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Surviving the crash: transitioning from effector to memory CD8⁺ T cell

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Abstract

One outcome of infection is the formation of long-lived immunological memory, which provides durable protection from symptomatic reinfection. In response to infection or vaccination, T cells undergo dramatic proliferation and differentiate into effector T cells that mediate removal of the pathogen. Following pathogen clearance, the majority of effector cells die, restoring lymphocyte homeostasis. However, a small number of antigen-specific cells survive and seed the memory T cell population. Here, we focus on recent advances in identifying the key proteins and transcription factors that allow a portion of effector CD8⁺ T cells to persist after contraction of the immune response, forming a memory cell population programmed for long-term self-renewal and survival. We will also examine new findings addressing the role of environmental cues such as cytokines and co-stimulatory molecules in CD8⁺ memory T cell formation and how the cell extrinsic cues influence the molecular players of intracellular pathways important for memory formation.

Keywords

infection; pathogen; transcription factor; immune response

CD8⁺ T cells and their response to infection

CD8⁺ T cells play an essential role in the control of infection by intracellular pathogens such as viruses, bacteria and protozoan parasites. The immune response is initiated when a naïve CD8⁺ T cell recognizes the presence of a pathogen and differentiates into an effector CD8⁺ T cell [1,2]. This activation results in both phenotypic and physiological changes for CD8⁺ T cells that include expression of molecules such as granzyme B and perforin, which mediate cytotoxicity. Alteration of adhesion molecules/chemokine receptors, such as CD62L and CCR7 allow egress from lymphoid organs and migration to the site of infection. Once at the site of infection, effector CD8⁺ T cells will perform their cytotoxic function, killing infected cells and will produce cytokines, recruiting and activate cells of the innate and adaptive immune system [3].

Once the pathogen has been cleared, the majority (~90%) of CD8⁺ effector T cells will die by apoptosis, while the remaining subset survives, protecting the host from re-infection. This contraction of the effector population restores homeostasis, preventing the dominance of a

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particular T cell clone within the T cell repertoire [4]. How it is determined which cells are fated to escape apoptosis and survive to join the memory compartment is not well understood. It is now recognized that the cytokine milieu under which this occurs has a significant impact on which effector CD8⁺ T cell will transition and survive to become memory T cells [5–7].

Once the long-term memory T cell population is established, these cells can exist for many months or years, undergoing slow basal homeostasis while at the same time maintaining the ability to proliferate extensively should their cognate antigen be re-encountered. The differentiation of effector cells to memory cells involves the progressive acquisition of these memory traits over time, generating heterogeneous phenotypic subsets [8]. Recently, it has been shown that the molecules KLRG1 and IL-7R α (CD127) can be used to differentiate between two types or subsets of differentiating cells during infection: KLRG1^{hi} CD127^{lo} or effector T cells which are rapidly produced during infection and can transiently occupy the memory compartment, and KLRG1^{lo} CD127^{hi} memory T cells which emerge later during infection and generate longer-lived memory cells [5,7]. Memory T cells distinguished on the basis of CD44 and CD62L have also been useful tools in certain experimental settings. CD44^{hi} CD62L^{lo} 'effector' memory CD8⁺ T cells reside primarily in peripheral tissues and provide a first line of defense against re-infection, and CD44^{hi} CD62L^{hi} 'central' memory CD8⁺ T cells which reside primarily in secondary lymphoid organs where they can reacquire full effector function upon re-infection. This secondary response, initiated by re-exposure to pathogen, is faster and of a greater magnitude than the primary infection and is the basis for efficacy of T cell specific vaccines.

Cell Intrinsic Factors

The differentiation of a naïve T cell to an effector cell armed with the ability to eliminate pathogen is initiated by T cell receptor recognition of antigen-derived peptides presented by MHC in the context of an inflammatory environment. This process is dependent on many transcription factors, which coordinate proliferation and the acquisition of cytolytic activity by expression of effector molecules such as IFN- γ , perforin and granzyme B [for review [9]]. Antigen recognition leads to progressive changes in gene expression and function that can be monitored through the course of the immune response and reveal the gradual adoption of the memory cell fate [10]. Recent studies evaluating naïve, effector and memory CD8⁺ populations conclude that memory cells display a unique gene-expression pattern, a portion of which is conserved among memory CD8⁺, memory CD4⁺ and memory B lymphocytes. These observations indicate that an understanding of the transcription factors that regulate memory cell formation is bound to shed light beyond the CD8⁺ memory subset population [10–13].

