD8 T cell memory in helpless hosts is not resolved. It is plausible that the requirement for CD4 T cell help is biphasic and that the presence of CD4 T cells during the induction phase may enhance the generation or “programming” of CD8 T cells which mature into memory CD8 T cells, and that CD4 T cells also subsequently operate to sustain the functional potential and numbers of these cells over time.

CD4 T cells are thought to deliver their help to CD8 T cells in several ways. CD4 T cells “license” antigen-presenting cells via CD40-CD40L interactions, empowering them to launch CD8 T cell responses [111–114] (Figure 2). It has also been proposed that the APC requirement can be bypassed, requiring direct delivery of the helper signal from the CD4 T cell to the CD8 T cell [115]. CD4 T cells not only play a role in activating dendritic cells but also produce the chemokines CCL3 and CCL4, which promote the migration of CD8 T cells toward these activated dendritic cells [116, 117]. Later in the response, CD4 T cells can stimulate the trafficking of CD8 T cells to sites of infection by the secretion of IFN $\gamma$  as well as the chemokines CXCL9 and CXCL10 [118].

Cytokine production is a cardinal trait of activated CD4 T cells and two related cytokines, IL-2 and IL-21, produced by these cells have been shown to impact gene regulation, function, and fate of responding CD8 T cells (Figure 2). IL-2 provides growth and differentiation signals to responding CD8 T cells [119–121], and IL-2 signals during priming are required for CD8 T cells to be subsequently capable of expanding upon rechallenge [122, 123]. Both IL-2 signaling and IL-21 signaling drive the upregulation of B lymphocyte-induced maturation protein 1 (Blimp-1/*Prdm1*), a transcription factor known to induce CD8 T cell differentiation and effector function (discussed further below) [124–126]. CD8 T cells are also able to produce IL-2 upon TCR ligation [127–129]; however after the initial activation the responding T cells enter a period termed “activation-induced nonresponsiveness” (AINR) [130–133]. During this time T cells are still able to lyse target cells and produce IFN $\gamma$  but are dependant on paracrine IL-2 supplied by CD4 T cells for further proliferation. After receiving these CD4 derived IL-2 signals, the responding CD8 T cells are rewired to allow IL-2 mRNA upregulation and proliferation in response to TCR signals in the absence of costimulation [132]. CD8 T cells primed in the absence of CD4 T cells are defective at mounting recall responses [92, 96, 97, 99, 100],

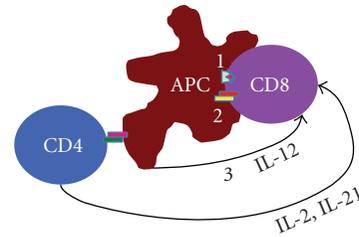


FIGURE 2: Successful activation of CD8 T cells requires three signals by APCs and CD4 T cell help. CD8 T cells engage cognate peptide-MHC complexes presented on APCs with their TCR (1). The APC also provides costimulation to the T cell via expression of CD80/CD86 molecules, which bind to CD28 on the T cell (2). Finally, the APC produces IL-12 after activation with inflammatory mediators or in response to CD40L interactions with CD4 T cells, and IL-12 provides signal 3 to the responding CD8 T cell. (3) CD4 T cells also produce cytokines that provide growth and differentiation signals to responding CD8 T cells.

and this defect mirrors what is observed if IL-2 signals are not available during priming [122, 123]. Accordingly, these findings support a model by which IL-2 production by CD4 T cells legitimizes the response and allows the CD8 T cells to become full-fledged memory cells.

