

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

Received 26 November 2009; Accepted 22 January 2010

Academic Editor: Zhengguo Xiao

Copyright © 2010 M. A. Cox and A. J. Zajac. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

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].

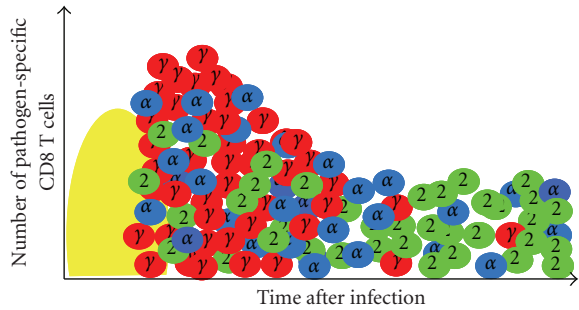
Although CD8 T cells can be highly effective at controlling acute infections and contribute to protective secondary responses, protracted and chronic infections do arise and are often associated with the development of phenotypically and functionally inferior responses [14, 27–46]. These types of infections 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 α production and IFN γ 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.

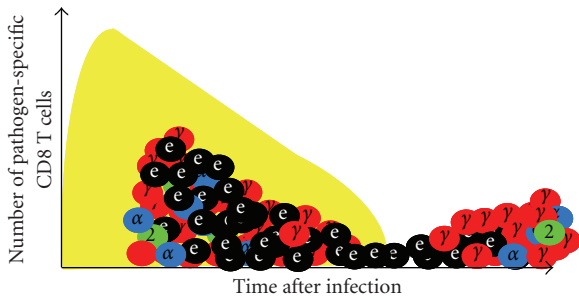
Despite the functional ineptness of exhausted CD8 T cells, they express patterns of surface receptors which are commonly associated with activated effector T cells. They express high levels of CD69 and CD43 (1B11) but low levels of CD62L and CD127 [29, 30, 42, 45, 48, 50]. Exhausted CD8 T cells also 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

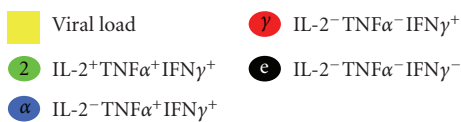
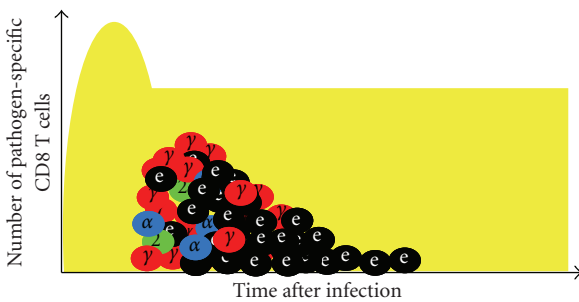
Naïve CD8 T cells become activated when they encounter professional antigen-presenting cells (APC) displaying cognate peptide antigen complexed with an appropriate MHC class I molecule. The ability of CD8 T cells to survey these MHC-peptide complexes allows these cells to fulfill their immunological 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].



(a) Rapidly controlled pathogen (acute)



(b) Slowly controlled pathogen (protracted)



(c) Pathogen never controlled (chronic)

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 γ , TNF α , and IL-2 (green circles) or IFN γ and TNF α (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 γ (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 γ . These two cytokines act in a positive feedback loop in which IFN γ activates the APC to produce IL-12, and then IL-12 induces more IFN γ production by the CD8 T cell [85, 86]. In addition to promoting IFN γ production, IL-12 and IFN α 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 γ , TNF α , 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 γ 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 γ 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],

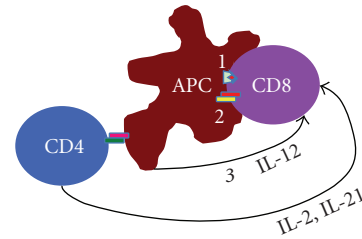


FIGURE 2: Successful activation of CD8 T cells requires three signals by APCs and CD4 T cell help. CD8 T cells engage cognate peptide-MHC complexes presented on APCs with their TCR (1). The APC also provides costimulation to the T cell via expression of CD80/CD86 molecules, which bind to CD28 on the 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 γ -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- γ 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

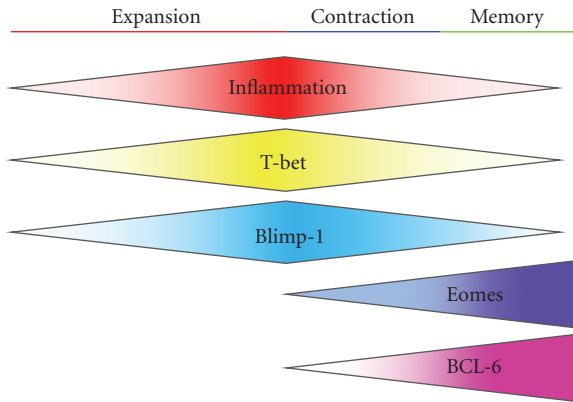


FIGURE 3: Expression of transcription factors in CD8 T cells is positively or negatively correlated with an inflammatory environment. Prototypic CD8 T cell responses that are induced following acute infection undergo three distinct phases: expansion, contraction, and the establishment of memory. Each 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.

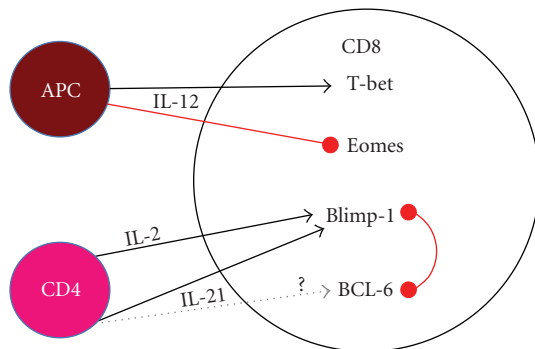


FIGURE 4: Regulation of transcription factors that impart CD8 T cell fate and function by external signaling. Black arrows indicate induction; red dots represent suppression. Blimp-1 and Bcl-6 are mutually repressive in CD8 T cells. IL-21 induces both Blimp-1 and Bcl-6 in B cells and is capable of inducing 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 γ 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 γ 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 γ and granzyme B in Th2 cells [149].