T-bet and eomesodermin

T-bet was originally identified as a T box transcription factor responsible for T-helper type I differentiation [14]. Subsequently, both T-bet and its sister protein, eomesodermin (eomes), have been implicated in a wide range of T cell-specific functions [5,15,16]. Of interest in the context of this review is the role that both proteins have been shown to play in CD8⁺ effector and memory T cell differentiation [15]. Compound deficiency in both T-bet and eomes was found to result in a near complete loss of CD8⁺ memory cells due to the fact that these two transcription factors are responsible for enhanced expression of CD122, the IL-2/15 β receptor expressed at high levels on the majority of CD8⁺ memory T cells which conveys IL-15 responsiveness [15]. T-bet and eomes also induced expression of granzyme B, perforin and IFN- γ in cytotoxic CD8⁺ T cells [9,16]. Given that T-bet and eomes were necessary for the up-regulation of CD122 and that T-bet/eomes compound mutants lack CD8⁺ memory T cells, it is now thought that both of these molecules play an important role in instigating and

sustaining the molecular programs that regulate effector to memory T cell differentiation [15,17].

Indeed, it has recently been shown that contraction of the KLRG1^{hi} CD127^{lo} and KLRG1^{lo} CD127^{hi} effector subsets is influenced by T-bet expression [5]. Joshi and colleagues found that high levels of T-bet (induced by inflammation and IL-12) enhanced differentiation of the shorter-lived effector KLRG1^{hi} CD127^{lo} CD8⁺ T cell population during the effector phase. In reciprocal experiments, the loss of T-bet lead to the absence of KLRG1^{hi} CD127^{lo} CD8⁺ T cells [5,18]. Interestingly, T-bet and eomes expression was regulated by IL-12, with IL-12 expression resulting in decreased levels of eomes while promoting T-bet expression [15,19]. This is consistent with the observation that generation of memory cells was increased in the absence of IL-12, in spite of a diminished effector response [20].

Together these data lead to the idea that the level of inflammation and the availability of certain cytokines (e.g. IL-12) influence the levels of T-bet and eomes available to the effector cell and that this in turn influences which cells will remain CD8⁺ effector T cells and which will transition to the memory CD8⁺ T cell pool [21]. Higher levels of inflammation would result in more T-bet expression and favor the generation of relatively short-lived KLRG1^{hi} CD127^{lo} CD8⁺ T cells while waning inflammation would support expression of eomes leading to the differentiation of longer-lived KLRG1^{lo} CD127^{hi} CD8⁺ memory T cells. Intlekofer et al. went on to further show that T-bet repressed CD127 expression on T cells transitioning to the memory T cell compartment and enhanced the size of the population destined to remain effector T cells [18], further highlighting the important role T-bet plays in the transition from effector to memory CD8⁺ T cell.

Inhibitor of DNA binding 2

E and Id proteins are key transcriptional regulators that control many aspects of lymphocyte development [22]. E proteins, of which there are four in mammals E47, E12, HEB and E2-2, bind as homo- or heterodimers to DNA at their canonical E box sites, where they act as transcriptional activators or repressors. Id proteins, of which there are also four Id1–Id4, heterodimerize with E proteins, preventing DNA binding at their target site [22–24]. Thus, E proteins are the natural dominant negative regulators of E protein activity.

Of the Id proteins, notably Id2 and Id3 are reported to play major roles in lymphocyte development and maintenance, and indeed, Id3-deficiency leads to a block in T cell development during the DP stage in the thymus [22,25]. Interestingly, thymocyte development appears normal in Id2-deficient animals with normal numbers and frequencies of CD4 and CD8 single positive cells produced [26]. However, populations of natural killer cell, CD8 α dendritic cell, Langerhans cell and intraepithelial cells are largely absent in Id2-deficient animals, underlining the importance of this protein in development and maintenance of a diverse set of immune cells [27–30].

