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REVIEW

Similarities and differences in CD4+ and CD8+ effector and memory T cell generation

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Naive CD4+ and CD8+ T cells undergo unique developmental programs after activation, resulting in the generation of effector and long-lived memory T cells. Recent evidence indicates that both cell-intrinsic and cell-extrinsic factors regulate memory T cell differentiation. This review compares and contrasts how naive CD4+ and CD8+ T cells make the transition to effector and/or memory cells and discusses the implications of these findings for vaccine development.

Antigen-specific CD4+ and CD8+ T cells exist at very low frequencies in naive hosts. After infection or immunization, naive T cells undergo clonal expansion, culminating in a higher frequency of antigen-specific cells with more rapid effector function. These quantitative and qualitative changes in T cell numbers and function constitute immune memory, and these memory T cells, in concert with antibody responses, form the basis for protective immunity against infection and disease. Although T cell immunity is mostly beneficial, T cell responses have the potential to cause immunopathology in certain situations. Thus, for both CD4+ and CD8+ responses, a careful balance between protective and proinflammatory effects must be maintained.

After being activated, naive CD4+ T cells differentiate into functional subsets called T helper type 1 (T_H1) and T helper type 2 (T_H2) cells, based on their production of cytokine interferon (IFN)-γ and interleukin (IL)-4, respectively[1]. T_H1 cells are essential for protection against a variety of intracellular infections, whereas T_H2 responses can be protective against certain extracellular infections. CD8+ T cells mediate their effector functions through production of cytokines such as IFN-γ and tumor necrosis factor (TNF)-α and/or by cytolytic mechanisms. Such responses are important in preventing or maintaining control against disease in a variety of intracellular infections and perhaps also against certain tumors. This review discusses the similarities and differences in the mechanisms regulating the generation and maintenance of CD4+ and CD8+ effector and memory T cells.

Initial activation of CD4+ and CD8+effector T cells

For both CD4+ and CD8+ T cells, transient exposure to antigen is sufficient to induce an antigen-independent program of proliferation and differentiation[2–5]; however, the strength and duration of antigenic plus costimulatory stimulation can affect the differentiation process and regulate the functional qualities of the effector and memory cells that develop[6,7]. Although both CD4+ and CD8+ T cells undergo an autonomous program of differentiation, the kinetics and efficiency of CD8+ T cell proliferation differ substantially from those of CD4+ T cell proliferation. The time of antigen exposure required to launch the proliferative program for naive CD8+ T cells seems to be less than that required for naive CD4+ T cells[3,4,8,9]. CD8+ T cells also divide sooner and have a faster rate of cell division than do CD4+ T cells[3,5,10,11]. There are also similarities and differences in their acquisition of effector functions. Both naive CD4+ (ref. 12) and CD8+ (ref. 13) T cells express little or no mRNA for effector molecules such as IFN-γ, IL-4, TNF or perforin. The differentiation of naive CD4+ T cells into cells with IFN-γ- or IL-4–producing capacity is a complex process involving the interaction of specific cytokines[1] with cell-signaling proteins[14] and transcriptional factors, with subsequent chromatin remodeling[15]. For T_H1 differentiation, IL-12 produced from antigen-presenting cells in response to Toll-like receptor stimulation acts in conjunction with signaling and transcription factors such as signal transducer and activator of transcription 4 (STAT4) and T-bet to induce a functional T_H1 response characterized by IFN-γ production[16,17]. Similarly, IL-4, STAT6 and the transcription factor GATA-3 are essential in controlling the generation of T_H2 cells[18]. These extensive regulatory mechanisms put considerable constraints on the relative efficiency of generating large numbers of IFN-γ- and IL-4-producing CD4+ T cells (**Fig. 1**).

In contrast to CD4+ T cells, naive CD8+ T cells more readily develop into effector cells after short-term primary stimulation (**Fig. 1**). A direct example that compared differences in the efficiency of generating cytokine-producing CD4+ and CD8+ T cells was the transfer of naive T cell receptor–transgenic CD4+ and CD8+ T cells specific for ovalbumin into normal mice subsequently infected with the bacterium *Listeria monocytogenes* expressing ovalbumin[19]. This model is useful for studying both CD4+ T_H1 and CD8+ T cell effector cell differentiation because of the potent induction of IL-12 and the efficient processing of antigen for major histocompatibility complex (MHC) class I presentation by the listeria infection. At 8 days after infection, >85% of ovalbumin-specific CD8+ T cells but <7% of ovalbumin-specific CD4+ T cells produced IFN-γ. In addition, CD8+ T cells underwent extensive proliferation, whereas CD4+ T cells divided a limited