T-bet is induced by both IFN γ 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^{hi}KLRG-1^{lo}) [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^{hi}KLRG-1^{lo}CD62L^{hi}) [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^{lo}KLRG-1^{hi} effector cells, but still develop protective memory cells capable of producing IFN γ , 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^{hi}KLRG-1^{lo}CD62L^{hi}Bcl-2^{hi}) [155]. In vitro, treatment with rapamycin abrogated IL-12-induced differentiation of naïve CD8 T cells, diminishing both IFN γ 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.

Acknowledgments

The authors wish to thank all of the members of the Zajac laboratory and also Laurie Harrington, Marla Hertz, Emily Hahn, and David Gaston for critical help and advice. This work was supported in part by Grants R01 AI049360, R01 AI067933, and U01 AI082966 from the National Institutes of Health.

References

- [1] F. Stoll-Keller, H. Barth, S. Fafi-Kremer, M. B. Zeisel, and T. F. Baumert, “Development of hepatitis C virus vaccines: challenges and progress,” *Expert Review of Vaccines*, vol. 8, no. 3, pp. 333–345, 2009.
- [2] F. Castellino, G. Galli, G. Del Giudice, and R. Rappuoli, “Generating memory with vaccination,” *European Journal of Immunology*, vol. 39, no. 8, pp. 2100–2105, 2009.
- [3] L. E. Brown and A. Kelso, “Prospects for an influenza vaccine that induces cross-protective cytotoxic T lymphocytes,” *Immunology and Cell Biology*, vol. 87, no. 4, pp. 300–308, 2009.
- [4] V. Appay, D. C. Douek, and D. A. Price, “CD8⁺ T cell efficacy in vaccination and disease,” *Nature Medicine*, vol. 14, no. 6, pp. 623–628, 2008.
- [5] S. M. Kaech, E. J. Wherry, and R. Ahmed, “Effector and memory T-cell differentiation: implications for vaccine development,” *Nature Reviews Immunology*, vol. 2, no. 4, pp. 251–262, 2002.
- [6] M. A. Williams and M. J. Bevan, “Effector and memory CTL differentiation,” *Annual Review of Immunology*, vol. 25, pp. 171–192, 2007.
- [7] P. C. Doherty and J. P. Christensen, “Accessing complexity: the dynamics of virus-specific T cell responses,” *Annual Review of Immunology*, vol. 18, pp. 561–592, 2000.
- [8] R. A. Seder and R. Ahmed, “Similarities and differences in CD4⁺ and CD8⁺ effector and memory T cell generation,” *Nature Immunology*, vol. 4, no. 9, pp. 835–842, 2003.
- [9] E. J. Wherry and R. Ahmed, “Memory CD8 T-cell differentiation during viral infection,” *Journal of Virology*, vol. 78, no. 11, pp. 5535–5545, 2004.
- [10] E. A. Butz and M. J. Bevan, “Massive expansion of antigen-specific CD8⁺ T cells during an acute virus infection,” *Immunity*, vol. 8, no. 2, pp. 167–175, 1998.
- [11] M. F. C. Callan, L. Tan, N. Annels, et al., “Direct visualization of antigen-specific CD8⁺ T cells during the primary immune response to Epstein-Barr virus in vivo,” *Journal of Experimental Medicine*, vol. 187, no. 9, pp. 1395–1402, 1998.
- [12] K. J. Flynn, G. T. Belz, J. D. Altman, R. Ahmed, D. L. Woodland, and P. C. Doherty, “Virus-specific CD8⁺ T cells in primary and secondary influenza pneumonia,” *Immunity*, vol. 8, no. 6, pp. 683–691, 1998.
- [13] K. Murali-Krishna, J. D. Altman, M. Suresh, et al., “Counting antigen-specific CD8 T cells: a reevaluation of bystander activation during viral infection,” *Immunity*, vol. 8, no. 2, pp. 177–187, 1998.
- [14] G. Pantaleo, J. F. Demarest, H. Soudeyns, et al., “Major expansion of CD8⁺ T cells with a predominant V β usage during the primary immune response to HIV,” *Nature*, vol. 370, no. 6489, pp. 463–467, 1994.
- [15] J. D. K. Wilson, G. S. Ogg, R. L. Allen, et al., “Oligoclonal expansions of CD8⁺ T cells in chronic HIV infection are antigen specific,” *Journal of Experimental Medicine*, vol. 188, no. 4, pp. 785–790, 1998.
- [16] S. Hou, L. Hyland, K. W. Ryan, A. Portner, and P. C. Doherty, “Virus-specific CD8⁺ T-cell memory determined by clonal burst size,” *Nature*, vol. 369, no. 6482, pp. 652–654, 1994.
- [17] J. M. Grayson, A. J. Zajac, J. D. Altman, and R. Ahmed, “Cutting edge: increased expression of Bcl-2 in antigen-specific memory CD8⁺ T cells,” *Journal of Immunology*, vol. 164, no. 8, pp. 3950–3954, 2000.