IL-21 is a 15 kDa member of the common  $\gamma$ -chain cytokine family and is most closely related to IL-2, which is encoded directly upstream of IL-21 on chromosomes 3 and 4, in mice and humans, respectively [134]. IL-21 is primarily produced by CD4 T cells and has been implicated in helping CD8 T cells [135, 136]. IL-21 binds to a heterodimeric receptor comprised of the common- $\gamma$  chain (CD132) and the unique IL-21 receptor, encoded on chromosome 7 and 16, in mice and humans, respectively [137]. The IL-21 receptor is widely distributed and is expressed by B cells, NK cells, dendritic cells, macrophages, keratinocytes, and CD4+ and CD8+ T cells [134, 137]. Overexpression of IL-21 in mice results in expansion of CD8 T cells with a memory phenotype at the expense of naïve CD8 T cells [138]. In vitro studies of T cell priming have shown that IL-21 can function as a “third” signal, acting in concert with TCR ligation and costimulation, to drive proliferation of naïve CD8 T cells and the development of cytolytic activity [139]. These studies both suggest a role for IL-21 in expanding responding CD8 T cell populations.

In the absence of CD4 T cell help, defects in the CD8 T cell responses manifest following acute infections; however, these impairments are even more severe during persistent infections. Several groups have recently demonstrated a critical role for IL-21 in helping CD8 T cell responses during chronic infections [140–143]. Both IL-21 and IL-21 receptor-deficient mice develop a chronic infection following inoculation with certain strains of LCMV, an outcome identical to that observed in CD4 deficient mice [140–142]. Using a series of mixed bone marrow chimeras, IL-21 was shown to act directly on CD8 T cells to support these responses during chronic infections. CD8 T cells that could not receive IL-21 signals exhibited exacerbated functional exhaustion and were rapidly deleted when in direct competition with IL-21

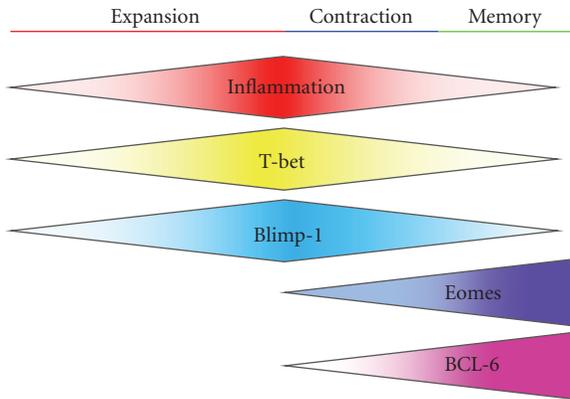


FIGURE 3: Expression of transcription factors in CD8 T cells is positively or negatively correlated with an inflammatory environment. Prototypic CD8 T cell responses that are induced following acute infection undergo three distinct phases: expansion, contraction, and the establishment of memory. Each of these phases is associated with shifts in the patterns of transcription factors expressed by the responding cells. Expression of T-bet and Blimp-1 correlates positively with inflammatory conditions, and their expression is reduced as the infection becomes resolved, and the memory pool develops. In contrast, Eomesodermin and Bcl-6 begin to be expressed as the infection is brought under control and exhibit highest expression in memory cells.

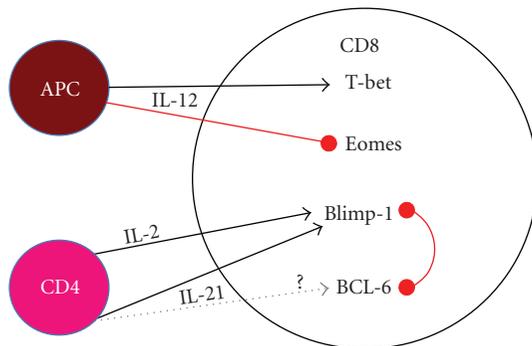


FIGURE 4: Regulation of transcription factors that impart CD8 T cell fate and function by external signaling. Black arrows indicate induction; red dots represent suppression. Blimp-1 and Bcl-6 are mutually repressive in CD8 T cells. IL-21 induces both Blimp-1 and Bcl-6 in B cells and is capable of inducing Blimp-1 in CD8 T cells. Induction of Bcl-6 in response to IL-21 in CD8 T cells has yet to be evaluated, as indicated by the dashed line with the question mark.