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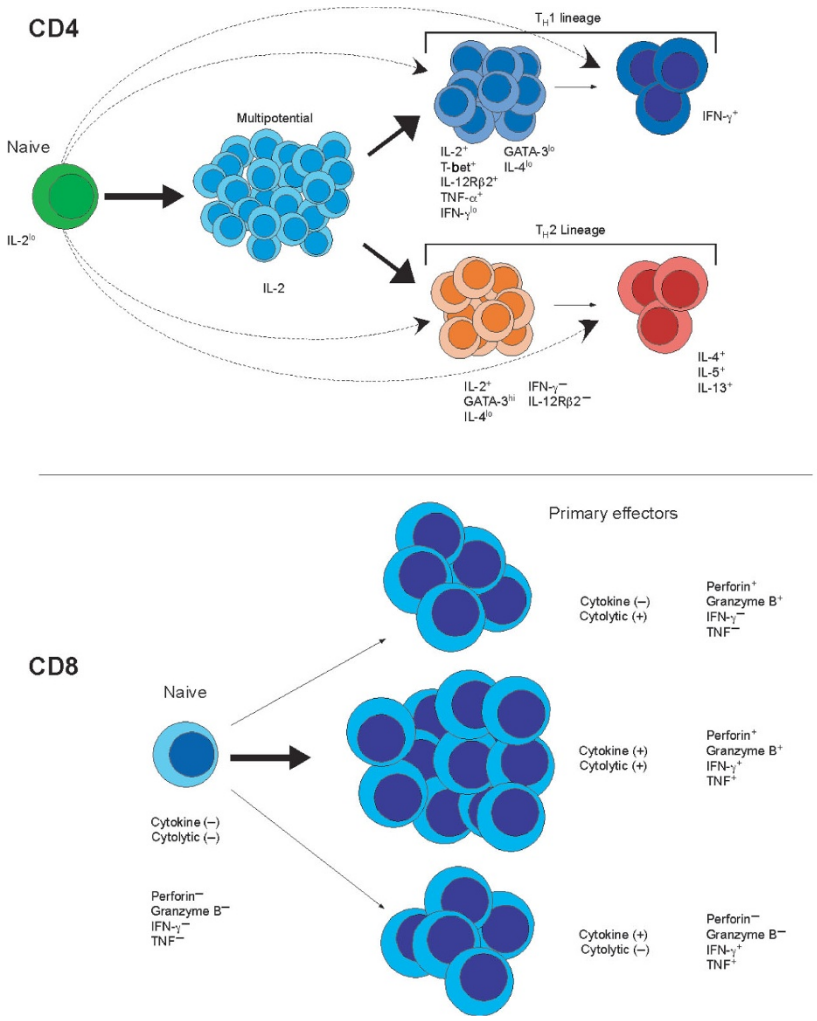

Figure 1 Generation of CD4+ and CD8+ effector T cells. Naive CD4+ T cells stimulated in T_H1- or T_H2-polarizing conditions undergo progressive differentiation toward becoming IFN-γ- or IL-4-producing cells. After activation, there is notable heterogeneity at the single-cell level in terms of cells producing IFN-γ or IL-4. Within a population of activated cells, a proportion of cells secrete IL-2 and have the capacity to develop into IFN-γ- and/or IL-4-producing cells (Multipotential). Another more differentiated population of cells has mRNA expression of markers denoting T_H1 or T_H2 cells but has low mRNA and protein secretion of the signature cytokines IFN-γ or IL-4, respectively. Such cells are committed toward a defined T_H1 or T_H2 lineage and have the capacity to become IFN-γ- or IL-4-producing cells after subsequent stimulation. The most differentiated cells can secrete IFN-γ or IL-4 immediately after stimulation. Naive CD8+ T cells efficiently develop into effector cells with cytolytic and/or cytokine-producing capacity after primary stimulation.

number of times. Another point regarding differences in the generation of effector cytokine production by CD4+ and CD8+ T cells is illustrated by an examination of how the factors involved in polarizing CD4+ T cell responses regulate the differentiation of CD8+ T cells. Similar to their effect on CD4+ T cells, IL-12 and IL-4 *in vitro* generate CD8+ effector T cells called T_c1 or T_c2 based on their production of IFN-γ or IL-4, respectively; however, in contrast to results with CD4+ T cells, there is less evidence demonstrating these cytokines are essential in polarizing CD8+ T cell responses *in vivo*. Also, it is notable that T-bet, the main transcription factor controlling T_H1 differentiation, has little effect on the generation of CD8+ IFN-γ-producing T cells[20]. These observations reflect substantial differences in the efficiency and factors regulating the induction of CD4+ and CD8+ cytokine-producing cells.

Finally, costimulatory requirements also seem to be different for the activation of naive CD4+ and CD8+ T cells. This has been demonstrated best in studies examining antiviral responses using mice deficient in various costimulatory pathways[21]. For example, lymphocytic choriomeningitis virus (LCMV)-specific CD8+ effector T cell responses were efficiently induced in mice deficient in CD40L, CD28 or OX-40, whereas in these same mice that had generated a potent effector CD8+ T cell response, virus-specific CD4+ T cell responses were severely compromised. In contrast, the reverse pattern was seen in 41BB-deficient mice: virus-specific CD4+ T cell responses were normal, whereas CD8+ T cell responses were slightly reduced. These crucial differences between CD4+ and CD8+ T cells must be considered in designing better T cell vaccines. Also, additional studies are needed to increase the understanding of the molecular basis for these distinct costimulatory requirements.