- [18] L. L. Lau, B. D. Jamieson, T. Somasundaram, and R. Ahmed, "Cytotoxic T-cell memory without antigen," *Nature*, vol. 369, no. 6482, pp. 648–652, 1994.
- [19] V. P. Badovinac, B. B. Porter, and J. T. Harty, "Programmed contraction of CD8⁺ T cells after infection," *Nature Immunology*, vol. 3, no. 7, pp. 619–626, 2002.
- [20] S. M. Kaech, J. T. Tan, E. J. Wherry, B. T. Konieczny, C. D. Surh, and R. Ahmed, "Selective expression of the interleukin 7 receptor identifies effector CD8 T cells that give rise to long-lived memory cells," *Nature Immunology*, vol. 4, no. 12, pp. 1191–1198, 2003.
- [21] A. J. Zajac, J. M. Dye, and D. G. Quinn, "Control of lymphocytic choriomeningitis virus infection in granzyme B deficient mice," *Virology*, vol. 305, no. 1, pp. 1–9, 2003.
- [22] D. Masopust, V. Vezys, A. L. Marzo, and L. Lefrançois, "Preferential localization of effector memory cells in nonlymphoid tissue," *Science*, vol. 291, no. 5512, pp. 2413–2417, 2001.
- [23] F. Sallusto, D. Lenig, R. Förster, M. Lipp, and A. Lanzavecchia, "Two subsets of memory T lymphocytes with distinct homing potentials and effector functions," *Nature*, vol. 401, no. 6754, pp. 708–712, 1999.
- [24] A. Lanzavecchia and F. Sallusto, "Understanding the generation and function of memory T cell subsets," *Current Opinion in Immunology*, vol. 17, no. 3, pp. 326–332, 2005.
- [25] R. Ahmed, M. J. Bevan, S. L. Reiner, and D. T. Fearon, "The precursors of memory: models and controversies," *Nature Reviews Immunology*, vol. 9, no. 9, pp. 662–668, 2009.
- [26] J. K. Northrop and H. Shen, "CD8⁺ T-cell memory: only the good ones last," *Current Opinion in Immunology*, vol. 16, no. 4, pp. 451–455, 2004.
- [27] C. Carini, M. F. McLane, K. H. Mayer, and M. Essex, "Dysregulation of interleukin-7 receptor may generate loss of cytotoxic T cell response in human immunodeficiency virus type 1 infection," *European Journal of Immunology*, vol. 24, no. 12, pp. 2927–2934, 1994.
- [28] H. Shin and E. J. Wherry, "CD8 T cell dysfunction during chronic viral infection," *Current Opinion in Immunology*, vol. 19, no. 4, pp. 408–415, 2007.
- [29] M. J. Fuller, A. Khanolkar, A. E. Tebo, and A. J. Zajac, "Maintenance, loss, and resurgence of T cell responses during acute, protracted, and chronic viral infections," *Journal of Immunology*, vol. 172, no. 7, pp. 4204–4214, 2004.
- [30] M. J. Fuller, D. A. Hildeman, S. Sabbaj, et al., "Cutting edge: emergence of CD127^{high} functionally competent memory T cells is compromised by high viral loads and inadequate T cell help," *Journal of Immunology*, vol. 174, no. 10, pp. 5926–5930, 2005.
- [31] A. Oxenius, R. M. Zinkernagel, and H. Hengartner, "Comparison of activation versus induction of unresponsiveness of virus-specific CD4⁺ and CD8⁺ T cells upon acute versus persistent viral infection," *Immunity*, vol. 9, no. 4, pp. 449–457, 1998.
- [32] V. Appay, D. F. Nixon, S. M. Donahoe, et al., "HIV-specific CD8⁺ T cells produce antiviral cytokines but are impaired in cytolytic function," *Journal of Experimental Medicine*, vol. 192, no. 1, pp. 63–75, 2000.
- [33] F. Lechner, D. K. H. Wong, P. R. Dunbar, et al., "Analysis of successful immune responses in persons infected with hepatitis C virus," *Journal of Experimental Medicine*, vol. 191, no. 9, pp. 1499–1512, 2000.
- [34] P. A. Goepfert, A. Bansal, B. H. Edwards, et al., "A significant number of human immunodeficiency virus epitope-specific cytotoxic T lymphocytes detected by tetramer binding do not produce gamma interferon," *Journal of Virology*, vol. 74, no. 21, pp. 10249–10255, 2000.
- [35] P. Champagne, G. S. Ogg, A. S. King, et al., "Skewed maturation of memory HIV-specific CD8 T lymphocytes," *Nature*, vol. 410, no. 6824, pp. 106–111, 2001.
- [36] N. H. Gruener, F. Lechner, M.-C. Jung, et al., "Sustained dysfunction of antiviral CD8⁺ T lymphocytes after infection with hepatitis C virus," *Journal of Virology*, vol. 75, no. 12, pp. 5550–5558, 2001.
- [37] M. P. Davenport, C. Fazou, A. J. McMichael, and M. F. C. Callan, "Clonal selection, clonal senescence, and clonal succession: the evolution of the T cell response to infection with a persistent virus," *Journal of Immunology*, vol. 168, no. 7, pp. 3309–3317, 2002.
- [38] V. Appay, P. R. Dunbar, M. Callan, et al., "Memory CD8⁺ T cells vary in differentiation phenotype in different persistent virus infections," *Nature Medicine*, vol. 8, no. 4, pp. 379–385, 2002.
- [39] M. J. Fuller and A. J. Zajac, "Ablation of CD8 and CD4 T cell responses by high viral loads," *Journal of Immunology*, vol. 170, no. 1, pp. 477–486, 2003.
- [40] L. G. Tussey, U. S. Nair, M. Bachinsky, et al., "Antigen burden is a major determinant of human immunodeficiency virus-specific CD8⁺ T cell maturation state: potential implications for therapeutic immunization," *Journal of Infectious Diseases*, vol. 187, no. 3, pp. 364–374, 2003.
- [41] E. J. Wherry, J. N. Blattman, K. Murali-Krishna, R. van der Most, and R. Ahmed, "Viral persistence alters CD8 T-cell immunodominance and tissue distribution and results in distinct stages of functional impairment," *Journal of Virology*, vol. 77, no. 8, pp. 4911–4927, 2003.
- [42] E. J. Wherry, D. L. Barber, S. M. Kaech, J. N. Blattman, and R. Ahmed, "Antigen-independent memory CD8 T cells do not develop during chronic viral infection," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 101, no. 45, pp. 16004–16009, 2004.
- [43] D. L. Barber, E. J. Wherry, D. Masopust, et al., "Restoring function in exhausted CD8 T cells during chronic viral infection," *Nature*, vol. 439, no. 7077, pp. 682–687, 2006.
- [44] E. J. Wherry, J. N. Blattman, and R. Ahmed, "Low CD8 T-cell proliferative potential and high viral load limit the effectiveness of therapeutic vaccination," *Journal of Virology*, vol. 79, no. 14, pp. 8960–8968, 2005.
- [45] S. Zhou, R. Ou, L. Huang, G. E. Price, and D. Moskophidis, "Differential tissue-specific regulation of antiviral CD8⁺ T-cell immune responses during chronic viral infection," *Journal of Virology*, vol. 78, no. 7, pp. 3578–3600, 2004.
- [46] E. S. Rosenberg, M. Altfeld, S. H. Poon, et al., "Immune control of HIV-1 after early treatment of acute infection," *Nature*, vol. 407, no. 6803, pp. 523–526, 2000.
- [47] D. Moskophidis, F. Lechner, H. Pircher, and R. M. Zinkernagel, "Virus persistence in acutely infected immunocompetent mice by exhaustion of antiviral cytotoxic effector T cells," *Nature*, vol. 362, no. 6422, pp. 758–761, 1993.
- [48] A. J. Zajac, J. N. Blattman, K. Murali-Krishna, et al., "Viral immune evasion due to persistence of activated T cells without effector function," *Journal of Experimental Medicine*, vol. 188, no. 12, pp. 2205–2213, 1998.
- [49] P. Agnellini, P. Wolint, M. Rehr, J. Cahenzli, U. Karrer, and A. Oxenius, "Impaired NFAT nuclear translocation results in split exhaustion of virus-specific CD8⁺ T cell functions

