Memories that last forever: strategies for optimizing vaccine T-cell memory

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For acute self-limiting infections a vaccine is successful if it elicits memory at least as good as the natural experience; however, for persistent and chronic infections such as HIV, hepatitis C virus (HCV), human papillomavirus (HPV), and human herpes viruses, this paradigm is not applicable. At best, during persistent virus infection the person must be able to maintain the integrity of the immune system in equilibrium with controlling replicating virus. New vaccine strategies are required that elicit both potent high-avidity CD8$^+$ T-cell effector/memory and central memory responses that can clear the nidus of initial virus-infected cells at mucosal surfaces to prevent mucosal transmission or significantly curtail development of disease. The objective of an HIV-1 T-cell vaccine is to generate functional CD8$^+$ effector memory cells at mucosal portals of virus entry to prevent viral transmission. In addition, long-lived CD8$^+$ and CD4$^+$ central memory cells circulating through secondary lymphoid organs and resident in bone marrow, respectively, are needed to provide a concerted second wave of defense that can contain virus at mucosal surfaces and prevent systemic dissemination. Further understanding of factors which can influence long-lived effector and central memory cell differentiation will significantly contribute to development of effective T-cell vaccines. In this review we will focus on discussing mechanisms involved in T-cell memory and provide promising new approaches toward expanding current vaccine strategies to enhance antiviral memory. (Blood. 2010;115:1678-1689)

Introduction

A common defining characteristic of immune memory is that it is both selective and parsimonious. After resolution of primary infection a small somewhat constant fraction of cells remain depending upon initial precursor frequency and the balance between T-cell receptor (TCR) signal strength and prosurvival signals received.[1,2] Memory cells are homeostatically maintained in a progrowth state poised to respond rapidly to secondary infection. The fidelity of memory and effectiveness to thwart disease is reflected in the multifunctional character of the recalled response. Memory T cells are heterogenous in terms of phenotype, function, and anatomical locations.[3-5] Understanding cytokine and costimulatory signals that influence transcriptional programs regulating T-cell differentiation and memory is key to manipulating vaccine responses. Furthermore, defining different subpopulations of memory CD8$^+$ T cells by differentiation and activation markers representative of transcriptional programs associated with protective recall responses will be key to predicting vaccine efficacy.

To date, strategies for targeted delivery of vaccines and inclusion of cytokines, chemokines, and immunomodulatory molecules for enhancing the magnitude of immune responses and memory have been mostly empirical. For HIV-1 vaccines this has involved the expression of HIV-1 genes, cytokines, and chemokines by in vivo delivery of plasmid DNA and recombinant viral vectors (both replicating and nonreplicating). Recombinant viral vectors that target antigen-presenting cells serve as a means to couple activation of the innate immune system to the induction of adaptive immunity and increase immunogenicity. It is assumed that the use of recombinant viral vectors could induce local innate responses that promote an adaptive immune response to recombinant antigens that might obviate the need for adjuvanting this category of vaccines. It is unclear how antiviral (vector) immunity competes with or skews long-term memory to recombinant antigens and which if any viral vector is capable of inducing the innate immune signature required for coupling adaptive humoral and cellular responses to recombinant antigens that will lead to protective memory responses against HIV-1 infection.

Although central memory cells are considered a renewable source of T effector cells responsible for protection from acute infections and are primed for a rapid effector response, arguably, their ability to exert full effector capacity against the initial infected cell population is delayed sufficiently until the antigen-specific memory population undergoes expansion approximately 3 days after activation.[6] In this regard, a number of studies have shown that effector memory cells at mucosal sites can proliferate and exercise a similar role to central memory in protection from disease.[7] In addition, a direct relationship between the magnitude and quality of effector CD8$^+$ cytotoxic T lymphocytes (CTL) at mucosal sites during acute simian immunodeficiency virus (SIV) infection was found to correlate with viral load in these animals.[8] In a recent study, complete protection from repeated low-dose SIVmac239 intrarectal challenge was achieved in 4 of 12 monkeys immunized with a persistent rhesus cytomegalovirus (RhCMV) expressing SIV Gag, Rev-Tat-Nef, and Env.[9] Low-level persistence of the CMV vector was responsible for maintaining a population of multifunctional effector memory SIV-specific CD4$^+$ T cells and activated CD8$^+$ effector T cells (CD28$^-$CCR7$^-$) in mucosal tissue (as measured in bronchoalveolar lavage) similar to the profile of immune responses directed against the virus during persistent RhCMV infection. Although a direct link between virus-infected cell killing and the specificities of CD8$^+$ CTL involved in the protective response was not determined, this study suggests that differentiated effector or effector memory CD8$^+$ CTL are durable and may play a significant role in extinguishing initial virus replication at local intestinal mucosal effector sites.[8]

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A recent study has provided evidence of an additional antiviral $CD8^+$ T-cell memory phenotype confined to the site of infection in latently infected HSV-1 trigeminal ganglion.[10] A population of tissue-resident $CD8^+$ effector/memory cells ($CD69^+$, $CD62L^{Low}CD122^{Low}$) were identified that expressed integrins $\alpha E\beta 7$ ($CD103^+$) and $\alpha 1\beta 1$ (very late activation antigen-1 [VLA-1]). $CD103^+$ can be induced locally by transforming growth factor-β (TGF-β) and binds E-cadherin on epithelial cells. CD103 is also expressed on interstitial epithelial lymphocytes in the intestine and tumor-infiltrating lymphocytes,[11] whereas VLA-1 recognizes collagen and ensures migration and retention of cells within the interstitium after resolution of inflammation. Tissue-resident memory cells can proliferate in response to $CD103^+$ dermal dendritic cells (DCs) and are more effective in local viral protection than $CD8^+$ CTL recruited into skin grafts during reactivation. Expression of VLA-1^+ is also found on a subset of human peripheral blood $CD4^+$ memory cells ($CD45RO^+CD62L^+CCR7^-CD25^-$) associated with delayed-type hypersensitivity (DTH) to recall antigens and in synovial fluid of arthritic patients.[12]

Different conclusions regarding the role of central and effector memory in protection from disease may in part be explained by differential transitional states and anatomical location of effector cells associated with different infectious agents in the different studies. The spectrum of effector and memory populations that develop after resolution of infection is a function of the infection potential, anatomical location, and survival niches in which responding cell populations anchor. Moreover, while the original paradigm suggests that the ability of $CCR7^+$ T cells to enter secondary lymph nodes in response to CCL19 and CCL21 provides a reliable marker of $CD44^+CCR7^+CD62L^+$ central memory cells (Tcm), more recent data suggest that for human $CD4^+$ T-cell memory that proliferative capability and much of the interleukin-2 (IL-2) production is actually derived from $CCR7^-CD4^+$ T cells. This is consistent with differential regulation of chemokine receptors during activation of $CD4^+$ and $CD8^+$ T cells, in part due to their different functional roles in response to infection and inflammation. Although this relatively simple classification of T-cell memory into Tcm and $CD44^+CCR7^-CD62L^-$ effector memory cells (Tem) will continue to be useful, it must be complemented by additional markers that reflect origin of priming and activation as well as functional analyses related to proliferative potential and role in protective outcomes. In this review we focus our attention on discussing signal transduction pathways responsible for induction and maintenance of T-cell memory formation and suggest strategies for enhancing vaccine induced memory for effective antiviral responses at mucosal surfaces.