The magnitude of CD4+ versus CD8+ T cell responses

The development of techniques to analyze responses at the single-cell level, such as MHC tetramers, enzyme-linked immunospot analysis and intracellular cytokine staining, has allowed more accurate quantification of antigen-specific T cells. Because of the much greater availability of reagents such as MHC class I tetramers and well defined immunodominant epitopes for viral infections, the preponderance of data comparing frequencies of CD4+ and CD8+ effector cytokine producing cells has been generated in mouse models of viral infection. The frequency of virus-specific CD8+ IFN-γ-producing cells is substantially higher than that for CD4+ IFN-γ-producing cells both at the peak and throughout the memory response in mice infected with LCMV[22], Sendai[23] or vaccinia[24]. Similarly, in human Epstein-Barr virus infection, the clonal expansion of Epstein-Barr virus–specific CD8+ T cells is greater than that for CD4+ T cells[25]. Comparable data were also obtained in mouse models of infection with *L. monocytogenes*[19]. These notable differences in the magnitude of CD4+ and CD8+ T cell responses to such infections could be due to many factors. First, because the intrinsic proliferative capacity of CD8+ T cells seems to be greater than that of CD4+ T cells, a small change in the number of cell divisions would have a substantial effect on the number of effector cells at the end of the response. For LCMV infection, it was estimated that CD4+ T cells underwent approximately 9 cell divisions, compared with 15 divisions for CD8+ T cells, during the first week after infection[22]. Similarly, for listeria infection, there was also a difference in cell division between CD4+ and CD8+ T cells, resulting in higher numbers of listeria-specific CD8+ T cells[19]. Another reason for the higher CD8+ responses may be that the efficiency of antigen presentation is better for CD8+ than for CD4+ T cells. This difference

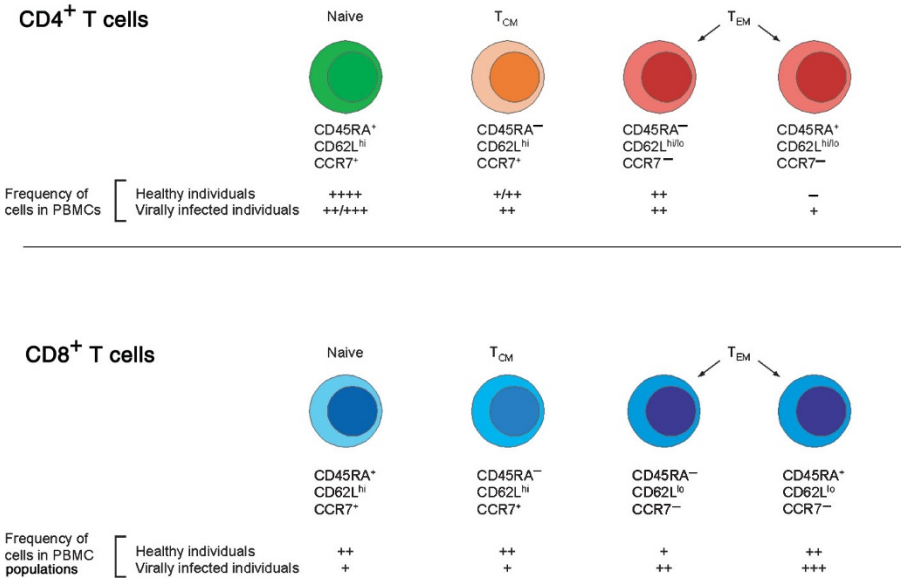

Figure 2 Subsets of memory T cells. Several subsets of CD4+ and CD8+ T cells based on differential expression of CD45, CD62L and CCR7 have been characterized from human PBMC populations. The relative frequencies of CD4+ and CD8+ T cells expressing such markers from PBMC populations of either healthy individuals or those exposed to chronic viral infection are shown. CCR7+ memory cells are T_{CM}; CCR7−memory cells are T_{EM}. In normal individuals, there are very few CD4+CD45RA+CCR7− cells compared with CD8+ T cells. 2°, secondary.

in antigen presentation efficiency could be because fewer cells express MHC class II compared with MHC class I. In addition, viral infections, through their normal endogenous processing, or listeria infection, through listeriolysin O, would allow for very efficient MHC class I antigen presentation. A third consideration for the better CD8+ responses is that cytokines such as IL-2 and IL-15 may have a more profound effect in sustaining the proliferative capacity of CD8+ T cells than for CD4+ T cells early in the course of activation[8]. Finally, regulatory mechanisms mediated by cytotoxic T lymphocyte antigen 4 (CTLA-4) or increased susceptibility to apoptosis could have a greater effect in limiting the expansion and promoting the death of CD4+ effector T cell responses[26].

Differences in the magnitudes of CD4+ and CD8+ effector responses described above with these acute viral and listeria infections might not be a general property of all infections. For example, in mouse models of *Leishmania major*[27,28] and *Mycobacterium tuberculosis*[29,30] infection, frequencies of CD8+ IFN-γ-producing cells were demonstrably less than those noted after viral or listeria infections. In addition, the frequencies of CD4+ IFN-γ-producing cells were higher[27,30,31] and comparable to those of CD8+ IFN-γ-producing cells. The limited frequency of CD8+ effector T cell responses seen for leishmania and tuberculosis infections could be due to less efficient processing of these pathogens for MHC class I presentation compared with that of viral or listeria infections. Thus, several factors, such as the type of pathogen, the nature of the infection (acute versus chronic) and the cells presenting antigen, will regulate the frequency of CD4+ and CD8+ effector T cells.