- during chronic viral infection,” *Proceedings of the National Academy of Sciences of the United States of America*, vol. 104, no. 11, pp. 4565–4570, 2007.
- [50] E. J. Wherry, S.-J. Ha, S. M. Kaech, et al., “Molecular signature of CD8⁺ T cell exhaustion during chronic viral infection,” *Immunity*, vol. 27, no. 4, pp. 670–684, 2007.
- [51] C. L. Day, D. E. Kaufmann, P. Kiepiela, et al., “PD-1 expression on HIV-specific T cells is associated with T-cell exhaustion and disease progression,” *Nature*, vol. 443, no. 7109, pp. 350–354, 2006.
- [52] S. Urbani, B. Amadei, D. Tola, et al., “PD-1 expression in acute hepatitis C virus (HCV) infection is associated with HCV-specific CD8 exhaustion,” *Journal of Virology*, vol. 80, no. 22, pp. 11398–11403, 2006.
- [53] V. Velu, K. Titanji, B. Zhu, et al., “Enhancing SIV-specific immunity in vivo by PD-1 blockade,” *Nature*, vol. 458, no. 7235, pp. 206–210, 2009.
- [54] S. D. Blackburn, H. Shin, W. N. Haining, et al., “Coregulation of CD8⁺ T cell exhaustion by multiple inhibitory receptors during chronic viral infection,” *Nature Immunology*, vol. 10, no. 1, pp. 29–37, 2009.
- [55] A. Gallimore, A. Glithero, A. Godkin, et al., “Induction and exhaustion of lymphocytic choriomeningitis virus-specific cytotoxic T lymphocytes visualized using soluble tetrameric major histocompatibility complex class I-peptide complexes,” *Journal of Experimental Medicine*, vol. 187, no. 9, pp. 1383–1393, 1998.
- [56] J. M. Moser, J. D. Altman, and A. E. Lukacher, “Antiviral CD8⁺ T cell responses in neonatal mice: susceptibility to polyoma virus-induced tumors is associated with lack of cytotoxic function by viral antigen-specific T cells,” *Journal of Experimental Medicine*, vol. 193, no. 5, pp. 595–606, 2001.
- [57] P. Krebs, E. Scandella, B. Odermatt, and B. Ludewig, “Rapid functional exhaustion and deletion of CTL following immunization with recombinant adenovirus,” *Journal of Immunology*, vol. 174, no. 8, pp. 4559–4566, 2005.
- [58] U. Dittmer, H. He, R. J. Messer, et al., “Functional impairment of CD8⁺ T cells by regulatory T cells during persistent retroviral infection,” *Immunity*, vol. 20, no. 3, pp. 293–303, 2004.
- [59] S. R. Nair, G. Zelinskyy, S. Schimmer, N. Gerlach, G. Kassiotis, and U. Dittmer, “Mechanisms of control of acute Friend virus infection by CD4⁺ helper T cells and their functional impairment by regulatory T cells,” *Journal of General Virology*, vol. 9, pp. 440–451, 2010.
- [60] G. Zelinskyy, S. J. Robertson, S. Schimmer, R. J. Messer, K. J. Hasenkrug, and U. Dittmer, “CD8⁺ T-cell dysfunction due to cytolytic granule deficiency in persistent friend retrovirus infection,” *Journal of Virology*, vol. 79, no. 16, pp. 10619–10626, 2005.
- [61] C. C. Bergmann, J. D. Altman, D. Hinton, and S. A. Stohlman, “Inverted immunodominance and impaired cytolytic function of CD8⁺ T cells during viral persistence in the central nervous system,” *Journal of Immunology*, vol. 163, no. 6, pp. 3379–3387, 1999.
- [62] H. Kloverpris, I. Karlsson, J. Bonde, et al., “Induction of novel CD8⁺ T-cell responses during chronic untreated HIV-1 infection by immunization with subdominant cytotoxic T-lymphocyte epitopes,” *AIDS*, vol. 23, no. 11, pp. 1329–1340, 2009.
- [63] K. Ladell, M. K. Hellerstein, D. Cesar, R. Busch, D. Boban, and J. M. McCune, “Central memory CD8⁺ T cells appear to have a shorter lifespan and reduced abundance as a function of HIV disease progression,” *Journal of Immunology*, vol. 180, no. 12, pp. 7907–7918, 2008.
- [64] S. A. Migueles, K. A. Weeks, E. Nou, et al., “Defective human immunodeficiency virus-specific CD8⁺ T-cell poly-functionality, proliferation, and cytotoxicity are not restored by antiretroviral therapy,” *Journal of Virology*, vol. 83, no. 22, pp. 11876–11889, 2009.
- [65] S. Reignat, G. J. M. Webster, D. Brown, et al., “Escaping high viral load exhaustion: CD8 cells with altered tetramer binding in chronic hepatitis B virus infection,” *Journal of Experimental Medicine*, vol. 195, no. 9, pp. 1089–1101, 2002.
- [66] S. Urbani, C. Boni, G. Missale, et al., “Virus-specific CD8⁺ lymphocytes share the same effector-memory phenotype but exhibit functional differences in acute hepatitis B and C,” *Journal of Virology*, vol. 76, no. 24, pp. 12423–12434, 2002.
- [67] H. Radziewicz, C. C. Ibegbu, M. L. Fernandez, et al., “Liver-infiltrating lymphocytes in chronic human hepatitis C virus infection display an exhausted phenotype with high levels of PD-1 and low levels of CD127 expression,” *Journal of Virology*, vol. 81, no. 6, pp. 2545–2553, 2007.
- [68] L. Klein, L. Trautman, S. Psarras, et al., “Visualizing the course of antigen-specific CD8 and CD4 T cell responses to a growing tumor,” *European Journal of Immunology*, vol. 33, no. 3, pp. 806–814, 2003.
- [69] S. Mumprecht, C. Schurch, J. Schwaller, M. Solenthaler, and A. F. Ochsenbein, “Programmed death 1 signaling on chronic myeloid leukemia-specific T cells results in T-cell exhaustion and disease progression,” *Blood*, vol. 114, no. 8, pp. 1528–1536, 2009.
- [70] T. Kozako, M. Yoshimitsu, H. Fujiwara, et al., “PD-1/PD-L1 expression in human T-cell leukemia virus type 1 carriers and adult T-cell leukemia/lymphoma patients,” *Leukemia*, vol. 23, no. 2, pp. 375–382, 2009.
- [71] P. P. Lee, C. Yee, P. A. Savage, et al., “Characterization of circulating T cells specific for tumor-associated antigens in melanoma patients,” *Nature Medicine*, vol. 5, no. 6, pp. 677–685, 1999.
- [72] S. K. Bromley, W. R. Burack, K. G. Johnson, et al., “The immunological synapse,” *Annual Review of Immunology*, vol. 19, pp. 375–396, 2001.
- [73] M. L. Dustin, “Stop and go traffic to tune T cell responses,” *Immunity*, vol. 21, no. 3, pp. 305–314, 2004.
- [74] P. A. van der Merwe, S. J. Davis, A. S. Shaw, and M. L. Dustin, “Cytoskeletal polarization and redistribution of cell-surface molecules during T cell antigen recognition,” *Seminars in Immunology*, vol. 12, no. 1, pp. 5–21, 2000.
- [75] M. F. Bachmann, K. McKall-Faienza, R. Schmits, et al., “Distinct roles for LFA-1 and CD28 during activation of naive T cells: adhesion versus costimulation,” *Immunity*, vol. 7, no. 4, pp. 549–557, 1997.
- [76] A. Scholer, S. Hugues, A. Boissonnas, L. Fetler, and S. Amigorena, “Intercellular adhesion molecule-1-dependent stable interactions between T cells and dendritic cells determine CD8⁺ T cell memory,” *Immunity*, vol. 28, no. 2, pp. 258–270, 2008.
- [77] P. Agarwal, A. Raghavan, S. L. Nandiwada, et al., “Gene regulation and chromatin remodeling by IL-12 and type I IFN in programming for CD8 T cell effector function and memory,” *Journal of Immunology*, vol. 183, no. 3, pp. 1695–1704, 2009.
- [78] W. Cui, N. S. Joshi, A. Jiang, and S. M. Kaech, “Effects of Signal 3 during CD8 T cell priming: bystander production of IL-12 enhances effector T cell expansion but promotes