competent CD8 T cells in the chimeras [140–142]. Yi et al. also made the intriguing observation that IL-21 treatment of chronically infected CD4 deficient mice can enhance the virus-specific CD8 T cell response and reduce the viral load [142], indicating that the levels of IL-21 may dictate both the quality of the ensuing CD8 T cell response and the outcome of the infection. Since CD4 T cells are a principle source of IL-21, it is likely that this cytokine functions as a helper factor that is vital for supporting CD8 T cell responses in chronically infected hosts.

## 5. Transcriptional Regulators in Effector, Memory, and Exhausted CD8 T Cells

The transcriptional program in effector, memory, and exhausted CD8 T cells is both unique and overlapping. Although the principal determinant of T cell differentiation is the strength and duration of the antigenic signals that the cells receive, a variety of other factors, including APC interactions, availability of costimulation, the inflammatory environment, and the presence of CD4 T cell help, act together to set the transcriptional landscape at each phase of the immune response. As outlined below, changes in the levels of several factors, such as T-bet, Eomesodermin (Eomes), Blimp-1, and Bcl-6, alter the transcriptional program of the responding cell, which in turn imparts both the functional capacity and fate of the pathogen-specific CD8 T cell.

T-box transcription factor family members T-bet and Eomes enforce the transcriptional program of effector and memory CD8 T cells. These transcription factors are related and, in the CD8 T cell lineage, can partially compensate for each other [144, 145]. T-bet deficiency in mice reduces but does not completely ablate IFN $\gamma$  production and cytolytic activity in CD8 T cells [146], and T-bet deficient mice are still able to control LCMV-Armstrong [147] and *Listeria monocytogenes* [148] infections. Deletion of both T-bet and Eomes in the CD8 T cell lineage eliminates IFN $\gamma$  production by the CD8 T cells and also dramatically shifts the function of the cells to IL-17 production resulting in massive inflammation and death after infection [147]. Thus, T-bet and Eomes operate to define the specific functional capabilities of CD8 T cells. The roles of T-bet and Eomes in dictating the “CD8” effector functions are further evidenced by the observation that both of these transcription factors are able to impart the capacity to produce IFN $\gamma$  and granzyme B in Th2 cells [149].

T-bet is induced by both IFN $\gamma$  and IL-12 signaling [85, 150, 151] and is upregulated in response to inflammatory signals (Figures 3 and 4). These signals, in conjunction with the levels of T-bet expression, influence the generation of effector CD8 T cells following infection. T-bet is expressed at higher levels in effector T cells than in cells with a “memory precursor” or memory phenotype (CD127<sup>hi</sup>KLRG-1<sup>lo</sup>) [83, 110]. In contrast, Eomes is induced later during the immune response and is expressed at a higher level in memory CD8 T cells compared to effector cells [152, 153] (Figure 3). In the absence of T-bet, reduced numbers of pathogen-specific CD8 T cells are detected in the blood, spleen, and tissues following infection with LCMV-Armstrong [110], *Listeria monocytogenes* [148], or *Trypanosoma cruzi* [154]. This reflects a defect in the generation of effector populations of CD8 T cells, distinguished by the expression of high levels of KLRG-1 and low levels of CD127 on their cell surface [83, 110]. The pathogen-specific cells in T-bet deficient mice instead adopt a “central memory” phenotype (CD127<sup>hi</sup>KLRG-1<sup>lo</sup>CD62L<sup>hi</sup>) [110, 148, 154] and elevated numbers of these CD8 T cell are present in the lymph nodes [110]. Collectively, these observations implicate T-bet as a factor necessary for the generation of terminally differentiated effector cells, while

lower T-bet expression is permissive for memory CD8 T cell generation.