Effector and central memory cell differentiation

On the basis of chemokine and homing receptors, proliferative capacity, and effector function, $CD8^+$ T-cell memory has been divided into 2 populations after infection, Tcm and Tem. Central memory cells preferentially localize in lymph nodes, secrete IL-2 after antigen stimulation yet exhibit reduced immediate effector function and cytotoxicity.[3] In contrast, effector memory cells preferentially localize in nonlymphoid peripheral tissue, have reduced proliferative potential, but exhibit immediate effector functions such as interferon-γ (IFN-γ) production and cytolytic capability. The contribution of Tcm and Tem in protective recall responses has been controversial. Proliferation and expansion of the population of $CD62L^{high}$ memory $CD8^+$ T cells following secondary systemic lymphocytic choriomeningitis virus (LCMV) infection was found to be responsible for virus clearance and resumption of functional memory.[13] In contrast, using a dual adoptive transfer model to follow both $CD62L^{hi}$ and $CD62L^{Lo}$ memory cells following intranasal Sendai virus infection, it was found that $CD62L^{Lo}CD8^+$ Tem cells proliferated as well as $CD62L^{High}CD8^+$ Tcm in all organs tested and contributed proportionally to the immediate memory pool.[14] However, over time the $CD62L^{high}$ Tcm population evolved under homeostatic maintenance to become the predominant responsive population.

Although Tem are not able to enter lymph nodes under homeostatic conditions and represent a transitional effector phenotype, a recent study showed $CXCR3^+$ Tem cells can migrate to reactive lymph nodes in response to CXCL9 and proinflammatory cytokines where they can undergo antigen-driven expansion and kill antigen-presenting cells.[15]

Consistent with this observation, adoptive transfer of a late stage melanoma antigen specific effector $CD8^+$ CTL line in humans ($CD28^{low}$, $CD27^{low}$, $CD45RA^-$, $CD62L^-$, $CCR7^{low}$IL-$7R^{low}$) exerted antitumor responses in vivo and evolved into a population of $CD28^+CD27^+CD62L^-$, $CCR7^{low}$IL-$7R^+$ effector memory cells.[16]

In summary, the relative role of Tcm to Tem in recall responses and protection seems to depend on whether the infection is systemic, sequestered in tissue parenchyma, or mucosal, and the ability of memory T cells to migrate to local reactive lymph nodes and respond to antigen.[17-19]

It is thought that the requirements for recalling memory responses are less stringent than those necessary for induction of primary responses. However, recent studies show that memory $CD8^+$ T cells require antigen presentation by lymph node resident $CD8\alpha^+$ DCs and CD70/CD27 costimulation for activation in response to secondary influenza challenge.[20] In contrast, naive T cells were immediately responsive to antigen presentation by tissue-resident $CD8\alpha^-$ DCs in the lung. Although $CD8a^+$ DCs in mice are responsible for priming $CD8^+$ CTL to viruses and intracellular bacteria, a distinct phenotypic counterpart to the mouse $CD8\alpha^+$ conventional DC responsible for cross presentation has not been identified in humans. There is some evidence that a C-type lectin, Clec9A or Clec12A, is a marker of an equivalent human DC subtype.

Compartmentalization between naive precursors and memory cells has 2 important implications for vaccine design. First, targeting antigen to specific DC subtypes provides the opportunity to increase the breadth of T-cell specificities achievable by delivering booster immunizations that target DCs locally at tissue sites of natural infection for de novo induction of new specificities and second, it may be possible to circumvent the immunodominant hierarchy that becomes fixed during boosting and cross-presentation.[21,22] One caveat in the interpretation of these results is that although naive $CD8^+$ T cells activated by viral antigens presented by tissue or extrafollicular DCs are capable of migrating into tissue spaces to exert immediate effector function, it is unclear if $CD8^+$ T cells primed in the absence of CD4-proficient help in T cell–rich zones of draining lymph nodes contribute to the long-term memory pool or serve as "kamikaze" cells in response to the immediate viral threat. It is also important to consider how the boosting immunogen and more importantly natural infection dictate the functional outcome of recalled $CD8^+$ CTL memory.[23]

In contrast to the simple classification of memory $CD8^+$ T cells based upon peripheral migration, an important new study in mice

suggests that the majority of $CD4^+$ T-cell memory is not found in either the Tcm or Tem cell populations but resides in bone marrow.[24] This population of "quiescent" $CD44^{hi}CD62L^-CD4^+$ T cells expressed Ly-6C, similar to memory $CD8^+$ T cells. Furthermore, expression of integrin α2 was required for homing to bone marrow niches where $CD4^+$ memory cells remained adherent to stromal cells or bone marrow DCs which ensure their survival by providing a continuous source of IL-7.[25] Upon recall, $CD4^+$ bone marrow–resident memory cells were shown to rapidly mobilize into secondary lymph nodes where they were activated to provide helper function for an anamnestic antibody response. It is interesting to speculate that this niche is reserved for $CD4^+$ memory T-cell maintenance since the size of the peripheral $CD4^+$ memory pool after resolution of infection contracts approximately 50-fold more than the $CD8^+$ T-cell memory population and declines over time as a result of attrition.[25] These new findings might require a reevaluation of mechanisms involved in homing and maintenance of long-term $CD4^+$ T-cell memory and have important implications for evaluating $CD4^+$ T helper function induced by vaccines.

Activation markers define functional fate of $CD8^+$ T cells

The observation that memory cell precursors display immediate effector function and the limitation of Tcm and Tem markers to identify early $CD8^+$ T-cell effector and memory cell precursors that develop during the first 4 days and 7 to 10 divisions after initial antigen encounter lead to the assignment of additional markers to define early lineage commitment. Within 48 hours after TCR activation of naive and memory T cells, CD62L is down-regulated and cleaved from the cell surface and IL-7R expression lost. The discovery that the IL-7Rα chain (CD127) was reacquired by a subset of cells that persisted into long-term memory distinguished 2 populations of cells: memory precursor effector cells (MPECs) and short-lived effector cells (SLECs).[26,27] The antigen-specific T-cell population that failed to up-regulate IL-7R expressed high levels of killer cell lectin-like receptor G1 (KLRG1) and showed little potential to develop into long-term memory was termed SLECs. KLRG1, a marker of replicative senescence, was recently shown to bind E cadherin expressed on epithelial cells and Langerhans cells (LCs) and inhibit antigen-induced proliferation in both mouse and human studies.[28,29] KLRG1 is a good discriminator of antigen experience and is found on a high percentage of $CD8^+$ T cells in persistent infections in humans such as HIV-1 but not hepatitis C virus (HCV).[30] For the most part, IL-7R and KLRG expression are inversely related on MPEC ($IL\text{-}7R^{high}KLRG^{low}$) and SLECs ($IL\text{-}7R^{low}KLRG^{low}$) although this distinction between MPECs and SLECs has not been confirmed in human studies. $KLRG1^+IL\text{-}7R^{lo}$ expression in $CD8^+$ effector cells is uniformly associated with Blimp-1 expression in both primary and recall responses to flu consistent with its role in regulating migration and terminal differentiation of effector cells.[31,32]

$CD4^+$ T-cell help is necessary for reexpression of IL-7R and IL-6Rα on the MPEC population suggesting an early imprint of $CD4^+$ T-cell help contributing to the survival of activated $CD8^+$ T cells.[27,33] It is important to note that IL-7R and KLRG are not sufficient to predict long-term functional memory as $CD8^+$ T cells are uniformly $IL\text{-}7R^{high}KLRG^{low}$ after DC immunization but few cells evolve into long-term memory.[34,35]