- terminal differentiation," *Vaccine*, vol. 27, no. 15, pp. 2177–2187, 2009.
- [79] C. S. Schmidt and M. F. Mescher, "Peptide antigen priming of naive, but not memory, CD8 T cells requires a third signal that can be provided by IL-12," *Journal of Immunology*, vol. 168, no. 11, pp. 5521–5529, 2002.
- [80] J. M. Curtsinger, C. S. Schmidt, A. Mondino, et al., "Inflammatory cytokines provide a third signal for activation of naive CD4⁺ and CD8⁺ T cells," *Journal of Immunology*, vol. 162, no. 6, pp. 3256–3262, 1999.
- [81] C. S. Schmidt and M. F. Mescher, "Adjuvant effect of IL-12: conversion of peptide antigen administration from tolerizing to immunizing for CD8⁺ T cells in vivo," *Journal of Immunology*, vol. 163, no. 5, pp. 2561–2567, 1999.
- [82] E. L. Pearce and H. Shen, "Generation of CD8 T cell memory is regulated by IL-12," *Journal of Immunology*, vol. 179, no. 4, pp. 2074–2081, 2007.
- [83] N. S. Joshi, W. Cui, A. Chandele, et al., "Inflammation directs memory precursor and short-lived effector CD8⁺ T cell fates via the graded expression of T-bet transcription factor," *Immunity*, vol. 27, no. 2, pp. 281–295, 2007.
- [84] Z. Xiao, K. A. Casey, S. C. Jameson, J. M. Curtsinger, and M. F. Mescher, "Programming for CD8 T cell memory development requires IL-12 or type I IFN," *Journal of Immunology*, vol. 182, no. 5, pp. 2786–2794, 2009.
- [85] C. S. Hsieh, S. E. Macatonia, C. S. Tripp, S. F. Wolf, A. O'Garra, and K. M. Murphy, "Development of TH1 CD4⁺ T cells through IL-12 produced by Listeria-induced macrophages," *Science*, vol. 260, no. 5107, pp. 547–549, 1993.
- [86] R. Manetti, P. Parronchi, M. G. Giudizi, et al., "Natural killer cell stimulatory factor (interleukin 12 [IL-12]) induces T helper type 1 (Th1)-specific immune responses and inhibits the development of IL-4-producing Th cells," *Journal of Experimental Medicine*, vol. 177, no. 4, pp. 1199–1204, 1993.
- [87] A. K. Abbas, K. M. Murphy, and A. Sher, "Functional diversity of helper T lymphocytes," *Nature*, vol. 383, no. 6603, pp. 787–793, 1996.
- [88] A. Oxenius, R. M. Zinkernagel, and H. Hengartner, "CD4⁺ T-Cell induction and effector functions: a comparison of immunity against soluble antigens and viral infections," *Advances in Immunology*, vol. 70, pp. 313–367, 1998.
- [89] C. King, S. G. Tangye, and C. R. Mackay, "T follicular helper (TFH) cells in normal and dysregulated immune responses," *Annual Review of Immunology*, vol. 26, pp. 741–766, 2008.
- [90] C. King, "New insights into the differentiation and function of T follicular helper cells," *Nature Reviews Immunology*, vol. 9, no. 11, pp. 757–766, 2009.
- [91] M. F. Bachmann, P. Wolint, K. Schwarz, and A. Oxenius, "Recall proliferation potential of memory CD8⁺ T cells and antiviral protection," *Journal of Immunology*, vol. 175, no. 7, pp. 4677–4685, 2005.
- [92] G. T. Belz, D. Wodarz, G. Diaz, M. A. Nowak, and P. C. Doherty, "Compromised influenza virus-specific CD8⁺-T-cell memory in CD4⁺-T-cell-deficient mice," *Journal of Virology*, vol. 76, no. 23, pp. 12388–12393, 2002.
- [93] M. J. Bevan, "Helping the CD8⁺ T-cell response," *Nature Reviews Immunology*, vol. 4, no. 8, pp. 595–602, 2004.
- [94] C. Bourgeois, H. Veiga-Fernandes, A.-M. Joret, B. Rocha, and C. Tanchot, "CD8 lethargy in the absence of CD4 help," *European Journal of Immunology*, vol. 32, no. 8, pp. 2199–2207, 2002.
- [95] E. M. Janssen, N. M. Droin, E. E. Lemmens, et al., "CD4⁺ T-cell help controls CD8⁺ T-cell memory via TRAIL-mediated activation-induced cell death," *Nature*, vol. 434, no. 7029, pp. 88–93, 2005.
- [96] E. M. Janssen, E. E. Lemmens, T. Wolfe, U. Christen, M. G. von Herrath, and S. P. Schoenberger, "CD4⁺ T cells are required for secondary expansion and memory in CD8⁺ T lymphocytes," *Nature*, vol. 421, no. 6925, pp. 852–856, 2003.
- [97] A. Khanolkar, M. J. Fuller, and A. J. Zajac, "CD4 T Cell-Dependent CD8 T Cell Maturation," *Journal of Immunology*, vol. 172, no. 5, pp. 2834–2844, 2004.
- [98] B. Rocha and C. Tanchot, "Towards a cellular definition of CD8⁺ T-cell memory: the role of CD4⁺ T-cell help in CD8⁺ T-cell responses," *Current Opinion in Immunology*, vol. 16, no. 3, pp. 259–263, 2004.
- [99] D. J. Shedlock and H. Shen, "Requirement for CD4 T cell help in generating functional CD8 T cell memory," *Science*, vol. 300, no. 5617, pp. 337–339, 2003.
- [100] J. C. Sun and M. J. Bevan, "Defective CD8 T cell memory following acute infection without CD4 T cell help," *Science*, vol. 300, no. 5617, pp. 339–342, 2003.
- [101] J. C. Sun, M. A. Williams, and M. J. Bevan, "CD4⁺ T cells are required for the maintenance, not programming, of memory CD8⁺ T cells after acute infection," *Nature Immunology*, vol. 5, no. 9, pp. 927–933, 2004.
- [102] M. G. von Herrath, M. Yokoyama, J. Dockter, M. B. A. Oldstone, and J. L. Whitton, "CD4-deficient mice have reduced levels of memory cytotoxic T lymphocytes after immunization and show diminished resistance to subsequent virus challenge," *Journal of Virology*, vol. 70, no. 2, pp. 1072–1079, 1996.
- [103] M. Matloubian, R. J. Concepcion, and R. Ahmed, "CD4⁺ T cells are required to sustain CD8⁺ cytotoxic T-cell responses during chronic viral infection," *Journal of Virology*, vol. 68, no. 12, pp. 8056–8063, 1994.
- [104] C. M. Smith, N. S. Wilson, J. Waithman, et al., "Cognate CD4⁺ T cell licensing of dendritic cells in CD8⁺ T cell immunity," *Nature Immunology*, vol. 5, no. 11, pp. 1143–1148, 2004.
- [105] U. Kumaraguru, K. Banerjee, and B. T. Rouse, "In vivo rescue of defective memory CD8⁺ T cells by cognate helper T cells," *Journal of Leukocyte Biology*, vol. 78, no. 4, pp. 879–887, 2005.
- [106] M. D. Hazenberg, D. Hamann, H. Schuitemaker, and F. Miedema, "T cell depletion in HIV-1 infection: how CD4⁺ T cells go out of stock," *Nature Immunology*, vol. 1, no. 4, pp. 285–289, 2000.
- [107] E. S. Rosenberg, J. M. Billingsley, A. M. Caliendo, et al., "Vigorous HIV-1-specific CD4⁺ T cell responses associated with control of viremia," *Science*, vol. 278, no. 5342, pp. 1447–1450, 1997.
- [108] H. M. L. Spiegel, G. S. Ogg, E. DeFalcon, et al., "Human immunodeficiency virus type 1- and cytomegalovirus-specific cytotoxic T lymphocytes can persist at high frequency for prolonged periods in the absence of circulating peripheral CD4⁺ T cells," *Journal of Virology*, vol. 74, no. 2, pp. 1018–1022, 2000.
- [109] S. A. Kalams and B. D. Walker, "The critical need for CD4 help in maintaining effective cytotoxic T lymphocyte responses," *Journal of Experimental Medicine*, vol. 188, no. 12, pp. 2199–2204, 1998.
- [110] A. M. Intlekofer, N. Takemoto, C. Kao, et al., "Requirement for T-bet in the aberrant differentiation of unhelped memory CD8⁺ T cells," *Journal of Experimental Medicine*, vol. 204, no. 9, pp. 2015–2021, 2007.