Following infection, CD8⁺ T cells showed dynamic regulation of Id2 mRNA levels; Id2 mRNA levels initially decrease (unpublished observation) and then dramatically increase at the peak of the response and were maintained into the memory phase [26]. We have observed this dynamic regulation of Id2 *in vitro* (unpublished observation) or *in vivo* during the course of infection [26], implicating a role for Id2 in the effector to memory T cell transition.

During infection, Id2-deficient CD8⁺ T cells initially generate a robust immune response, reaching their peak of expansion sooner than their wild type counterparts. However, as the response proceeds, far fewer Id2-deficient effector cells are generated and the few cells that are present are rapidly culled during contraction, resulting in 5–10-fold fewer memory T cells [26]. The rapid loss of Id2-deficient cells during infection indicated two major possibilities:

either Id2 deficiency led to loss of the cells ability to proliferate during infection or to greater cell death or apoptosis as the cells responded to the infection. We showed that while Id2-deficient T cells are able to proliferate as well as wild type cells, they demonstrated increased apoptosis resulting in fewer effector and memory T cells being formed [26]. Given that Id2-deficient cells died more rapidly during infection, it was not surprising that clearance of infection was hindered in an Id2-deficient environment [26]. How Id2 protects effector cells from death is not yet known. Gene-expression analysis comparing Id2-deficient and -sufficient CD8⁺ effectors revealed increased mRNA levels of pro-apoptotic molecules such as Bim and Ctla-4 and a decrease in anti-apoptotic molecules such as Bcl-2 and Spi6 in the former population [26].

Aside from its key role in effector T cell survival, Id2 has an effect on both the size and composition of the memory T cell compartment [26]. Far fewer memory CD8⁺ T cells are formed from Id2-deficient cells and they are skewed toward a CD44^{hi} CD62L^{hi} central memory phenotype. We have found that Id2-deficiency also leads to almost complete loss of KLRG1^{hi} CD127^{lo} short-term effector/memory cells in the course of infection, while KLRG1^{lo} CD127^{hi} precursor memory cells are still formed, although at lower frequencies than in wild-type sufficient cells (unpublished observation). Thus, the Id2-deficient CD44^{hi} CD62L^{lo} or KLRG1^{hi} CD127^{lo} effector memory T cells are either lost more rapidly, or are less likely to be formed in the first place [26]. The latter hypothesis, that Id2 dependency dictates memory T cell subset formation, seems unlikely as both effector memory and central memory T cells have equal Id2 mRNA expression levels (unpublished observation). As Id2 is up-regulated late in the effector phase this suggests that not all memory programming occurs at the earliest stages of the immune response.

The clear role of Id2 in the CD8⁺ T cell response evokes the hypothesis that their targets, the E proteins will also function during T cell activation. Indeed, we find enhanced E protein DNA binding activity in OT-I CD8⁺ T cells stimulated *in vitro* [26] and in CD8⁺ effectors generated *in vivo* (unpublished observation). It is interesting to speculate on the role E proteins will play in a healthy immune response. It has previously been shown in thymocyte development, that Id3 is increased, negatively regulates E47 and HEB activity once signaling through the pre-TCR or TCR occurs, so influencing both DN to DP and DP to SP transitions [22]. Thus, it is thought that Id3 expression is necessary for proliferation and maturation, perhaps mirroring what we see with Id2 in effector T cell transition during infection. Indeed, over-expression of Id2 or Id3 can lead to tumor development [31,32], indicating its role in promoting cell survival and proliferation. If Id2 is essential to dampen E protein activity during late infection, it begs the question as to what genes may be regulated or influenced by E proteins early in the immune response and how these genes will impact efficient CD8⁺ T cell memory formation.