Heterogeneity of CD4+ and CD8+ effector T cell responses

In a developing immune response, essentially all activated CD8+ T cells that have proceeded through a full proliferative cycle (>8–10 divisions) can be classified as effectors based on their production of cytokines (for example, IFN-γ or TNF) and/or secretion of perforin or granzyme (**Fig. 1**). Because CD8+ T cells can mediate effector function through cytokines and/or cytolytic activity, there can be heterogeneity among CD8+ T cells in terms of the mechanisms by which they mediate their effector function[32]. In addition, evidence indicates that CD4+ T cells can regulate the 'quality' of the CD8+ T cells generated[33–35]. Whereas the frequency and effector function of CD8+ T cells generated in the absence of CD4+ T cells are not altered during a primary response, such cells are demonstrably impaired in their capacity to proliferate and mediate effector function in a secondary challenge. It has been proposed that CD4+ T cells, through CD40L, may imprint a unique 'molecular signature' on effector CD8+ T cells, endowing them with their capacity for improved memory cell function[36].

In studies of the factors regulating T cell differentiation, naive CD4+ T cells stimulated *in vitro* in optimal stimulatory conditions favoring T_H1 or T_H2 differentiation show comparable activation based on cell size, cell division and surface markers after short-term stimulation, and have been referred to as effectors based on their enhanced capacity to more rapidly produce IFN-γ or IL-4 compared with naive cells. However, within such a population of comparably activated cells there is considerable heterogeneity at the single-cell level in terms of cells producing the T_H1- and T_H2-defining cytokines IFN-γ and IL-4, respectively (refs. 12,37–40). Given this heterogeneity of the CD4+ T cell response[41,42], we propose the existence of several distinct types of cells within a population of activated CD4+ T cells. The first population would be cells that spontaneously produce cytokines without further antigenic stimulation *in vitro*. Such cells are rare and usually detected only in situations in which there is active antigenic stimulation *in vivo*, such as during an ongoing infection. The second population would be cells that produce IFN-γ or IL-4 after short-term *in vitro* stimulation. A third population would consist of cells that do not yet express IFN-γ or IL-4 protein but have an mRNA expression pattern consistent with commitment of the cells toward a T_H1 (IL-2^{hi}IFN-γ^{lo}T-bet^{hi}IL-12RB2^{hi}GATA-3^{lo}IL-4^{lo}) or T_H2 (IL-2^{lo}IFN-γ^{lo}IL-12RB2^{lo}GATA-3^{hi}IL-4^{lo}) phenotype[12]. Such cells have the capacity to produce exclusively IFN-γ or IL-4 depending on the conditions of the subsequent stimulation and would be called T_H1- or T_H2-lineage cells. In terms of lineage commitment toward a fixed T_H1 or T_H2 phenotype, CD4+ T cells need to undergo at least three cell divisions[43]. The fourth population would be cells that have not committed toward a T_H cell lineage but have the capacity to produce IFN-γ and/or IL-4 after further stimulation. Such cells are at the earliest stage of differentiation and are most prevalent when naive CD4+ T cells are activated in nonpolarizing conditions[44,45]. The relative frequency of cells at each stage of the differentiation pathway will be determined by the strength and duration of the signal delivered by antigen and/or antigen-presenting cells. This progressive model of T_H cell differentiation has important implications for which populations of activated CD4+ T cells become long-term memory cells and will thus help determine the type of vaccine formulations most optimal for diseases requiring T_H1 responses. Finally, this model of differentiation focuses on how CD4+ T cells develop into IFN-γ- or IL-4-producing cells. Although these clearly represent true effector cells, activated CD4+ T cells secreting neither IFN-γ nor IL-4 may exert effector and/or