- [111] J. P. Ridge, F. Di Rosa, and P. Matzinger, "A conditioned dendritic cell can be a temporal bridge between a CD4⁺ T-helper and a T-killer cell," *Nature*, vol. 393, no. 6684, pp. 474–478, 1998.
- [112] S. P. Schoenberger, R. E. M. Toes, E. I. H. van Dervoort, R. Offringa, and C. J. M. Melief, "T-cell help for cytotoxic T lymphocytes is mediated by CD40-CD4OL interactions," *Nature*, vol. 393, no. 6684, pp. 480–483, 1998.
- [113] S. R. M. Bennett, F. R. Carbone, F. Karamalis, R. A. Flavell, J. F. A. P. Miller, and W. R. Heath, "Help for cytotoxic-T-cell responses is mediated by CD40 signalling," *Nature*, vol. 393, no. 6684, pp. 478–480, 1998.
- [114] A. Lanzavecchia, "Immunology: licence to kill," *Nature*, vol. 393, no. 6684, pp. 413–414, 1998.
- [115] C. Bourgeois, B. Rocha, and C. Tanchot, "A role for CD40 expression on CD8⁺ T cells in the generation of CD8⁺ T cell memory," *Science*, vol. 297, no. 5589, pp. 2060–2063, 2002.
- [116] F. Castellino, A. Y. Huang, G. Altan-Bonnet, S. Stoll, C. Scheinecker, and R. N. Germain, "Chemokines enhance immunity by guiding naive CD8⁺ T cells to sites of CD4⁺ T cell-dendritic cell interaction," *Nature*, vol. 440, no. 7086, pp. 890–895, 2006.
- [117] F. Castellino and R. N. Germain, "Chemokine-guided CD4⁺ T cell help enhances generation of IL-6Rα^{high}IL-7Rα^{high} prememory CD8⁺ T cells," *Journal of Immunology*, vol. 178, no. 2, pp. 778–787, 2007.
- [118] Y. Nakanishi, B. Lu, C. Gerard, and A. Iwasaki, "CD8⁺ T lymphocyte mobilization to virus-infected tissue requires CD4⁺ T-cell help," *Nature*, vol. 462, no. 7272, pp. 510–513, 2009.
- [119] W. N. D'Souza, K. S. Schluns, D. Masopust, and L. Lefrançois, "Essential role for IL-2 in the regulation of antiviral extralymphoid CD8 T cell responses," *Journal of Immunology*, vol. 168, no. 11, pp. 5566–5572, 2002.
- [120] W. N. D'Souza and L. Lefrançois, "IL-2 is not required for the initiation of CD8 T cell cycling but sustains expansion," *Journal of Immunology*, vol. 171, no. 11, pp. 5727–5735, 2003.
- [121] L. P. Cousens, J. S. Orange, and C. A. Biron, "Endogenous IL-2 contributes to T cell expansion and IFN-γ production during lymphocytic choriomeningitis virus infection," *Journal of Immunology*, vol. 155, no. 12, pp. 5690–5699, 1995.
- [122] M. A. Williams, A. J. Tzysnik, and M. J. Bevan, "Interleukin-2 signals during priming are required for secondary expansion of CD8⁺ memory T cells," *Nature*, vol. 441, no. 7095, pp. 890–893, 2006.
- [123] M. F. Bachmann, P. Wolint, S. Walton, K. Schwarz, and A. Oxenius, "Differential role of IL-2R signaling for CD8⁺ T cell responses in acute and chronic viral infections," *European Journal of Immunology*, vol. 37, no. 6, pp. 1502–1512, 2007.
- [124] H. Kwon, D. Thierry-Mieg, J. Thierry-Mieg, et al., "Analysis of interleukin-21-induced Prdm1 gene regulation reveals functional cooperation of STAT3 and IRF4 transcription factors," *Immunity*, vol. 31, no. 6, pp. 941–952, 2009.
- [125] D. Gong and T. R. Malek, "Cytokine-dependent Blimp-1 expression in activated T cells inhibits IL-2 production," *Journal of Immunology*, vol. 178, no. 1, pp. 242–252, 2007.
- [126] G. A. Martins, L. Cimmino, J. Liao, E. Magnusdottir, and K. Calame, "Blimp-1 directly represses Il2 and the Il2 activator Fos, attenuating T cell proliferation and survival," *Journal of Experimental Medicine*, vol. 205, no. 9, pp. 1959–1965, 2008.
- [127] M. T. Kasaian and C. A. Biron, "The activation of IL-2 transcription in L3T4⁺ and LYT-2⁺ lymphocytes during virus infection in vivo," *Journal of Immunology*, vol. 142, no. 4, pp. 1287–1292, 1989.