In contrast to the role of T-bet in effector CD8 T cell development, Eomes is associated with memory CD8 T cell generation. T-bet deficient mice fail to generate CD127<sup>lo</sup>KLRG-1<sup>hi</sup> effector cells, but still develop protective memory cells capable of producing IFN $\gamma$ , and possess cytotoxic activity by virtue of Eomes expression [144, 145, 147, 149, 152]. In vitro, IL-12 has been shown to reduce Eomes levels [153] (Figure 4), supporting a model in which inflammation induces effector CD8 T cell generation, and resolution of the infection results in environmental signals which favor Eomes upregulation and the emergence of memory T cells (Figure 3).

In addition to regulation of T-bet and Eomes, IL-12 has recently been shown to enhance activation of the mammalian target of rapamycin (mTOR) after T cell activation. Inhibition of mTOR by the drug rapamycin mimics cell starvation and results in cell cycle arrest and, at high doses, rapamycin treatment is used to prevent immune-mediated rejection of solid organ transplants. In light of its immunosuppressive qualities, it is surprising that low doses of rapamycin have been shown to improve generation of memory phenotype CD8 T cells following infection or immunization [155, 156]. Inhibition of mTOR during either the priming or contraction phase of the immune response enhanced the number and fraction of responding T cells which exhibited a memory type phenotype (CD127<sup>hi</sup>KLRG-1<sup>lo</sup>CD62L<sup>hi</sup>Bcl-2<sup>hi</sup>) [155]. In vitro, treatment with rapamycin abrogated IL-12-induced differentiation of naïve CD8 T cells, diminishing both IFN $\gamma$  production and T-bet expression. Instead, these cells exhibited higher Eomes expression and were better able to persist in vivo, expand upon a secondary challenge, and protect against a tumor challenge, demonstrating their enhanced memory potential [157]. Additionally, the drug metformin has been shown to influence memory CD8 T cell differentiation in a similar manner to rapamycin treatment [156]. Metformin activates AMPK, an inhibitor of mTOR, and may therefore be acting in a similar manner to rapamycin treatment. In addition to the recently described role in the regulation of T-bet and Eomes expression, mTOR and AMPK may also function as a metabolic switch to revert the anabolic metabolism of an effector cell (largely driven by glycolysis) to the catabolic metabolism characteristic of naïve and resting memory cells (oxidative phosphorylation) [158]. These metabolic shifts may act on multiple pathways in the T cell to enforce the memory phenotype.

Blimp-1 is a transcription factor that promotes differentiation in the CD8 T cell lineage as well as other cell types [159]. Blimp-1 was first identified in the B cell lineage, where it drives the terminal differentiation of germinal center B cells into plasma cells [160, 161]. Blimp-1 is expressed in antigen experienced CD8 T cells, with higher expression in effector cells than memory T cells [162]. Deletion of Blimp-1 in mice leads to lymphoproliferative dysregulation, resulting in death due to autoimmunity [162, 163], indicating a role for Blimp-1 in restricting T cell responses. A system in which

Blimp-1 is deleted only from responding T cells by virtue of granzyme B driven Cre expression has allowed the role of Blimp-1 in T cell development and function to be elucidated. Deletion of Blimp-1 in CD8 T cells responding to acute LCMV-Armstrong [164] or influenza [165] infection led to greater generation of virus-specific CD8 T cells. Although the virus-specific response was larger with Blimp-1 deficiency, these cells were less effective at controlling infection and had lower levels of effector molecules such as granzyme B. These Blimp-1 deficient T cells could not fully differentiate into effector cells and instead formed an “altered” memory pool of poorly functional cells, which were defective in their capacity to proliferate in a secondary response [164, 165]. Shin et al. have demonstrated that during a chronic infection Blimp-1 expression is highly upregulated, correlating with its high expression in effector cells. In a chronic infection, CD8 T cells that lack Blimp-1 display a memory cell phenotype and are partially protected from deletion despite high viral loads in the conditional Blimp-1 knockout mice [166]. Intriguingly, Blimp-1 haploinsufficiency allowed accelerated control of chronic LCMV clone-13 infection, indicating that the level of Blimp-1 influences the functional capacity of the T cells; without it T cells cannot execute effector functions, but if too highly expressed then the cells succumb to exhaustion and are deleted. Thus the levels of Blimp-1 must be tightly regulated to ensure a successful immune response (Figure 3).