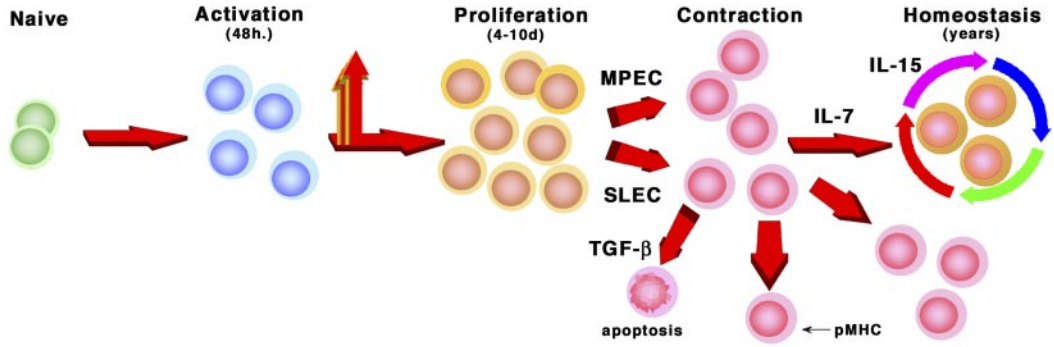
A recent study showed that activation markers may more accurately predict long-term memory.[36] One month after Sendai virus infection, 3 equally represented antigen-specific populations of memory cells could be identified based upon expression of CD27 and T-cell activation marker CD43 (which also binds E cadherin). Although all 3 subsets displayed similar IFN-γ, tumor necrosis factor-α (TNF-α), and IL-2 multifunctional cytokine responses 1 month after infection and similar granzyme B expression during recall responses, the $CD44^{high}CD27^{high}CD43^{low}$ antigen-specific $CD8^+$ T cells mounted the strongest recall response in lung and airways. After 2 years 90% of long-term memory cells expressed $CD27^{high}CD43^{low}$. In contrast, peptide/complete Freund adjuvant (CFA)–vaccinated animals displayed the same 3 phenotypes yet the relative proportion of each population was strongly skewed toward the $CD27^{low}CD43^{high}$ and an additional $CD27^{low}CD43^{low}$ subset which expressed KLRG1. The progressive loss of poorly proliferating memory cells associated with the activation marker CD43 indicate poor long-term vaccine efficacy relative to protection afforded by natural infection.

These studies indicate that memory $CD8^+$ T cells with the highest proliferative capacity upon recall express $CD62L^{high}$, $CD127^{high}$ (IL-7R), $KLRG1^{low}$, $CD27^{high}$, $CD43^{low}$, $CD122^{high}$ (IL-2/ IL-15Rβ), and $Bcl\text{-}2^{high}$. Thus, the relative proportion of long-lived antigen-specific peripheral blood $CD8^+$ T cells expressing these markers could be a useful predictor of vaccine efficacy (Figure 1).

Models of T-cell memory formation

The factors recognized to affect T-cell memory lineage decisions are precursor frequency, duration of infection, proinflammatory cytokines, expression of activation markers and common γ-chain receptors, and anatomic location. Several models have been proposed to describe the events leading to memory cell formation. The first model proposes that memory cells arise during the contraction phase of the immune response and develop directly from effector cells. Thus, all cells are equipotential and effectors either die or dedifferentiate acquiring memory potential over time as a stochastic function of cytokine deprivation. The linear differentiation model is supported to a certain degree by the observation that activated precursors at some point express effector molecules during transition to memory cells.[38] A number of studies have shown that highly activated multiple cytokine-producing $CD4^+$ T cells also transit directly into memory cells during the first 3 to 5 days after antigen encounter suggesting a common developmental program for both $CD4^+$ and $CD8^+$ T cells.[39-41]

A second model developed to help explain the phenotypic and functional heterogeneity of effector cells during priming of the immune response is based on the observation that activated $CD8^+$ T cells progress in a stepwise manner gradually losing their memory potential as they differentiate toward a more terminally differentiated phenotype. The decreasing potential model implies that naive cells progress from weakly differentiated to terminally differentiated states as a result of the cumulative signals they receive during infection (eg, TCR-pMHC affinity, antigen dose and duration, cytokine and costimulatory signals). The corollary is that naive T cells entering lymph nodes late during infection (or circumstances in which infection is truncated or inflammation is limited) experience overall weaker signal strength and preferentially contribute to the antigen-specific memory precursor pool.[42-44] One caveat of this approach for enhancing memory responses to vaccines is that compromising clonal expansion also severely



	Naive	Activated	MPEC	SLEC	Teff	Tem	Tcm	Tscm
CD45RA	+++	+/-	+/-	-	-	+	++	++
CD44	-	-	+/-	+/-	+++	+++	+++	+/-
CCR7	+++	+/-	+++	-	-	+/-	+++	
CD62L	+++	+/-	+++	+/-	-	+/-	+++	+++
CD127	+ ⇄ +++	+/-	+++	-	+/-	+	+++	+++
CD122	+	++	++	++	+/-	+	+++	+++
CD28	++	+++	+++	+	-	+	+++	+++
CD27	+/-	++	++	-	+/-	++	++	
CD43	-	++	++	+++	+++	+/-	-	-
CD95	+/-	++	++	+++	+++	++	+/-	
KLRG1	-	++	+	+++	+++	++	-	-
Peforin	-	-	+	+++	+++	++	-	-
GranzymeB	-	-	-	+++	+++	+*	-	-

* Sip6 maintains integrity of cytotoxic granules in late Teff and Tem

Figure 1. Phenotypic markers associated with antigen-induced differentiation of naive CD8⁺ T cells. Within 48 hours after TCR activation, naive and memory CD8⁺ T cells up-regulate activation markers including IL-2R, Ki67, PCNA, and respond to signals delivered through JAK/STAT pathways to undergo changes in chemokine and homing receptors. CD62L is down-regulated and cleaved from the cell surface and IL-7Rα (CD127) expression is lost. During the proliferative phase a small subset of cells defined as memory precursor effector cells (MPECs) reacquire the IL-7Rα and have the potential to persist into long-term memory whereas the population that fails to up-regulate this receptor represent short-lived effector cells (SLECs).[26,27,37] The antigen-specific T-cell population that fails to up-regulate IL-7R expresses high levels of killer cell lectin-like receptor G1 (KLRG1). Although all antigen-activated CD8⁺ T cells are thought to express immediate effector function and proliferative capacity, transition to memory is dependent upon multiple sequential signals received by the T cell including the intensity and duration of TCR activation, CD4⁺-proficient help, costimulation, and cytokines that regulate survival. Transition to memory after natural infection is more dependent on IL-7, whereas T cells that receive weaker signals in the case of soluble protein antigens delivered with adjuvants are equally dependent on IL-7 and IL-15 for survival and transition to memory. Defining phenotypic and functional markers that characterize different transitional phases of CD8⁺ T cells induced by different viral vectors and other delivery systems will be instrumental in advancing strategies for effective T-cell vaccines for HIV-1, cancer, and other infectious diseases. CD45RA also known as leukocyte common antigen, highly glycosylated protein tyrosine phosphatase regulating TCR, BCR activation and found on naive/resting T cells; CD44, family of cell-surface glycoproteins involved in leukocyte attachment and rolling on endothelial cells and homing to peripheral lymphoid organs by binding mucosal addressin on high endothelial venules. Marker for antigen-experienced cells; CCR7, CC-chemokine G protein–coupled receptor guides cells from peripheral tissue into lymph nodes binding CCL19 (Mip3β) and CCL21(SLC) deposited on HEVs and reticular network; CD62L, L-selectin binds to CD34 and mediates lymphocyte homing through high endothelial venules of peripheral lymphoid tissue and inflamed tissue; CD127, also known as IL-7R; CD122, (IL-2β chain) pairs with γ common chain. Critical component of IL-2 and IL-15–mediated signaling; CD28, constitutive, low-affinity costimulatory signal induces T-cell activation, IL-2 production, and survival; CD27, TNFR superfamily member 7 binds to CD70. Costimulatory signal helps differentiate memory-type CD8⁺ T cells (CD27⁺) from effector-type CD8⁺ T cells (CD27⁻); CD43, leukosialin ligand-receptor complex involved in T-cell activation. Ligand for E-selectin and may regulate T-cell trafficking; CD95, TNF receptor superfamily member 6 also known as Fas. Cell-surface membrane receptor that activates apoptotic pathways when bound by Fas ligand (FasL, CD178); KLRG1, killer cell lectin-like receptor G1, senescence-associated inhibitory receptor binds E cadherin and inhibits AKT phosphorylation; Perforin, is indispensable for granule-mediated cell death by CD8⁺ CTL; Granzyme B, serine protease–inducing caspase-dependent apoptosis. Performs a key role in the cytotoxic activity mediated by CD8⁺ CTL (http://www.pathologyoutlines.com/cd100247.html).