Bim and Fas

The abrupt loss of effector cells at the conclusion of the immune response ensures that the lymphocyte pool is not dominated by cells responsive to one pathogen [4]. This dramatic contraction is thought to involve apoptosis and a number of pro- and anti-apoptotic molecules have been implicated in this process. The combined effect of these molecules allows the survival of a small memory population of antigen-experienced CD8⁺ T cells that remain to protect against subsequent infection. Apoptosis can occur through two major pathways [for review [33]]. The first, the intrinsic pathway is controlled at the mitochondrial membrane and is activated by the presence of cellular stress or damage. Initiation of this pathway occurs when the mitochondrial membrane is disrupted by the expression/activation of proteins belonging to the BH3-only family such as Bim, which results in the release of cytochrome c from the mitochondria. This, in turn results in the generation of active caspases 3 and 7, which mediate death through several mechanisms including the initiation of double-stranded DNA breaks

[34]. The second pathway, the extrinsic pathway, is initiated by cell surface death receptors of the tumor necrosis factor (TNF) family such as Fas upon their binding to extracellular ligands [35,36]. Following interaction of the death receptor with its ligand, the death-inducing signaling complex is formed, which in turn results in activation of caspases 3 and 7 and apoptosis [35,36]. Recent results suggest both pathways influence CD8⁺ T cell death following infection.

The molecule Bcl-2, a pro-survival molecule in the intrinsic apoptotic pathway, was initially shown to be down-regulated at the peak of *in vivo* infection in activated T cells [37]. It was subsequently shown that transgenic expression of Bcl-2 rescued these dying cells [38], implicating the down-regulation of Bcl-2 in the T cell contraction phase. However, Bcl-2 does not act alone, and Hildeman et al. went on to show that contraction of T cells in response to staphylococcal enterotoxin B (SEB) was mediated by the pro-apoptotic molecule Bcl-2-interacting mediator of death (Bim) [38]. Pellegrini and colleagues showed, using Bim-deficient mice, that Bim is essential for T cell contraction although removal of the antigen (in this case, herpes simplex virus) was Bim-independent [39]. Interestingly, Bim-deficiency was also shown to increase memory T cell formation [40]; when Bim-deficient CD127^{lo} LCMV-specific CD8⁺ T were tracked, they were able survive the contraction phase and re-express CD127 [40]. However, over time these cells were lost due to their inability to undergo basal proliferation, suggesting that Bim may play a role in limiting the numbers of antigen-specific cells that can initially enter the memory T cell compartment and that different mechanisms influence memory T cell homeostasis [40]. Bim-deficiency has also been shown to enhance protection against challenge with *Leishmania major*, again indicating that Bim plays a role in limiting memory T cell formation [41] but that other cell death pathways are responsible for a gradual decline in memory T cells [42]. Ultimately, it is the balance between pro-apoptotic molecules like Bim (e.g. Bad, Bid, Puma) and anti-apoptotic molecules like Bcl-2 (e.g. Bcl-XL, MCL-1) that dictates whether the effector cell will initiate an apoptotic program, and as such many of these molecules have been found to influence T cell survival during infection [43].

Fas-deficient mice display an abundance of activated/memory-like T cells over time [44], although the effect of Fas/Fas-L on contraction of CD8⁺ T cells after infection was initially thought to be minimal [39]. Recent publications have implicated Fas in 'backing up' or supporting Bim in certain scenarios during T cell contraction [42,45–47]. Weant and colleagues found in acute LCMV infection that both Fas and Bim play a role in T cell contraction and compound mutants showed dramatically increased numbers of memory T cells. Interestingly, Hughes et al. rather found a role for Fas and Bim in the case of chronic MHV infection while acute infection required only Bim for contraction [42,46]. As discussed by Green, these data suggest that antigen persistence (either in acute or chronic infection), can lead to expression of FasL, which in turn can interact with Fas expressed on both T cells and dendritic cells. Death of dendritic cells and removal of stimulation signals to T cells would then result in Bim-dependent death once the system senses the antigen has been cleared [45].

It is apparent that regulation of T cell contraction is key in the building of adequate T cell memory and that certain factors that contribute to contraction, namely Bim and Bcl-2, will also play a role in dictating which cells will differentiate into memory T cells and so escape death by apoptosis.