There are multiple repressors of Blimp-1 in the B cell lineage; however, only one of these repressors is known to be expressed in T cells, and that is B cell CLL/lymphoma 6 (Bcl-6) (Figure 4). Bcl-6 is involved in driving the homeostatic proliferation of CD8 T cells and the expansion and maintenance of memory cells. Ordinarily, Bcl-6 is expressed highly in central memory T cells, is moderately expressed in effector and effector memory cells, and is not expressed by naïve T cells [167] (Figure 3). Mice deficient in Bcl-6 develop smaller responses to immunization compared to wild type. By contrast, overexpression of Bcl-6 enhanced the size of the antigen specific T cell response [167, 168], which were primarily polarized to a central memory phenotype and became distributed in the secondary lymphoid organs. So it is plausible that Bcl-6 supports the differentiation of central memory CD8 T cells. Bcl-6 also downregulates Blimp-1 expression (Figure 4); so the enhanced numbers of central memory cells generated in Bcl-6 transgenic mice may be a result of lowered Blimp-1 expression. However, unlike Blimp-1 deficient T cells, effector cells are still generated in normal numbers in Bcl-6 transgenic mice. Thus both Bcl-6 and Blimp-1 can be expressed in responding T cells, and their relative levels may dictate the function and fate of the T cell.

## 6. Future Directions

Many advances have been made in our understanding of CD8 T cell differentiation and how these responses contribute to infection control; nevertheless, more progress

is needed. Although early activation events and the APC-T cell interaction are an essential first step in priming CD8 T cell responses, how exactly these events fully shape the development of remarkably heterogeneous responses remains unclear. Elucidating whether or not polarization of the responding CD8 T cell and potential asymmetric division of the activated naïve CD8 T cell truly dictates the subsequent differentiation of the daughter cells is clearly of interest [25, 169]. Although development of CD8 T cell exhaustion is increasingly well described, we still do not fully understand all of the factors that drive these inferior responses, and importantly, we need to determine better strategies for reversing or circumventing the exhausted state. This is necessary if curative immune-based approaches are to be developed for persistent infections, and it will also be essential to devise methods that promote control of these infections while avoiding widespread immunopathology. Our understanding of the requirements for CD4 T cell help for CD8 T cell responses has improved in recent years and the importance of IL-21 in this process has been highlighted. Additional studies will be required to better understand exactly how IL-21 functions to promote CD8 T cell responses and what the key differences are between CD8 T cells which do and do not receive IL-21 signals.

Given the pivotal importance of transcriptional regulators in dictating CD8 T cell fate, a better understanding of the signals that induce expression of these factors, their exact function, and how these factors integrate their transcriptional program is warranted. T-bet and Blimp-1 appear to operate in a “rheostat” mechanism, where little to no expression depreciates effector capabilities, while too high expression results in terminally differentiated cells and a loss of proliferative capacity. We will need to know not only the appropriate levels of these transcription factors that elicit long lived central and effector memory populations, but also what signals induce expression of these molecules, and whether they can control the expression of one another. Expression of the transcription factors Eomes and Bcl-6 are also associated with memory T cell generation; however, little is known about how these molecules are induced or regulated in the T cell lineage. Thus, it will be interesting to discover what nonoverlapping functions these two transcription factors have with T-bet and Blimp-1, which allow the high proliferative potential and maintenance of effector functions in memory CD8 T cells. Given the multitude of gaps in our understanding of CD8 T cell biology it is likely that this area of research will continue to be exciting and surprising.

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