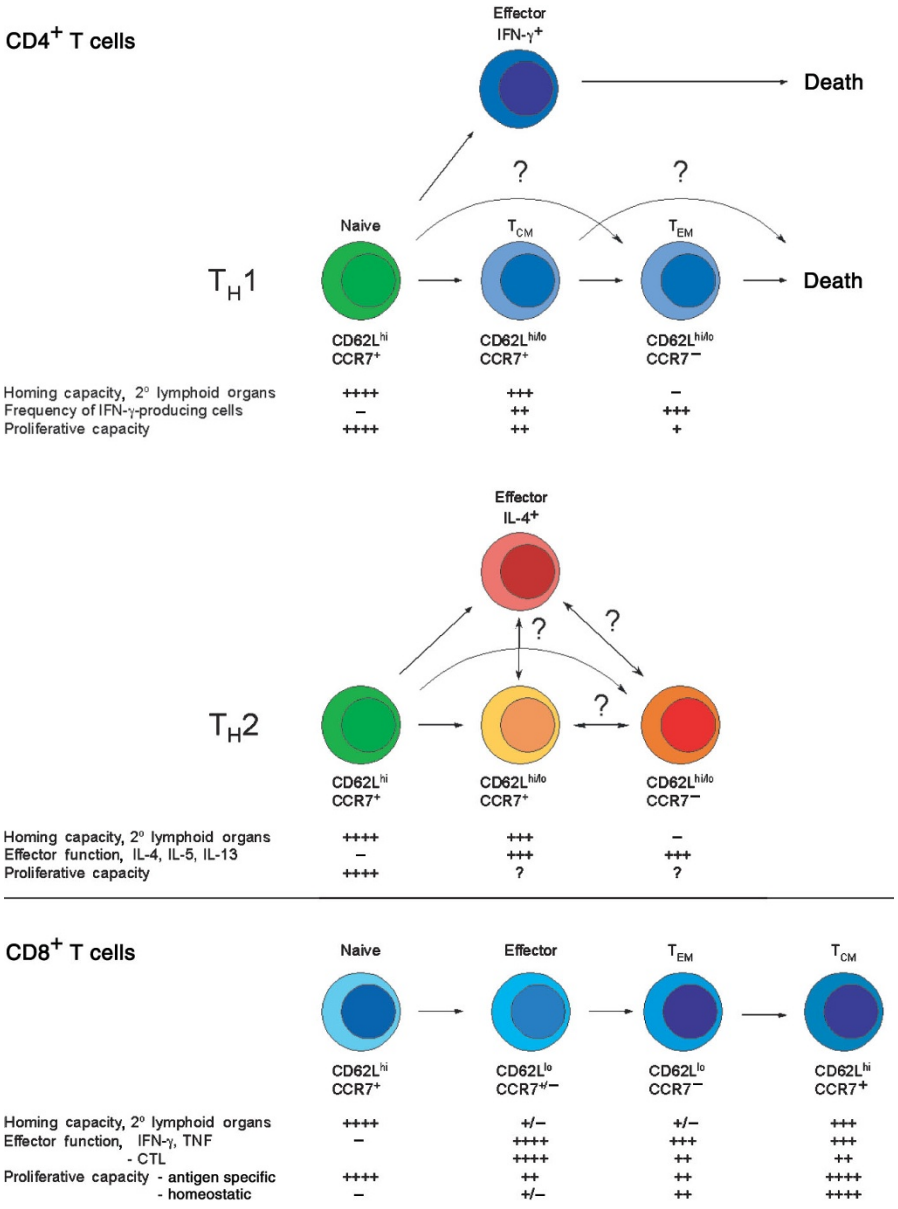

Figure 3 Lineage differentiation of memory T cells. Naive CD4+ T cells have multiple potential pathways for differentiating from naive to effector and then to memory cells. For T_H1 cells, because CD4+ IFN-γ-producing effector cells are relatively short-lived *in vivo* and do not efficiently develop into memory cells, differentiation is likely to follow a progressive and linear pathway. CD4+ IL-4-producing T cells may have multiple potential pathways of memory cell differentiation. CD8+ T cells readily develop into effector cells after activation. After the contraction phase of the response, CD8+ T cells seem to proceed to effector T_{EM} and then T_{CM} cells. T_{EM} and T_{CM} cells have comparable cytolytic and cytokine-producing capacity, but the T_{CM} population has enhanced proliferative capacity.

regulatory effects through a variety of other soluble factors and cell surface molecules. Given the considerable heterogeneity within a population of activated CD4+ T cells, future efforts should focus on characterizing the functional aspects of such populations at the single-cell level to better define their effector and/or regulatory functions and their capacity to become memory cells.

The type of tissue-homing molecules expressed by activated T cells can also manifest heterogeneity among effector T cell populations. *In vitro* stimulation of naive CD8+ T cells by antigen-pulsed dendritic cells derived from Peyer's patches selectively up-regulated expression of gut-homing integrin α4β7 on activated T cells[46]. In contrast, antigen presentation by dendritic cells derived from peripheral lymph nodes or spleen was not as efficient in inducing expression of α4β7 on activated CD8+ T cells. These *in vitro* observations need to be confirmed *in vivo*, but this study establishes, in principle, a mechanism for generating heterogeneity among effector T cell populations.

Factors regulating the death of effector T cells

Because of the potent proinflammatory effects elicited by activated T cells, there are multiple control mechanisms to regulate the magnitude of the effector T cell response. The initial activation and expansion phase of both CD4+ and CD8+ T cell responses is invariably followed by a death phase during which most (~90%) effector cells are eliminated. This is a complex process; multiple pathways seem to be involved in the apoptosis of effector T cells[32,47]. These include interactions between Fas and Fas ligand, and TNF and TNF receptors I and II, as well as costimulatory molecules such as CD40 and CD40 ligand. Cytokine withdrawal also is involved in this 'downsizing', as IL-2 treatment during the contraction phase can result in both increased proliferation and survival of effector CD4+ and CD8+ T cells[48]. In addition to the aforementioned mechanisms, effector molecules such as perforin and IFN-γ seem to be involved in regulating the effector T cell response. For example, mice deficient in perforin or IFN-γ have increased numbers of CD8+ T cells in the expansion and/or contraction phase[49]. Moreover, IFN-γ-deficient mice also have a 30–50% increase in the number of activated CD4+ T cells in mouse models of mycobacterial infection[50] and experimental autoimmune encephalitis[51]. An essential function for IFN-γ in the regulation of CD4+ T cell responses was further substantiated in studies showing that a homogeneous population of CD4+ IFN-γ-producing cells was short lived *in vivo* but could be rescued if IFN-γ was inhibited at the time of activation[12,52]. The mechanism by which IFN-γ limits CD4+ T cell expansion is through caspase-8-dependent apoptosis[53]. Thus, whereas IFN-γ is obviously an important regulator of cell death for both CD4+ and CD8+ effector T cells, it is notable that CD8+ effector T cells have a much greater capacity to develop into stable long-term resting memory cells. This indicates a fundamental characteristic unique to CD4+ cells that renders them more susceptible to cell death when they become IFN-γ-producing cells[54].