Blimp and Bcl-6

The transcription factor B lymphocyte-induced maturation protein-1 or Blimp-1 was initially described as a transcriptional repressor capable of binding the IFN β promoter [48]. The protein Blimp-1 is encoded by the gene *Prdm-1* and contains a SET domain and zinc finger domain

allowing it to modify chromatin structure and repress transcription [49,50]. One of its most crucial roles is its ability to drive differentiation of B cells to antibody-secreting plasma cells and for maintenance of these cells in the bone marrow [51]. However, more recently it has come to light that Blimp-1 is also required for homeostasis of CD8⁺ T cells [52,53] and that it may impact which cells are destined for memory CD8⁺ T cell formation.

Blimp-1 is expressed in T cells, and similarly to Id2, its expression is higher in effector or antigen-experienced T cells, while lower in peripheral naïve cells and reduced, when compared to effector cells, in central memory T cell [52,53]. Using a Blimp-1 GFP knock-in mouse, which expresses GFP and a truncated Blimp-1 protein, it could be further shown that antigen-specific cells responding to herpes simplex virus infection *in vivo* were GFP⁺ CD8⁺ T cells [52]. Given that these GFP⁺ CD8⁺ T cells were actually Blimp-1-deficient, it appears that Blimp-1-deficiency gives antigen-specific effector and memory T cells a survival advantage [52]. Moreover, deficiency in Blimp-1 leads to altered T cell homeostasis in the periphery and eventually development of spontaneous inflammatory disease [52,53]. Even in a non-infectious scenario, it can be seen that Blimp-1 deficiency leads to an increase in effector and memory T cell subsets [52,53], indicating that when present, the protein may negatively regulate memory T cell differentiation, perhaps by reducing the ability of effector T cells to proliferate and survive the effector phase. It will be of interest to identify which genes may be repressed by Blimp-1, especially as they may directly impact memory T cell formation [49]. Bcl-6, as mentioned above, is important for memory T cell formation and it has been shown in B and T cells that Blimp-1 targets Bcl-6 expression [54,55] so it may be that these two proteins work in concert to affect memory T cell differentiation.

IL-2 production and Blimp-1 are intimately linked during the effector T cell response, as it was initially shown that IL-2 expression by activated T cells is inversely co-related to Blimp-1 expression [56]. Furthermore, ectopic over-expression of Blimp-1 leads to down-regulation in IL-2 production upon TCR stimulation [56]. In fact, it has been suggested that IL-2 negatively regulates its own expression in activated T cells by the induction of Blimp-1 [56] and more recently, it was shown that IL-2 induced Blimp-1 expression in activated T cells. Following that, Blimp-1 repressed IL-2 transcription both directly, and indirectly through repression of Fos transcription (a member of AP-1, an activator of IL-2 transcription), and this autoregulatory loop is thought to influence the contraction phase of the immune response [50]. How IL-2 regulates Blimp-1 expression has not yet been determined, but it is likely to include STAT5 as this lies directly downstream of the IL-2 signaling pathway [49]. Surprisingly, IL-2 levels do eventually decrease in Blimp-1-deficient cells suggesting that additional mechanisms control IL-2 levels during T cell contraction [50]. However, Blimp-1 deficiency does lead to altered T cell homeostasis and an abundance of effector T cells under infectious conditions [52], highlighting the importance in T cell contraction and suggesting a role for Blimp-1 in regulating which effector T cells are destined to transition to memory T cell.

The transcriptional repressor Bcl-6 has also been shown to be important for CD8⁺ memory T cell differentiation [54,55]. Bcl-6 was initially found to be up-regulated in germinal center B cells and essential for the formation of memory B cells in germinal centers [57,58]. Infection with vaccinia virus showed a reduction in the frequency and number of memory CD8⁺ T cells in Bcl-6 deficient mice, while the opposite was true for mice expressing a Bcl-6 transgene [54]. It was further shown that Bcl-6 is particularly important for generation of central (CD44^{hi} CD62L^{hi}) memory T cell subset and for their secondary response to infection [55]. Subsequently, Bcl-6 was found to be expressed early in infection by effector T cells [59] rather than at late time points and so it will be important to understand where in the effector to memory T cell transitional pathway Bcl-6 may intersect with other factors.