- [128] L. Andrus, S. J. Prowse, and K. J. Lafferty, "Interleukin 2 production by both Ly2⁺ and Ly2⁻ T-cell subsets," *Scandinavian Journal of Immunology*, vol. 13, no. 3, pp. 297–301, 1981.
- [129] W. N. D'Souza and L. Lefrançois, "Frontline: an in-depth evaluation of the production of IL-2 by antigen-specific CD8 T cells in vivo," *European Journal of Immunology*, vol. 34, no. 11, pp. 2977–2985, 2004.
- [130] M. J. Deeths, R. M. Kedl, and M. F. Mescher, "CD8⁺ T cells become nonresponsive (anergic) following activation in the presence of costimulation," *Journal of Immunology*, vol. 163, no. 1, pp. 102–110, 1999.
- [131] E. L. Tham and M. F. Mescher, "The poststimulation program of CD4 versus CD8 T cells (death versus activation-induced nonresponsiveness)," *Journal of Immunology*, vol. 169, no. 4, pp. 1822–1828, 2002.
- [132] E. L. Tham, P. Shrikant, and M. F. Mescher, "Activation-induced nonresponsiveness: a Th-dependent regulatory checkpoint in the CTL response," *Journal of Immunology*, vol. 168, no. 3, pp. 1190–1197, 2002.
- [133] E. L. Tham and M. F. Mescher, "Signaling alterations in activation-induced nonresponsive CD8 T cells," *Journal of Immunology*, vol. 167, no. 4, pp. 2040–2048, 2001.
- [134] J. Parrish-Novak, S. R. Dillon, A. Nelson, et al., "Interleukin 21 and its receptor are involved in NK cell expansion and regulation of lymphocyte function," *Nature*, vol. 408, no. 6808, pp. 57–63, 2000.
- [135] W. J. Leonard and R. Spolski, "Interleukin-21: a modulator of lymphoid proliferation, apoptosis and differentiation," *Nature Reviews Immunology*, vol. 5, no. 9, pp. 688–698, 2005.
- [136] W. J. Leonard, R. Zeng, and R. Spolski, "Interleukin 21: a cytokine/cytokine receptor system that has come of age," *Journal of Leukocyte Biology*, vol. 84, no. 2, pp. 348–356, 2008.
- [137] K. Ozaki, K. Kikly, D. Michalovich, P. R. Young, and W. J. Leonard, "Cloning of a type I cytokine receptor most related to the IL-2 receptor β chain," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 97, no. 21, pp. 11439–11444, 2000.
- [138] E.-L. Allard, M.-P. Hardy, J. Leignadier, et al., "Overexpression of IL-21 promotes massive CD8⁺ memory T cell accumulation," *European Journal of Immunology*, vol. 37, no. 11, pp. 3069–3077, 2007.
- [139] K. A. Casey and M. F. Mescher, "IL-21 promotes differentiation of naive CD8 T cells to a unique effector phenotype," *Journal of Immunology*, vol. 178, no. 12, pp. 7640–7648, 2007.
- [140] H. Elsaesser, K. Sauer, and D. G. Brooks, "IL-21 is required to control chronic viral infection," *Science*, vol. 324, no. 5934, pp. 1569–1572, 2009.
- [141] A. Frohlich, J. Kisielow, I. Schmitz, et al., "IL-21R on T cells is critical for sustained functionality and control of chronic viral infection," *Science*, vol. 324, no. 5934, pp. 1576–1580, 2009.
- [142] J. S. Yi, M. Du, and A. J. Zajac, "A vital role for Interleukin-21 in the control of a chronic viral infection," *Science*, vol. 324, no. 5934, pp. 1572–1576, 2009.
- [143] L. D. S. Johnson and S. C. Jameson, "A chronic need for IL-21," *Science*, vol. 324, no. 5934, pp. 1525–1526, 2009.
- [144] S. J. Szabo, B. M. Sullivan, C. Sternmann, A. R. Satoskar, B. P. Sleckman, and L. H. Glimcher, "Distinct effects of T-bet in Th1 lineage commitment and IFN-γ production in CD4 and CD8 T cells," *Science*, vol. 295, no. 5553, pp. 338–342, 2002.
- [145] A. M. Intlekofer, N. Takemoto, E. J. Wherry, et al., "Effector and memory CD8⁺ T cell fate coupled by T-bet and eomesodermin," *Nature Immunology*, vol. 6, no. 12, pp. 1236–1244, 2005.