In addition to understanding the mechanisms controlling the death of antigen-specific effector CD4+ and CD8+ T cells generated after infection or immunization, it is instructive to review additional factors that regulate the survival of activated T cells in response to environmental or endogenous self antigens. Mice lacking IL-2, IL-2 receptor, Fas, Fas ligand or CTLA-4 (ref. 47) have manifestations of

autoimmune disease. For most of these deficient mice, there is selective dysregulation and expansion of activated $CD4^+$ T cells. These data provide additional evidence for multiple checkpoints for the elimination of activated $CD4^+$ T cells versus $CD8^+$ T cells.

Memory T cell subsets

During the past few years, two parallel but independent lines of investigations, one in humans and the other in mice, have had a profound effect on our thinking about memory T cells. Subsets of memory $CD4^+$ and $CD8^+$ T cells in peripheral blood mononuclear cells (PBMCs) of humans were identified based on cell surface markers, in particular, the expression of CCR7, a molecule that mediates homing to lymph nodes through high endothelial venules[55]. Differences were also noted in the ability of these memory T cell subsets to produce effector cytokines, with the $CCR7^-$ subset being more efficient. Based on these findings, two functionally distinct memory T cell subsets were proposed: $CCR7^-$ effector memory T cells (T_{EM} cells) present in the blood, spleen and nonlymphoid tissues that will rapidly respond to antigen by producing effector molecules, and $CCR7^+$ central memory T cells (T_{CM} cells)in lymph nodes, spleen and blood (but not in nonlymphoid tissues) that are slower in making cytokines or becoming killer cells than are T_{EM} cells. It was also postulated that T_{CM} cells located in secondary lymphoid organs after stimulation with antigen would decrease CCR7 expression, migrate to peripheral organs, and become T_{EM} cells. Thus, this model indicated that CCR7 expression could be used to distinguish the functional capacity and pathway of differentiation for memory T cells. Around this time, several groups studying T cell immunity in mouse models showed that a substantial number of memory $CD4^+$ and $CD8^+$ T cells was present in nonlymphoid tissues and persisted at these sites for extended periods[56,57]. These studies also suggested that these nonlymphoid memory T cells respond faster to antigen (become effectors more rapidly) than do memory T cells residing in lymphoid tissues; in addition, their cell surface phenotype ($CD62L^{lo}CCR7^-$) was consistent with the T_{EM}-versus-T_{CM} hypothesis. Several studies in both humans[58] and mice[59,60] have shown, however, that CCR7 and CD62L expression does not correlate with the effector functions of memory $CD8^+$ T cells. These studies found that both memory $CD8^+$ T cell subsets were equally efficient in producing effector cytokines or becoming killer cells after restimulation with antigen. In studies assessing functional differences in $CD4^+$ T cells based on expression of CCR7, $CD4^+CCR7^-$ cells were enriched for production of IFN-γ and IL-4 from human PBMCs[55,61]; however, because of the much higher frequency of memory $CD4^+$ T cells expressing CCR7 (~80%) than those not expressing CCR7 (~20%) in normal individuals, the total number of effector $CD4^+$ T cells in humans is greater among $CCR7^+$ than $CCR7^-$ cells[61]. Similarly, there is evidence in mice that memory T_H1 cells are mainly $CCR7^+$ (ref. 60). It thus seems that CCR7 and CD62L may not be useful markers for distinguishing effector function for $CD4^+$ and $CD8^+$ T cells, although these markers are valuable for defining anatomic location (particularly homing to lymph nodes).

Additional studies have been done in humans using a more extensive panel of markers to characterize memory and effector T cells present in PBMCs of both normal individuals and those infected with human immunodeficiency virus (HIV; **Fig. 2**). These results emphasize the heterogeneity of memory T cells and also demonstrate differences between $CD4^+$ and $CD8^+$ T cells. A notable finding is that in healthy individuals, a relatively large number of $CD8^+$ cells are $CCR7^-CD45RA^+$, compared with $CD4^+$ T cells[55]. This may reflect differences in the inherent stability of $CD4^+$ and $CD8^+$ memory T cells that are $CCR7^-$ in normal steady-state conditions. HIV-infected individuals do have an increased frequency of cells expressing $CD8^+CD45RA^+CCR7^-$, which may have functional consequences in HIV pathogenesis[62]. These $CD8^+CD45RA^+CCR7^-$ T cells may represent terminally differentiated HIV-specific effector T cells with limited potential for proliferation and expansion after antigenic stimulation[63].

Models of memory T cell differentiation

The transition of effector $CD4^+$ and $CD8^+$ T cell responses to resting memory cells occurs after massive contraction of the response and is coincident with clearance of antigen[11,64,65]. $CD8^+$ effector cells that survive apoptosis during this contraction phase develop into durable long-term resting memory cells[22]. The initial 'burst size' of the $CD8^+$ effector T cell response correlates with the magnitude of the long-term memory response[66]. However, the larger primary burst size seen with $CD8^+$ T cells in response to viral infections is associated with a more profound decay than is noted for $CD4^+$ T cells, probably reflecting a homeostatic mechanism to maintain a certain number of $CD8^+$ memory cells[67]. As discussed above, $CD4^+$ IFN-γ-producing cells do not efficiently develop into resting memory cells, although activated $CD4^+$ T cells stimulated in T_H1-polarizing conditions that have not yet become IFN-γ-producing cells (T_H1-lineage cells or uncommitted) are able to develop into long-term memory cells. For T_H2 cells, a heterogeneous population of activated $CD4^+$ T cells cultured in T_H2 conditions develops into resting memory cells[68]. These observations indicate that $CD4^+$ IFN-γ^+ cells differ from $CD8^+$ effector T cells in their transition to resting memory cells.