Environmental Cues/Extrinsic Factors

The expression and activity of intrinsic factors that control survival during the immune response such as transcriptional regulators and proteins that influence apoptotic pathways are subject to cues delivered by the environment including cytokines, antigen, and inhibitory receptors. Of course, T cell receptor mediated recognition of antigen is the primary and crucial trigger of the immune response and memory formation. How the abundance and the duration of antigenic signaling influence the formation of effector and memory CD8⁺ T cells and how chronic infection may impact this process has been extensively studied [60–62]. In the subsequent section we will address recent studies focusing on the role of cytokines in the effector to memory cell transition.

Common γ -chain cytokines

Cytokines play an important role in fine-tuning the ability of effector CD8⁺ T cells to seed the memory compartment following antigenic challenge. For individual CD8⁺ T cells, the impact of extrinsic factors can be dramatic, and often dictates life and death decisions. Perhaps most studied of these extrinsic factors are cytokines, the small proteins used to communicate between cells of the immune system. Members of the common gamma-chain (γ_c) cytokine family, including IL-2, IL-7 and IL-15, modulate CD8⁺ T cell survival and proliferation, particularly during the contraction phase [63–65].

The most studied cytokines that modulate the transition from effector to memory CD8⁺ T cells are IL-7 and IL-15. In the absence of either of these two cytokines, the loss of CD8⁺ T cells after antigen stimulation is considerably exaggerated and leads to a deficient CD8⁺ memory T cell compartment [6,66–71]. This loss is even more exaggerated in the absence of both IL-7 and IL-15 [6,68,72] suggesting that they together support the survival and maintenance of CD8⁺ T cells following antigen stimulation. A role for a dual dependence on these two cytokines has also been reported in the homeostatic survival of CD8⁺ memory T cells [66, 73], indicating that IL-7 and IL-15 are not only important for survival of the contraction phase but also for maintenance of the memory T cell compartment.

While the removal of endogenous IL-7 or IL-15 clearly demonstrates a role for these cytokines in enhancing the accumulation of CD8⁺ T cells following antigen stimulation, similar conclusions are drawn from studying the effects of exogenously administered recombinant cytokines. Injection of IL-7 or IL-15 dramatically reduced the loss of CD8⁺ T cells following antigen-specific stimulation [70,74–78]. An advantage of using exogenous cytokines is that it allows a clear determination of the timeframe within the immune response that cytokines are most effective at enhancing the maintenance of CD8⁺ T cells. In this regard, the effects of both IL-7 and IL-15 are much more dramatic when administered after the peak CD8⁺ T cell response as opposed to early after antigenic challenge [70,75]. These findings suggest that early on in the immune response, the proliferation and survival of CD8⁺ T cells may be more cytokine-independent, while later in the response, effector CD8⁺ T cells become more cytokine-dependent for memory cell formation.

A second advantage associated with the use of recombinant cytokines to study CD8⁺ T cell immune responses is that it allows the evaluation of IL-2, another γ_c -family member. The role of this cytokine has been difficult to discern as IL-2-deficient mice develop autoimmunity due to a deficiency in CD4⁺CD25⁺ regulatory T cells [64,79]. However, as purified CD8⁺ memory T cells proliferate in a similar manner to both IL-2 and IL-15 [80], and IL-2 is produced by effector T cells during infection, there may be an important role for endogenous IL-2 in modulating the response of CD8⁺ T cells. In support of this possibility, the administration of IL-2 enhances the maintenance of CD8⁺ T cells when administered during the contraction phase of the immune response [81]. Thus, IL-2, IL-7, and IL-15 appear to play overlapping

and complimentary roles in assisting effector CD8⁺ T cells transitioning to memory cells, particularly late in the immune response.