limits the magnitude of memory cell development and in one study did not influence programming for secondary expansion.[2]

A recent study in support of this model identified a population of antigen-specific CD8⁺ cells 4.5 days (8-10 cell divisions) after LCMV infection that exhibited full effector function, expressed KLRG1^{int} yet were unique in their ability to produce IL-2.[45] This KLRG1^{int} population displayed greater potential for homeostatic proliferation and recall proliferation than KLRG1^{high}CD8⁺ T cells. Importantly, during resolution of infection at day 6, KLRG1^{int} memory precursors were proliferating less and down-regulating granzyme B expression whereas KLRG1^{high} continued to proliferate and express granzyme B. Manipulating the duration of antigen stimulation and inflammation resulted in the largest number of KLRG^{low}CD62L, CD127^{high}, CD27^{high} IL-2–producing memory cells. Thus, the degree which naive CD8 ⁺ T cells are activated by "licensed" DCs, and the extent of clonal expansion before exiting lymph nodes and further encounters with antigen-presenting cells, most likely correlate with the quality and magnitude of the memory cell pool[46] (Figure 2). In contrast to the studies that show excessive antigenic stimulation and inflammatory mediators drive CD8⁺ T-cell terminal differentiation and impairment of memory, evidence suggests that CD4⁺ T cells require more prolonged antigenic stimulation to drive cellular programs leading to the formation of long-term high-avidity memory.[48]

A third model proposed to explain the origin of T-cell diversification into memory and effector cells suggests that memory is predetermined by numeric differences in TCR clonotypic precursor frequencies and intraclonal diversification. A number of studies