$CD8^+$ memory T cell differentiation and the lineage relationships between T_{CM} and T_{EM} cells *in vivo* were analyzed in mice after infection with LCMV[13,59]. In this model, T_{EM} cells converted to T_{CM}, but not vice versa, when cells were monitored in the absence of antigen. In the presence of antigen, T_{CM} cells did convert to T_{EM}. These data provided strong evidence that T_{CM} cells derive from T_{EM} cells, at least in the setting of an acute viral infection that is readily cleared. In addition to establishing a lineage relationship between T_{EM} and T_{CM} cells, these studies also showed that memory $CD8^+$ T cell qualities and gene-expression patterns continued to change for several weeks after resolution of the virus infection. In this model of linear and progressive differentiation (**Fig. 3**), several key memory $CD8^+$ T cell qualities were acquired only gradually after antigen clearance. These included the ability to rapidly proliferate after re-exposure to pathogen, the ability to produce IL-2 and the ability to persist long-term *in vivo* by undergoing homeostatic proliferation in response to IL-15 and IL-7. The ability to rapidly exert effector functions (killing and IFN-γ production) after re-exposure to antigen did not diminish with time; hence, these 'late' memory $CD8^+$ T cells were very effective in conferring protective immunity.

To conclude, there are several potential models for the generation of memory $CD4^+$ and $CD8^+$ T cells. $CD8^+$ T cells seem to follow a linear differentiation pathway from naive → effector → T_{EM} → T_{CM} → T_H1 and T_H2 memory differentiation seems more complex and potentially different from $CD8^+$ T cells. The memory differentiation models described here (**Fig. 3**) are based on studies in mice, in which it is easier to track antigen-specific T cells and, more importantly, in which it is possible to do adoptive-transfer experiments with defined T cell populations to establish lineage relationships between various memory T cell subsets. Studies examining memory $CD8^+$ T cell differentiation have also been done in humans. One study has suggested that T_{EM} and T_{CM} are largely independent subpopulations, whereas other studies have suggested that T_{EM}-like cells are the end product of memory development[62,63,69]. At face value, these findings seem to be at odds

with the mouse studies describing a naive → effector → T_{EM} → T_{CM} pathway for $CD8^+$ T cells; however, the human studies analyzed $CD8^+$ memory T cells generated to persistent viral infections such as HIV, cytomegalovirus and Epstein-Barr virus, whereas the mouse studies were done after an acute LCMV or *L. monocytogenes* infection. Thus, it is likely that the differences in the findings are not because of differences between mice and humans but result from the examination of $CD8^+$ T cell differentiation during acute versus chronic infection. This issue needs to be addressed in future studies using mouse models and also in humans. A better definition of the quality of memory T cells generated during acute versus chronic infections will provide a better understanding of the immunopathogenesis of persistent infections and also help in vaccine design.

Requirements for maintenance of $CD4^+$ and $CD8^+$ memory T cells

What is required to sustain $CD4^+$ and $CD8^+$ memory T cells has been reviewed[70]. For $CD4^+$ and $CD8^+$ memory cells, antigen and MHC class I or II are not essential for survival. For $CD8^+$ memory T cells, IL-7 and IL-15 are important in regulating T cell survival and turnover, respectively. $CD4^+$ memory T cells do not require expression of the common cytokine receptor γ chain for survival, providing indirect evidence that cytokines such as IL-2, IL-4, IL-7, IL-9 and IL-15 may not be required for the maintenance of $CD4^+$ memory cells[71]; however, IL-7 may indeed be important in sustaining the survival of memory $CD4^+$ T cells[72]. With regard to costimulatory molecules, there is evidence that OX-40 and OX-40 ligand may be involved in sustaining $CD4^+$ T cell survival[73].

Differences in stability of $CD4^+$ and $CD8^+$ memory T cells

When the stability of $CD4^+$ and $CD8^+$ memory cells was directly compared, mice infected with LCMV were found to have $CD8^+$ memory responses that were sustained at a steady state for 3 years, whereas memory $CD4^+$ T cells gradually decreased over this 3-year period[22]. This is the clearest evidence that, during a viral infection, there is a demonstrable difference in the longevity of $CD4^+$ and $CD8^+$ memory cells. The considerable stability noted for memory $CD8^+$ T cell responses after viral infection in naive mice needs to be considered in a more physiologic context in which multiple exposures to different viral infections occur over a lifetime in the host. Exposure to heterologous viral infections substantially diminished the frequency of $CD8^+$ T cells from a previous viral infection[74]. Finally, when the frequency of virus-specific $CD4^+$ and $CD8^+$ T cells after heterologous viral infections or protein antigen immunizations was compared, again there was reduction of memory $CD8^+$ T cells but not $CD4^+$ T cells specific to viruses from earlier infections[75]. Thus, although $CD4^+$ and $CD8^+$ memory pools seem to be regulated independently, the history of prior antigenic exposure and homeostatic mechanisms may serve to regulate the magnitude of memory $CD8^+$ responses.