While it appears IL-7, IL-15 and IL-2 do have overlapping effects, we recently demonstrated that there are important distinctions in how individual cytokines affect different CD8⁺ T cell subsets. Some of these differences emanate from the unique receptor subunit usage of these different cytokines. While IL-2, IL-7, IL-15 all share use of the IL-2R γ subunit (CD132) for intracellular signaling, IL-7 uses the IL-7R α (CD127) subunit, while IL-2 and IL-15 share the use of the IL-2R β (CD122) subunit for intracellular signaling [64]. This receptor subunit usage predicts that the effects of IL-2 and IL-15 with each other may be more similar than with the effects of IL-7. This prediction is consistent with our findings [6] demonstrating differential regulation of CD8⁺ T cell subsets defined by the expression of KLRG1 and CD127 [5,7,68]. Thus, we found that administration of IL-2 and IL-15 during contraction induced the preferential accumulation of shorter-lived effector/memory cells (KLRG1^{hi}CD127^{lo}) while provision of IL-7 favored accumulation of long-lived memory cells (KLRG1^{lo}CD127^{hi}). Thus, as IL-2 and IL-15 are known to be increased during periods of inflammation, our results suggest that an inflammatory environment may be uniquely suited for allowing elevated numbers of these short-lived KLRG1^{hi}CD127^{lo} CD8⁺ T cells while IL-7 will be necessary for effector CD8⁺ T cells transitioning to CD8⁺ memory T cells.

The requirement for IL-7 mediated signals in CD8⁺ memory formation appears to be influenced by the context of infection including lymphopenia and the specific pathogen, perhaps an indication that the specific cytokine milieu dictates IL-7 dependence. In lymphoreplete hosts, T-cells lacking IL-7R α were unable to differentiate into long-term resting T cells but could do so in a lymphopenic environment [72]. In a model of viral infection, IL-7R α ^{-/-} CD8⁺ T cells failed to differentiate into memory T cells [82]. However, the transfer of T cells into IL-7-deficient mice or use of IL-7R antibody blockade, resulted in impaired but not absent memory formation [6,68,83]. The ability of IL-7 and IL-15 to compensate for one another and the fact that thymic stromal lymphopoietin (TSLP) can signal CD8⁺ T cells through the IL-7R α subunit [84] may explain these observations.

In the broader context of these findings, and the knowledge that expression of IL-7R α identifies those CD8⁺ T cells destined to seed the memory compartment, Hand et al. asked whether surface expression of IL-7R α is instructional or passive in dictating the fate of these cells [85]. They found that forced transgenic expression of IL-7R α on effector CD8⁺ T cells that would normally be IL-7R α ^{low} did not bestow the ability to seed the memory compartment. Thus, they elegantly demonstrated that IL-7R α expression is not instructional, but a consequence of more fundamental changes within the effector CD8⁺ T cell that perhaps may be initiated by other intercellular signaling mechanisms [85].

Non-common γ -chain cytokines

While the γ_c -cytokine family members are essential in the regulation of CD8⁺ T cell maintenance, other cytokines produced during the response to infection are also important [60]. In IFN γ -deficient mice, antigen-specific CD8⁺ T cells responding to infection with either *Listeria* or LCMV failed to undergo contraction [86,87]. Importantly, CD8⁺ T cells also did not contract in *Listeria*-infected mice treated with antibiotics [88,89], thus providing evidence that the failure to contract in IFN γ -deficient mice was not the result of persistent infection. However, persistent infection was detected in LCMV-infected IFN γ -receptor-deficient mice [90,91]. When wildtype and IFN γ -receptor-deficient CD8⁺ T cells were co-transferred, wildtype cells had a 100-fold increase in ability to generate antigen-specific CD8⁺ memory T cells [90,91]. Although there remain unanswered questions, these findings demonstrate that IFN γ can impact the transition from effector to memory CD8⁺ T cell.