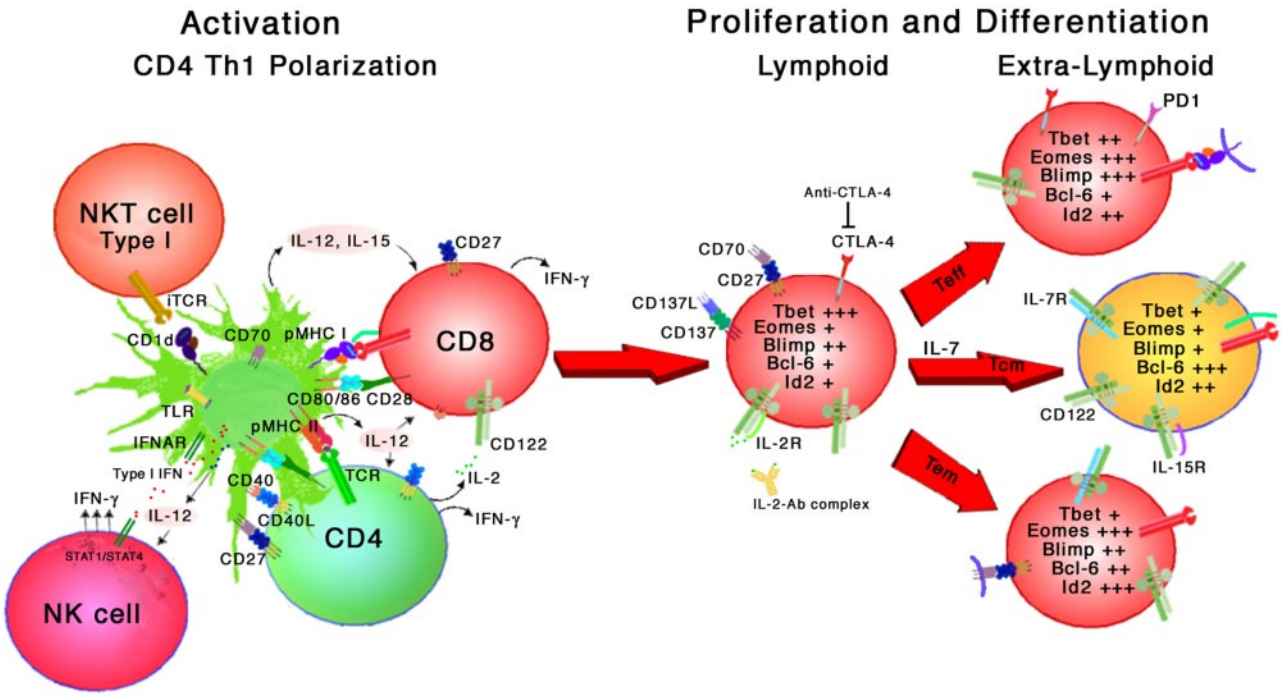


Figure 2. Spatial-temporal signals determine the fate of activated CD8⁺ T cells. The balance between TLR-induced proinflammatory and apoptotic signals via STAT1 pathways in activating DCs and subsequent induction of negative feedback loops that initiate anti-inflammatory signals via the STAT3 axis define the narrow window of DC competency for polarizing CD4⁺ Th1 responses and cross-presentation of antigen to CD8⁺ T cells. TCR recognition of pMHC results in rapid down-regulation of CD62L, SIP1, and IL-7R, up-regulation of activation markers, and acquisition of effector function. During the next 3 to 4 days, Th1 CD4⁺ and CD8⁺ T cells proliferate in response to autocrine and paracrine IL-2, produce IFN-γ, and up-regulate CTLA-4. CTLA-4 ligation on DCs leads to indoleamine 2,3-dioxygenase (IDO) up-regulation, Foxo3-mediated inhibition of IL-6 production, and a shift from competency to induction of regulatory T cells, anergy, and cell death. This is the cue for activated CD4⁺ and CD8⁺ T cells to egress from secondary lymphoid organs. Foxo1 and KLF2 transcription factors, regulated by posttranscriptional modifications, coordinate renewed expression of the IL-7R, CD62L, CCR7, and S1P1, and down-regulate inflammatory chemokine receptors, respectively, on cells destined to become T central memory (Tcm) cells. In contrast, both IL-2 and IL-4 suppress Kruppel-like factor 2 (KLF2) expression and signals transmitted through IL-4R/ STAT6 up-regulate Eomes which induces expression of CXCR3 on CD8⁺ T cells. Survival after cytokine deprivation is dependent on costimulatory and other survival signals received before egress from lymphoid tissue. Limiting growth factors cause activated T cells to shutdown growth and proliferative programs sustained through TCR/CD28, and IL-2 and the P13K/AKT/mTOR pathway and to up-regulate autophagy pathways during transit into nonactivated lymphoid tissue and tissue niches. Here, T central memory (Tcm) and T effector memory (Tem) cell populations become dependent upon cytokine and tissue-specific interactions for maintenance and homeostasis. CD8⁺ T cells that receive CD4-proficient help in T cell–rich zones of draining lymph nodes may be more destined for long-term survival. The balance between costimulatory signals that up-regulate antiapoptotic factors, and negative costimulatory molecules up-regulated during the effector phase such as CTLA-4, BTLA-4, and PD1 (during chronic stimulation) that block effector function ultimately determine the population of cells that survive and transit into the memory pool. Expression of CD8⁺ effector function is regulated by 2 T box–binding transcription factors, Tbet and eomesodermin (Eomes). Although both Tbet and Eomes are essential inducers of CD8⁺ T-cell IFN-γ, perforin, granzyme B, and cytolytic capability, how these 2 transcription factors are coordinately regulated to control effector function and transition to long-term memory is unclear. During the inductive phase of the immune response, IL-12 and IFN-γ drive the differentiation and expansion of CD4⁺ Th1 and CD8⁺ T effector cells. This is reflected in the levels of T-bet expression and secreted osteopontin. During late-stage effector differentiation, inhibitor of DNA binding 2 (Id2), an antagonist of E protein transcription factors is up-regulated by CD8⁺ T cells and maintained in effector memory cells.[47] E proteins are basic helix-loop-helix family of transcriptional activators and repressors, which bind specifically to DNA sequences containing the E-box consensus sequence. E protein homodimers regulate a complex array of genes during T-cell differentiation including expression of CD127 and CD27. Members of helix-loop-helix (HLH) protein family of Id (inhibitor of differentiation) dimerize with bHLH transcription factors and function as negative regulators of differentiation and promote progression of cells into S phase. E-Id2 heterodimer formation leads to diminished E-box–mediated gene expression such as *Ctla4 and Bcl2l11* (BimEL) associated with reduced survival of T effector cells and relieves E protein repression of *Serpinb9* coding for the serine protease inhibitor (Spi-6) thought to protect cytolytic effector cells from programmed cell death. Thus, Id-2 regulates the size of the Tem (CD62L^{low}, CD122^{low}CD127^{low}CD27^{high}) subset in addition to regulating the survival of effector CD8⁺ T cells. Understanding transcriptional programs that control proliferation, acquisition of effector function, and survival of CD4⁺ and CD8⁺ T cells during the immune response will be key to developing new vaccine strategies.

have shown that T-cell clonotypes are represented and persist long-term in both subsets representing the immunodominant hierarchy.[49] In addition, an important recent study disputing this model found that memory precursors diverge from a single effector cell through a process of asymmetric division assigning different fates to each daughter cell.[50] This finding has lead to the concept that the self-renewal properties of antigen-specific T-cell memory is maintained through asymmetrical mitoses similar to pluripotent stem cells and that a complex interplay of physiologic signals direct the differentiation fates of T cells derived from a limited number of multipotent memory cells. Furthermore, evidence from a growing number of studies suggest that both memory CD4⁺ and CD8⁺ T-cell and B-cell lineages share common transcriptional programs with inducible pluripotent stem cells (iPSC) that is evolutionarily conserved between humans and mice.[51,52] Integrating knowledge of physiologic signals that regulate cell-cycle controls and signal transduction pathways, epigenetic states, and noncoding microRNAs (miRNAs) is crucial for understanding how cells escape replicative senescence and achieve the balance between self-renewal and cell differentiation.

Cell-cycle control and self-renewal pathways regulating CD8⁺ T-cell memory

A self-renewing cell must avoid replicative senescence and divide indefinitely. In addition to the 2 complementary survival signaling pathways: MAPK (Ras/Raf/Mek/Erk) and PI3K/AKT/mTOR activated by growth factors and costimulatory molecules that control the balance between proapoptotic and antiapoptotic factors, cell-cycle controls are integral for maintaining a pluripotential cell. The

hallmark of a pluripotential stem cell is an unusual cell-cycle structure lacking a G1-G_o restriction point and with a shortened G1 and extended S phase. This is believed to shield cells from the influence of differentiation cues in G1 yet maintain differentiation competency. A recent study found memory $CD8^+$ T cells dwell in G1 phase that is maintained by costimulatory signals.[53] Cell-cycle progression is regulated by the balance between cyclins/cyclin-dependent kinases (Cdk) and expression of Cdk inhibitory proteins $p21^{Cip}$, $p27^{Kip1}$, and the Ink4/Arf family of tumor suppressors. Antigen activation results in a hyperproliferative state, generation of reactive oxygen species (ROS) and hypoxia inducible factor 1α (HIF1-α) and a DNA-damage response that triggers antiproliferative tumor suppressor pathways leading to p53-mediated apoptosis and $p21^{Cip}$ and $p16^{INK4a}$-Rb–mediated senescence. During the contraction period, drivers of the PI3K/AKT pathway which integrate cell proliferation with cell growth, and cMyc, an epigenetic licensing factor, are central to establishment of a metastable multipotent state. Activated AKT phosphorylates the constitutively active serine theronine kinase GSK3β leading to its inactivation. The inactivation of GSK3β/destruction complex is a critical control point in the physiologic regulation of hematopoietic stem cell (HSC) renewal pathways Wnt and Notch. The canonical Wnt pathway leads to inactivation of the GSK3β complex, accumulation of high levels of β-catenin in the cytosol and translocation to the nucleus where it binds Tcf/Lef transcription factors to regulate multiple downstream target genes including cyclin D1, cMyc, and vascular endothelial growth factor (VEGF). Inactivation of GSK3β prevents proteasomal degradation of β-catenin and Smad-1, a cofactor in bone morphogenic protein receptor (Bmpr) signaling. Bmpr and leukemia inhibitory factor receptor (Lifr) signals are essential for stem cell renewal and differentiation and have been shown to represent distinct signaling pathways of self-renewal operative in memory B cells.[52]

A seminal study recently showed that activating the Wnt/β-catenin pathway of self-renewal leads to arrested differentiation in activated $CD8^+$ T cells in vitro yet these cells possessed high proliferative capability and function after adoptive transfer and reexposure to antigen in vivo (10-fold > Tcm upon adoptive transfer).[54] Stem cell memory (Tscm) could be induced by direct activation of the Wnt pathway by Wnt3a protein or inhibitors of GSK3-β. It is important to note that Wnt-mediated inhibition of differentiation is dependent upon up-regulation of Notch target genes (eg, *Hes1, Hey1*, and *Deltex1*) which inhibit Cdk inhibitors mediating the balance between proliferation and differentiation. Further investigation of signaling pathways regulating gene expression by histone modifications and methyl transferases, and noncoding miRNAs will be important in understanding global mechanisms regulating memory cell formation.