- [146] B. M. Sullivan, A. Juedes, S. J. Szabo, M. von Herrath, and L. H. Glimcher, "Antigen-driven effector CD8 T cell function regulated by T-bet," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 100, no. 26, pp. 15818–15823, 2003.
- [147] A. M. Intlekofer, A. Banerjee, N. Takemoto, et al., "Anomalous type 17 response to viral infection by CD8⁺ T cells lacking T-bet and eomesodermin," *Science*, vol. 321, no. 5887, pp. 408–411, 2008.
- [148] S. S. Way and C. B. Wilson, "Cutting edge: immunity and IFN- γ production during *Listeria monocytogenes* infection in the absence of T-bet," *Journal of Immunology*, vol. 173, no. 10, pp. 5918–5922, 2004.
- [149] E. L. Pearce, A. C. Mullen, G. A. Martins, et al., "Control of effector CD8⁺ T cell function by the transcription factor eomesodermin," *Science*, vol. 302, no. 5647, pp. 1041–1043, 2003.
- [150] M. Afkarian, J. R. Sedy, J. Yang, et al., "T-bet is a STAT1-induced regulator for IL-12R expression in naive CD4⁺ T cells," *Nature Immunology*, vol. 3, no. 6, pp. 549–557, 2002.
- [151] A. A. Lighvani, D. M. Frucht, D. Jankovic, et al., "T-bet is rapidly induced by interferon- γ in lymphoid and myeloid cells," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 98, no. 26, pp. 15137–15142, 2001.
- [152] F. Cruz-Guilloty, M. E. Pipkin, I. M. Djuretic, et al., "Runx3 and T-box proteins cooperate to establish the transcriptional program of effector CTLs," *Journal of Experimental Medicine*, vol. 206, no. 1, pp. 51–59, 2009.
- [153] N. Takemoto, A. M. Intlekofer, J. T. Northrup, E. J. Wherry, and S. L. Reiner, "Cutting edge: IL-12 inversely regulates T-bet and eomesodermin expression during pathogen-induced CD8⁺ T cell differentiation," *Journal of Immunology*, vol. 177, no. 11, pp. 7515–7519, 2006.
- [154] D. Cobb, S. Guo, A. M. Lara, P. Manque, G. Buck, and R. B. Smeltz, "T-bet-dependent regulation of CD8⁺ T-cell expansion during experimental *Trypanosoma cruzi* infection," *Immunology*, vol. 128, no. 4, pp. 589–599, 2009.
- [155] K. Araki, A. P. Turner, V. O. Shaffer, et al., "mTOR regulates memory CD8 T-cell differentiation," *Nature*, vol. 460, no. 7251, pp. 108–112, 2009.
- [156] E. L. Pearce, M. C. Walsh, P. J. Cejas, et al., "Enhancing CD8 T-cell memory by modulating fatty acid metabolism," *Nature*, vol. 460, no. 7251, pp. 103–107, 2009.
- [157] R. R. Rao, Q. Li, K. Odunsi, and P. A. Shrikant, "The mTOR kinase determines effector versus memory CD8⁺ T cell fate by regulating the expression of transcription factors T-bet and eomesodermin," *Immunity*, vol. 32, no. 1, pp. 67–78, 2010.
- [158] M. Prlic and M. J. Bevan, "Immunology: a metabolic switch to memory," *Nature*, vol. 460, no. 7251, pp. 41–42, 2009.
- [159] S. Crotty, R. J. Johnston, and S. P. Schoenberger, "Effectors and memories: Bcl-6 and Blimp-1 in T and B lymphocyte differentiation," *Nature Immunology*, vol. 11, no. 2, pp. 114–120, 2010.
- [160] G. Martins and K. Calame, "Regulation and functions of Blimp-1 in T and B lymphocytes," *Annual Review of Immunology*, vol. 26, pp. 133–169, 2008.
- [161] D. Savitsky, L. Cimmino, T. Kuo, G. A. Martins, and K. Calame, "Multiple roles for Blimp-1 in B and T lymphocytes," *Advances in Experimental Medicine and Biology*, vol. 596, pp. 9–30, 2007.
- [162] G. A. Martins, L. Cimmino, M. Shapiro-Shelef, et al., "Transcriptional repressor Blimp-1 regulates T cell homeostasis and function," *Nature Immunology*, vol. 7, no. 5, pp. 457–465, 2006.
- [163] A. Kallies, E. D. Hawkins, G. T. Belz, et al., "Transcriptional repressor Blimp-1 is essential for T cell homeostasis and self-tolerance," *Nature Immunology*, vol. 7, no. 5, pp. 466–474, 2006.
- [164] R. L. Rutishauser, G. A. Martins, S. Kalachikov, et al., "Transcriptional repressor Blimp-1 promotes CD8⁺ T cell terminal differentiation and represses the acquisition of central memory T cell properties," *Immunity*, vol. 31, no. 2, pp. 296–308, 2009.
- [165] A. Kallies, A. Xin, G. T. Belz, and S. L. Nutt, "Blimp-1 transcription factor is required for the differentiation of effector CD8⁺ T cells and memory responses," *Immunity*, vol. 31, no. 2, pp. 283–295, 2009.
- [166] H. Shin, S. D. Blackburn, A. M. Intlekofer, et al., "A role for the transcriptional repressor Blimp-1 in CD8⁺ T cell exhaustion during chronic viral infection," *Immunity*, vol. 31, no. 2, pp. 309–320, 2009.
- [167] H. Ichii, A. Sakamoto, Y. Kuroda, and T. Tokuhisa, "Bcl6 acts as an amplifier for the generation and proliferative capacity of central memory CD8⁺ T cells," *Journal of Immunology*, vol. 173, no. 2, pp. 883–891, 2004.
- [168] H. Ichii, A. Sakamoto, M. Hatano, et al., "Role for Bcl-6 in the generation and maintenance of memory CD8⁺ T cells," *Nature Immunology*, vol. 3, no. 6, pp. 558–563, 2002.
- [169] J. T. Chang, V. R. Palanivel, I. Kinjyo, et al., "Asymmetric T lymphocyte division in the initiation of adaptive immune responses," *Science*, vol. 315, no. 5819, pp. 1687–1691, 2007.