Several groups have also found a role for type I interferons (IFN-I; either IFN α or IFN β) in enhancing the generation of CD8⁺ memory T cells [92–94]. Notably, Kolumam et al. co-transferred wildtype and IFN-I receptor-deficient LCMV-specific CD8⁺ T cells into mice [92]. Upon LCMV infection, lack of IFN-I signaling lead to a 99% reduction in the capacity of the CD8⁺ T cells to expand and generate memory T cells. Unlike previous studies, which arrived at different conclusions, the current study involved the cotransfer of purified wildtype and IFN-I receptor-deficient CD8⁺ T cells, and thus avoided the possibility of chronic infection induced by the lack of IFN-I and/or the action of IFN-I on non-CD8⁺ T cells. The role of IFN-I, however, remains complicated, as in addition to acting on multiple cell types, depending on the state of T cell activation IFN-I can have different effects *in vitro* [95,96]. In the context of CD8⁺ T cells, it is also relevant that IFN-I is a potent inducer of IL-15 [97].

Additional cytokines also influence memory cell formation during the contraction phase. IL-12 is a pro-inflammatory cytokine that can be produced by phagocytes during early infection [98] and acts as a third signal for T cell activation [3,99]. It was recently shown that IL-12 influences memory CD8⁺ T cell formation [20]. Here, IL-12-deficient mice were used to show that a greater population of memory T cells are formed after infection with *Listeria*-OVA relative to that of wild-type mice [20]. Interestingly, these mice were more resistant to subsequent infection but more susceptible to primary infection owing to their inability to produce an adequate number of effector CD8⁺ T cells [20]. The authors speculate that in the absence of IL-12, CD8⁺ T cells were driven to become memory T cells rather than effector T cells, and indeed their *in vitro* studies show that addition of IL-12 increases IFN γ production, an effector T cell characteristic [20]. IL-12 has also been shown to down-regulate expression of eomes in CD8⁺ effector T cells during infection [19] while up-regulating the expression of T-bet [5,19], indicating the role IL-12 may play in shaping the memory CD8⁺ T cell compartment.

Another cytokine recently found to impact CD8⁺ T cell responses is IL-10 [100,101]. Here, reduced numbers of CD8⁺ memory T cells in IL-10-deficient mice were seen, even when mice were treated with antibiotics 24 hours post-infection [101]. In contrast, a negative role for IL-10 in the generation of CD8⁺ memory T cells has also been observed [100]. Specifically, these authors co-transferred wild-type and IL-10 receptor-deficient antigen-specific CD8⁺ T cells, and observed that upon *Listeria* infection, the receptor-deficient T cells generated higher frequencies of memory cells. To explain these different results, it was speculated that despite treatment with ampicillin 24 hours post-infection, *Listeria* may have persisted at higher levels in the IL-10-deficient mice [100,101]. In addition to IL-10, recent evidence also indicates a role for tumor necrosis factor (TNF) in down-regulating the CD8⁺ T cell immune response against LCMV [102]. These and other findings indicate the increasing importance of cytokines in regulating the response of CD8⁺ T cells [103] and these external factors play an important role in influencing the transition from effector to memory CD8⁺ T cells.

Concluding remarks

An understanding of both the cell intrinsic and extrinsic factors related to the generation of CD8⁺ memory T cells allows one to begin consideration of how these different factors interrelate, and in particular, how cytokines such as IL-2, IL-7, and IL-15 may mediate life and death signals to CD8⁺ T cells. For example, signaling by γ_c -family cytokine members induce activation of the Jak/Stat and PI3K survival pathways. These cytokine-dependent intracellular signaling events occur concomitantly with regulation of factors such as Bcl2 and Bim leading to enhanced survival, among other functional outcomes [104,105]. There are however both positive and negative regulators of this process. For example, continued IL-2 signaling initially activates Blimp during the immune response, which in turn represses additional IL-2 transcription [50]. In contrast, IL-12 signaling regulates T-bet and eomes, which, leads to

upregulation of the IL-2/IL-15 receptor subunit, CD122, and thus, enhances IL-2 and IL-15 signaling. Given the similarity between the role of T-bet and Id2 in the effector to memory T cell transition, it is tempting to consider that similar cytokines may also influence Id2 expression. These examples illustrate the complex molecular pathways that integrate the interaction between intrinsic and extrinsic factors to attenuate and drive the generation of CD8⁺ memory T cells. An enhanced understanding of these complex factors will be instrumental in understanding not only CD8⁺ memory T cell but also other memory cells of the immune system.

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