The reader is referred further to a recent discussion of the models of $CD8^+$ T-cell differentiation and memory.[55]

Subset diversification patterns and implications for vaccines

A number of questions regarding the origin of memory precursors and the interpretation of different experimental protocols was resolved in an elegant series of experiments that showed that subset diversification can occur from a single naive precursor during the effector phase.[56] Importantly, the pattern of subset diversification was the same when 100 precursors were adoptively transferred and reproduced the diversity seen in the endogenous response. Moreover, by introducing a single naive precursor after infection to reproduce "late comer" cells into the immune response it was shown that a similar pattern of subset diversification synchronized with cells primed earlier with no trend toward preferential development of $CD62L^+$ Tcm populations. In contrast to the pattern of diversification seen by adoptive transfer of a single transgenic T cell followed by infection with *Listeria monocytogenes* expressing ovalbumin (L.m-Ova), the phenotypic pattern was substantially skewed toward a $CD62L^{low}CD127^{low}$ effector cell phenotype in animals that were immunized subcutaneously with Ova and CpG oligodeoxynucleotide (ODN). Thus, cumulative signals received by antigen-specific T cells during clonal expansion profoundly shape and synchronize the diversification patterns of effector and memory phenotypes that develop after resolution of infection. Furthermore, cells entering the immune response late during infection or responding to residual antigen depots that persist after resolution of infection will synchronize into similar diversification patterns.

These findings reinforce the importance of phenotypic and functional markers to define heterogenous patterns of multifunctional cells that predict the quality and magnitude of memory T-cell responses elicited by infection or vaccines[57] (Figure 1). Extrapolating these findings to defining individual specificities which become representative of the memory cell pool with time is complex. The kinetics of transition to long-term memory will also differ for different infections, vectors, and immunization strategies. Vaccine strategies must be able to deliver the full complement of signals to T cells in a sequential manner to induce clonal expansion of antigen-specific T cells, control the duration of antigen expression and proinflammatory cytokines, and support the expansion and survival of memory precursor cells.

A recent study showed that the T effector memory pool to a single specificity can be expanded by a heterologous prime-boost strategy whereas T central memory is tightly regulated during secondary expansion.[58] This study corroborates previous reports that show limiting antigen duration during boosting, including delivery of protein antigens by repeated short interval antigen pulses[59] progressively skews the ratio of Tem/Tcm by converting the Tcm population attained after priming toward predominantly Tem cells. The general rule that the size of the Tcm compartment is under exquisite physiologic control begs the question of whether it is possible to significantly expand the antigen-specific Tcm compartment by repeated immunization; and if so, to what extent expansion of one or multiple new specificities compromise preexisting T-cell memory to the detriment of the host.

A balance between T-cell specificities occupying both T effector memory and T central memory pools could conceivably enable better control of virus during acute infection. For HIV vaccines this will require immunization strategies that generate sufficient numbers of high-avidity effector memory $CD8^+$ CTL armed and ready at mucosal sites of infection and $CD4^+$ and $CD8^+$ central memory T cells capable of being rapidly recalled in a second wave of attack to curtail primary infection and prevent dissemination from mucosal compartments.[8,60-65]

The role of cytokines, type I and type II IFNs in regulating effector function and transition to memory

The production of inflammatory cytokines and in particular the induction of the IFN-γ/IL-12–signaling axis plays a key role in the

coordinate regulation of the transcriptional programs that guide $CD4^+$ Th1 and $CD8^+$ T-cell effector and effector/memory differentiation.[66] IL-12 levels shift Tbet expression into high gear driving $CD4^+$ Th1 differentiation and cytolytic effector functions of $CD8^+$ T and NK cells. Late in the effector phase as IL-12 levels fall, Tbet expression declines in activated $CD8^+$ T cells while Eomes expression increases. $CD8^+$ T cells induced in the absence of $CD4^+$ T helper cells express high levels of Tbet and fail to develop into effective memory populations.[37,67,68] Notably, Tbet represses IL-2 transcription and IL-7R expression. Eomes is expressed mainly in $CD8^+$ T cells and was shown to control IFN-γ and perforin expression in tumor-infiltrating lymphocytes which correlated with positive antitumor responses in colorectal cancer patients.[69] Eomes up-regulates IL-2Rβ/IL-15R (CD122) and IL-7R (CD127), and is positively regulated by TGF-β and IL-4R/signal transducer and activator of transcription 6 (STAT6) signals which confer CXCR3 expression on activated $CD8^+$ T cells that can migrate to tissue effector sites. Eomes is negatively regulated by IL-12, CTLA-4, and IL-21.[70] Attenuating IFN-γ production by CTLA-4 ligation could prevent cell death of effector/memory cells migrating to tissue effector sites and increase secondary $CD8^+$ CTL responses.[71] In summary, temporal regulation of IFN-γ and IL-12 produced by activated DCs during the inductive phase and cytokine and costimulatory signals received during contraction may be critical for determining the population of cells capable of entering the effector/memory cell pool in response to infection or vaccination (Figure 2).

Type I IFNs in the induction of $CD8^+$ T-cell effector and memory responses

The differential roles of type I and type II IFNs in influencing DC function and $CD8^+$ T-cell responses is complex. Low levels of IFN-α can up-regulate CD40L on DCs and potently enhances $CD8^+$ T-cell expansion and cytotoxicity to cross-presentation of antigen.[72,73] However, STAT1 signals driven by high levels of IFN-α and IFN-γ inhibit CD40L induction of IL-12 by DCs and $CD8^+$ T-cell proliferation. Type I IFNAR/STAT1 signals also up-regulate IL-15 production by DCs and have been shown to fuel a feed-forward loop required for $CD8^+$ T-cell survival during the contraction phase and T-cell memory cell formation.[41,74,75] It was recently shown that the accelerated secondary responsiveness of $CD8^+$ T cells to antigen is also mediated by DC-produced IL-18 induced through a type I IFNAR-positive regulatory loop.[76] Conversely, IFN-α up-regulation of CXCL-9 and CXCL10 on high endothelial venules (HEVs) may contribute to the infiltration $CD8^+$ CXCR3 into lymph nodes (LNs) killing antigen-presenting DCs.

Coordinate functions of IL-2, IL-7, and IL-15 in regulating T-cell memory

Although the nonredundant role of IL-2 in expansion of effector cells in peripheral tissues and during recall responses is well understood, its role during priming and formation of $CD8^+$ T-cell memory is less clear.[77,78] The finding that $CD8^+$ cells generated in the absence of IL-2 signals are strongly impaired in recall responses but not expansion suggest that IL-2 could exert an imprint on early memory progenitor survival programs before reexpression of IL-7R and CD62L.[79] Strong TCR/CD28 signals alone are sufficient to initiate G1-S cell-cycle progression. TCR signals up-regulate IL-2 expression while CD28 costimulation up-regulates Aurora B and Survivin downstream of the PI3K pathway. IL-2–PI3K signals activate the survivin–aurora mTOR complex to inactivate $p27^{kip1}$ allowing Cdk1/2 to stimulate cell-cycle progression.