Consequences for vaccine development

The factors regulating the generation and maintenance of $CD4^+$ and $CD8^+$ memory/effector T cells have practical relevance for the design of vaccines against infections requiring such responses. For infections such as *M. tuberculosis* or *L. major*, T_H1 cells are necessary and may be sufficient to mediate protection after vaccination. Given the factors regulating T_H1 effector and memory differentiation discussed above, there are two main considerations in designing vaccines against diseases in which T_H1 cells are required. First, a vaccine must be able to direct differentiation of naive $CD4^+$ T cells into T_H1 cells. This can be done by targeting the vaccine to host antigen-presenting cells through specific Toll-like receptors to produce a favorable cytokine environment (IL-12, IFN-γ and IFN-α) and/or provide T_H1-polarizing cytokines exogenously as part of the vaccine. Although such approaches have been validated in mouse models, current non-live vaccine formulations and the types of adjuvant (such as alum) used in humans have not been effective at inducing potent and sustained T_H 1 responses. With an improved understanding of the cellular and molecular mechanisms of antigen presentation *in vivo*, more directed and effective approaches for inducing such responses in humans should be forthcoming. The second main factor is sustaining T_H1 responses *in vivo*. It may be difficult for a non-live vaccine to induce lifelong protection against diseases like *M. tuberculosis* and *L. major* through T_H1 cells without continuous boosting. Live attenuated vaccines against *M. tuberculosis* and *L. major* remain the 'gold standards' for protection against such diseases in both mouse models and in humans. Whether the long-lived protection conferred by live vaccines is due to a higher frequency of antigen specific T cell or to the continuous generation of cells from persistence of antigen remains an unanswered question. Finally, live vaccines also elicit $CD8^+$ T cell responses. Because of the relative durability of $CD8^+$ T cell memory responses and their potential effector function in mediating protection against tuberculosis and leishmania infection, vaccines for such infections should generate both T_H1 and $CD8^+$ T cells. At present, non-live vaccine regimens capable of inducing both T_H1 and $CD8^+$ T cell responses include prime-boost approaches, with DNA or protein plus adjuvant as a prime followed by a recombinant replication–deficient viral boost.

For vaccines against HIV and malaria, it is apparent that both humoral and cellular immune responses can be important in preventing infection and/or disease. For the optimization of $CD8^+$ T cell responses, there are qualitative and quantitative considerations. From a quantitative perspective, as the magnitude of the peak $CD8^+$ effector T cell response correlates to the long-term memory response, vaccines should be designed to maximize this peak response. Either live attenuated or replication-deficient recombinant viral vectors seem to be the most potent type of vaccines for inducing a high frequency of $CD8^+$ T cells responses. From a qualitative point of view, as $CD4^+$ T cells are involved in programming $CD8^+$ T cells for subsequent expansion in a secondary response, vaccines designed to elicit $CD8^+$ T cell responses should also have the capacity to induce $CD4^+$ T cell responses. As the peak of the $CD8^+$ T cell response is followed by the contraction phase, in which >90% of the effector cells die, interventions that could potentially limit the massive contraction phase may lead to enhanced memory responses[48]. In addition, in terms of boosting existing $CD8^+$ T cell responses, $CD8^+$ T cells expressing CCR7 and CD62L (T_{CM}) have much more proliferative capacity than do $CCR7^-CD62L^{lo}$(T_{EM}) cells. Hence, the boosting of the $CD8^+$ T cell response should be timed to occur when the number of T_{CM} cells is highest. This timing in turn will depend on the potency and type of response elicited by the primary immunization. At present, the vaccine regimens used in human clinical trials for HIV and malaria include prime-boost immunization with plasmid DNA immunization followed by boosting with replication-deficient adenovirus or modified vaccinia virus. It is hoped that with improved capacity to measure antigen-specific T cell responses, these initial clinical studies should be helpful in defining immune correlates of protection that would allow further refinement and optimization of future vaccines.

It is important to consider the issue of vaccine-induced systemic versus mucosal T cell immunity. This is a crucial topic that merits far more detailed discussion in a separate review. Nevertheless, a few questions should be raised about mucosal and systemic responses in the context of vaccination. Perhaps the 'central' question is whether a vaccine must be administered mucosally for protection against a

mucosal infection or whether systemic immunization will also work. Some studies have suggested that mucosal delivery is necessary for optimal mucosal immunity, whereas others have documented excellent protection against mucosal challenge after parenteral immunization in a primate model of HIV infection. Clearly, the requirement for a mucosal- versus systemic-based vaccine is likely to vary depending on the pathogen. Overall, the potential importance of mucosal immune responses in mediating protection against infection raises specific questions about systemic and mucosal effector and memory T cells. For example, are qualitatively different effector T cells induced depending on the route of immunization? Will these cells differ in effector function and potential to generate memory T cells? Is the memory differentiation program same or different after mucosal versus systemic immunization? Can memory T cells persist at mucosal sites? If so, are cytokines like IL-15 and IL-7 involved in mucosal immunity, as they are in the maintenance of systemic memory T cells? Or will mucosal immunity be more dependent on antigen or another cytokine? In addition, do memory T cells move back and forth between mucosal and systemic sites? These are just a few of the many salient questions that need to be addressed about mucosal versus systemic T cell immunity. An understanding of these issues is essential for rational design of T cell–based vaccines.

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