The transcription factor Blimp-1 is a sequence-specific recruitment factor for chromatin-remodeling enzymes and is well known for its role in silencing plasma cell transcriptional programs and promoting differentiation of B cells into antigen-secreting cells (ASC) and T effector cells.[80] Blimp-1 has also been shown to play a pivotal role in T cells by suppressing IL-2 production and limiting proliferation of antigen-activated T cells during contraction of the immune response, increasing IL-10 production, and antigen-induced cell death (AICD).[81] Blimp-1 expression is elevated in $IL\text{-}7R^{low}$ Tem cells and unhelped $CD8^+$ T cells concordant with Tbet, $KLRG1^{high}$ expression.[67] Alternately, expression of Bcl-6, a transcriptional repressor and antiapoptotic factor is necessary for generation of antigen-specific $CD8^+$ T-cell memory and may function by suppressing Blimp-1 AICD similar to its reciprocal repression in B cells.[82]

When IL-2 and IL-15 were complexed to antibody or soluble membrane IL-15Rα–Fc, respectively, and administered during the contraction phase of the immune response, $KLRG1^{high}CD127^{low}$ effector $CD8^+$ T cells preferentially accumulated whereas IL-7 antibody complexes given during the contraction phase skewed $KLRG1^{low}CD127^{high}$ memory $CD8^+$[83] and $CD4^+$ T cells. Elevated levels of IL-2 were interpreted as sustaining effector responses that might be required in conditions of prolonged inflammation associated with pathogen persistence while IL-15 could prevent activation-induced cell death during the contraction phase. Although IL-15 shares the IL-2Rβ (CD122) and γc (CD132) with IL-2, these 2 cytokines have distinct and often competing roles. IL-2 plays a unique role in activation-induced cell death of self-reactive T cells and maintenance of peripheral $CD4^+CD25^+$ regulatory T cells (Tregs). In contrast, IL-15 is important for homeostatic maintenance, proliferation, and survival of NK cells and long-term high-avidity $CD8^+CD44^{hi}CD122^{high}$ T-cell memory. We recently showed that the differential effects on T-cell function could be attributed to different signal transduction pathways initiated by the intracellular domains of IL-15Rα and IL-2Rα chains.[84] Both IL-15 and IL-15R are coordinately up-regulated on DCs and monocytes by type I and type II IFNs in concert with NFκβ activation triggered by ligation of CD40 or Toll-like receptor 4 (TLR4). Furthermore, IL-15 enhances DC survival. Transpresentation of IL-15 by membrane-bound IL-15Rα on DCs is required for survival and maintenance of $CD8^+$ T-cell memory as well as secondary expansion.[85] The receptor undergoes endocytosis upon binding and is recycled back to the cell surface prolonging its expression and availability for transpresentation.

IL-7 is also critical for the survival, homeostatic maintenance, and clonal turnover of antigen-activated memory T cells. TCR avidity, CD28 costimulation, and coreceptor expression have long been known to determine signal strength, up-regulation of CD25 (IL-2Rα), and functional outcome of the T-cell response.[48,86] Strong TCR signals up-regulate expression of activation markers including CD5 which inversely correlates with CD8α coreceptor expression and IL-7 responsiveness.[87] Scalable signal strengths were shown to regulate the IL-7 responsiveness of $CD4^+$ T cells and influence memory and effector cell fate through increased survival signals transmitted downstream of the PI3K/AKT pathway.[88] Costimulatory signals transmitted in parallel through the RAF/MEK/ERK pathway enforce the antiapoptotic program for cell survival of antigen-activated cells.

A recent study showed that administering IL-7 during the contraction phase (days 7-14) but not during the expansion phase is most effective in enhancing the magnitude of the antigen-specific $CD8^+$ memory cell pool after virus infection or DNA immunization.[89] In addition to increased survival via STAT5 up-regulation of Bcl-2, IL-7 has also been shown to enhance effector function and resistance to TGF-β–mediated suppression during the contraction phase of the immune response.[90] These studies underscore the importance of timing the delivery of IL-7 after T-cell activation and reexpression of IL-7R in promoting T-cell transition to memory.

IL-21 shares the common γ-chain cytokine receptor with IL-2, IL-7, and IL-15 but signals through STAT1 and STAT3 where IL-2 and IL-15 primarily signal through STAT5. In contrast to IL-2 and IL-15, IL-21 limits activated T-cell proliferation. Naive $CD4^+$ T cells stimulated by IL-6, Th17, T follicular helper (Tfh), and NKT cells secrete IL-21 and naive $CD8^+$ T cells and DCs express the IL-21R. The inhibitory effect of IL-21 on T-cell responses is largely due to its potent effect in up-regulating SOCS-1 and SOCS-3 gene expression in DCs and macrophages, and inhibition of TLR2 and TLR4 induced maturation, cytokine production, and IFN-β–mediated signaling.[91] IL-21 down-regulates Eomes expression on $CD8^+$ CTL resulting in inhibition of IFN-γ production[70] and has been shown to up-regulate perforin production without activation of $CD8^+$ T cells from HIV-infected patients.[92] Another study showed that IL-21 repressed CD44, Eomes, and granzyme B expression, and up-regulation of the high-affinity IL-2Rα subunit.[93] Interestingly, adoptive transfer of $CD8^+$ T cells primed with antigen in the presence of IL-21 were significantly more effective in undergoing secondary in vivo expansion and mediating tumor regression than either IL-2– or IL-15–primed cells. The enhanced in vivo efficacy of IL-21 versus IL-2– and IL-15–primed $CD8^+$ CTL in tumor rejection was attributed to the ability of IL-21–primed $CD8^+$ cells to express CD62L and maintain a less differentiated state. Primed $CD8^+$ T cells could enter draining lymph nodes and undergo secondary expansion in vivo. IL-2 more than IL-15 is known to down-regulate CD62L on mature antigen-activated $CD8^+$ T cells and this may be reversed by PI3K inhibitors. IL-21 has also been shown to synergize with both IL-7 and IL-15 in boosting $CD44^{hi}CD8^+$ T cells[94] and enhanced antitumor functions.[95] Thus, IL-21 seems to have a profound effect in inhibiting DC activation and in arresting $CD8^+$ T-cell differentiation yet antigen-activated cells retain the ability to enter antigen-draining lymph nodes and undergo expansion and full effector maturation in vivo.

The role of costimulation in T-cell memory formation

Although CD28 provides the primary costimulatory signal for T-cell activation and proliferation, several members of the TNFR superfamily, such as CD40, CD27, CD30, 4-1BB, OX40, and TNFR2, provide additional signals for induction of $CD8^+$ T-cell effector and memory differentiation. Reciprocal signaling through TNF receptor superfamily (TNFRSF) molecules and ligands expressed on activated DCs and T cells is not only important for influencing the cytokines secreted by DCs but also for shaping $CD4^+$ T-cell lineage commitment and $CD8^+$ T-cell effector function and memory (Figure 2). We previously showed that coimmunization with vaccinia virus expressing a triad of costimulatory molecules (TRICOM), B7-1, ICAM-1, and LFA-3 could induce high-avidity $CD8^+$ CTL responses.[96] However, costimulation alone is not sufficient for the development of $CD8^+$ T-cell memory and soluble or membrane-bound ligands have not been able to recapitulate the intimate interactions between DCs, $CD4^+$ Th and $CD8^+$ T cells necessary for imparting long-term memory and secondary expansion to $CD8^+$ T cells.

CD40L continues to be a mainstay in vaccine strategies in combination with TLRs for induction of IL-12 and other TNFSF members on DCs that provide costimulation for augmenting T-cell help and induction of memory $CD8^+$ T-cell responses[27,97-100] (Table 1). Although interaction of CD40L, up-regulated on activated $CD4^+$ Th1 cells with CD40 expressed on DCs is considered a primary mechanism for the up-regulation of costimulatory molecules and licensing DCs for $CD8^+$ T-cell priming, a recent study showed that transient CD40L up-regulation on DCs is responsible for CD4-independent priming of $CD8^+$ T cells seen in many virus infections.[126]

Reciprocal signaling through up-regulated 4-1BBL/4-1BB interactions on activated DCs and antigen-activated $CD4^+$ T cells can suppress IL-10 production and Treg effects thus potentiating Th1 polarization and $CD8^+$ CTL induction.[127] The mechanism of 4-1BB ligation in enhancing survival of activated $CD8^+$ T cells was shown to involve activation of TNFR-associated factors TRAF-1 and TRAF-2. TRAF-1 up-regulated prosurvival Bcl-2 family member Bcl-xL and survival in conjunction with ERK-dependent proteasomal degradation of proapoptotic Bcl-2 family member Bim.[128] A comparison of the relative role costimulatory molecules CD27, 4-1BB, and OX40 play in antiviral $CD8^+$ T-cell responses showed that both CD27 and 4-1BB were necessary and nonredundant for the induction of $CD8^+$ T-cell responses to influenza infection while OX40 had little effect.[129] Collectively, costimulatory signals delivered by all 3 molecules during priming contributed to maximum secondary clonal expansion.

CD70/CD27 costimulation of antigen-activated $CD4^+$ T cells is also a requirement for competent helper function.[110,130] CD27 costimulation of activated $CD4^+$ T cells skews the cell population toward a Th1 phenotype and up-regulates expression of a unique membrane marker confined to thymocytes and naive T cells, MS4A4B.[110] Interestingly, MS4A4B was also identified as a differentially expressed marker on activated $CD8^+$ T cells receiving CD27-"proficient" $CD4^+$ T-cell help. Moreover, type 1 NKT cells stimulated by α gal-cer have been shown to up-regulate CD70 expression on DCs and effectively prime CD27-dependent $CD8^+$ T-cell responses and effector memory.[115,131] Thus, CD27 costimulation during priming appears to exert a strong influence on long-term survival of $CD8^+$ effector memory.[118]

The CD28 family members (CD28, CTLA-4, ICOS, PD-1) contain PI3K-binding motifs in their cytoplasmic tail and upon ligation result in strong PI3K/AKT signal transduction in lymphocytes in which the receptor is up-regulated.[132] In contrast, TNFRSF members require TRAF adaptor molecules and are dependent upon both TCR signals and CD28 costimulation to deliver their costimulatory signals. CD28 family members CTLA-4, PD-1 and PD-2, T-cell immunoglobulin and mucin domain-containing molecule (TIM-3), and B and T-cell lymphocyte attenuator (BTLA, a member of the immunoglobulin [Ig] superfamily of proteins), are important coinhibitory receptors of TCR activation and effector function.[133] Blocking PD-1 and CTLA-4 has been shown to restore T-cell responses in anergic T cells from patients with chronic HCV and HIV infections.[120] Blocking the inhibitory receptor BTLA-4 has also been shown to enhance the development of $CD8^+$ T-cell memory.[121] A recent study demonstrated the effectiveness of combining anti–4-1BB agonist and anti–CTLA-4 blocking antibodies for induction of effector $CD8^+$ T cell–mediated rejection of an established tumor and long-lasting memory responses. Importantly,

Table 1. Experimental strategies to optimize vaccine T-cell memory

Approach	CD8 T-cell memory		References
	Tem	Tcm	
Heterologous prime boost*			58
DNA prime viral vector boost†	++	++	61, 101-103
Multiple heterologous recombinant viral vector	+++	++	104-107
Mucosal heterologous prime boost	+++	++	61, 108
Persistent replicating vectors	+++	+	9, 109
Concurrent heterologous vaccination	++	++	
Maximize CD4$^+$-proficient help			
Epitope enhancement, CD40L, agonist CD27 Ab	++	+++	97, 98, 100, 110
Cytokines and immunomodulatory molecules			
IL-12	+++	+	111
IL-7	++	+++	89, 90
IL-15	++	++	112-114
IL-7 or IL-15 plus IL-21	+	++	94, 95
α-galactosylceramide (αGalCer) activation of NKT cell type I	+++	++	115
Synergistic TLR combinations in prime boost	+++	+	116, 117
Dendritic cell targeting combined with synergistic TLR ligands	+++	++	
Wnt 3a or GSK3β inhibitors		Tscm	54
Provide effective costimulation			
TNFSF: 41BBL, OX40L, CD70	++	++	118, 119
Block negative costimulation			
Anti–CTLA-4, anti–BTLA-4, anti-PD1	+++	++	120, 121
Push-pull approaches			
GM-CSF and CD40L, plus IL-13Rα2–Fc	+++	+++	97
41BBL plus anti–CTLA-4,	+++	+++	119
TLR combinations plus anti–IL-10	+++	+	
Dendritic cell immunization (Flt3L in vitro expansion)			34
TLR 3 plus TLR7/8 activation	+++	+	116, 122
Adenovirus transfection	++	++	123
miRNA–SOCS-1 inhibition	+	++	
Repeated immunization at short intervals with multiepitope fusion peptides (*Bacillus anthracis* lethal factor Lfn) plus protective antigen (PA)	++	++	59

*Although DNA prime and recombinant viral vector boost regimens induce enhanced cell-mediated and humoral immune responses in preclinical models, the results have not yet translated to clinical use. Ongoing efforts to develop new vaccine delivery platforms including DNA electroporation are moving forward toward clinical trial.[124]

†The cultured ELISPOT assay provides a more sensitive method to enumerate antigen-specific cells not detected in overnight ex vivo assays. This assay may better reveal long-term CD8$^+$ T-cell memory responses achieved by prime boost and highlights differences in measuring human CD4$^+$ and CD8$^+$ T-cell memory.[125]

the combination ameliorated autoimmune sequelae associated with administration of either antibody alone.[119]

Further understanding of the expression kinetics, signal intensity, and dynamic interactions of activating and inhibitory costimulatory signals will better inform vaccine strategies that optimize the magnitude and quality of protective effector/memory and memory T-cell responses while avoiding induction of autoimmune responses.

Concluding remarks

The emerging view, and one to which we subscribe, of immune memory as an inducible quasi-potent stem cell–like state in which differentiation fates are determined by signaling pathways that regulate transcription factors, epigenetic modifications, and miRNAs is rapidly becoming the new paradigm that links together current research ideas in the fields of stem cell biology and cancer ontogeny. Since the original classification of memory CD8$^+$ cell subsets into Tcm and Teff/mem, additional subsets (eg, memory precursor effector cell [MPEC]), tissue-resident memory, stem cell memory [Tscm]) with distinct phenotypes, anatomical locations, transcriptional programs, and function have been added to the list. Collectively, combination strategies and push-pull approaches that modulate Treg and anergic mechanisms may provide optimum immunization strategies capable of skewing adaptive T-cell responses toward high-avidity effector memory and central memory T-cell populations. New prime boost strategies that enhance the quantity and quality of CD4$^+$ and CD8$^+$ T-cell effector/memory cells in combination with mucosal delivery strategies that provide potent recall responses at mucosal sites of viral infection will greatly advance vaccine strategies against HIV and other intracellular pathogens. In summary, the overall understanding reached is to see the ongoing immune response as a dynamic process of activation and regulation that will require combination strategies and temporal spatial intervention to skew effective T-cell memory formation and augment protective immunity induced by vaccines (see supplemental text for additional information, available on the *Blood* website; see the Supplemental Materials link at the top of the online article).

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