Published in final edited form as:

Immunol Rev. 2010 May ; 235(1): 190–205. doi:10.1111/j.0105-2896.2010.00899.x.

Plasticity in programming of effector and memory CD8⁺ T-cell formation

Ramon Arens¹ and Stephen P. Schoenberger¹

¹Laboratory of Cellular Immunology, The La Jolla Institute for Allergy and Immunology, La Jolla, CA, USA.

Summary

CD8⁺ T cells (also called cytotoxic T lymphocytes) play a major role in protective immunity against many infectious pathogens and can eradicate malignant cells. The path from naive precursor to effector and memory CD8⁺ T-cell development begins with interactions between matured antigen-bearing dendritic cells (DCs) and antigen-specific naive T-cell clonal precursors. By integrating differences in antigenic, costimulatory, and inflammatory signals, a developmental program is established that governs many key parameters associated with the ensuing response, including the extent and magnitude of clonal expansion, the functional capacities of the effector cells, and the size of the memory pool that survives after the contraction phase. In this review, we discuss the multitude of signals that drive effector and memory CD8⁺ T-cell differentiation and how the differences in the nature of these signals contribute to the diversity of CD8⁺ T-cell responses.

Keywords

T cells; effector; memory

Introduction

Dissection of the CD8⁺ T-cell response

The ontogeny of CD8⁺ T cells occurs by a discrete series of developmental steps. After migration of T-cell progenitors from the bone marrow to the thymus, developing T cells are positively selected on the basis of the affinity threshold of their T-cell receptors (TCRs) for self-peptides presented by major histocompatibility complex (MHC) molecules displayed on thymic cortical epithelial cells. This is followed by the removal of potentially self-reactive thymocytes (negative selection) bearing high affinity TCRs for self-peptides displayed on medullary thymic epithelial cells and thymic dendritic cells (1). The naive T-cell population that exits the thymus after this selection expresses a broad array of unique TCRs that are able to detect a wide range of foreign antigens. In steady state, the survival of naive peripheral CD8⁺ T-cell pools depends on interleukin-7 (IL-7) and interaction with MHC class I molecules (2).

Once released into the periphery, naive T cells constantly survey and sample antigen-presenting cells (APCs) in secondary lymphoid tissues in search of cognate peptide-MHC (pMHC) molecules. Steady-state presentation of antigen by APCs induces CD8⁺ T-cell tolerance by

default, but in the context of infection or inflammation, T cell-APC interactions drive the clonal expansion and effector and memory cell formation. The precursor frequency of naive CD8⁺ T cells that recognize a specific antigen presented by MHC class I molecules has been estimated to be 1–5 in 10⁵ (3,4). Thus, in a mouse spleen that contains about 2×10^7 total number of CD8⁺ T cells with a diversity of $1-5 \times 10^5$ different clono-types, about 40–200 epitope-specific cells should exist. Recently, this number has been directly determined in mice using pMHC class I tetramers and magnetic-bead enrichment and demonstrated that the precursor frequency for a particular antigen ranged from approximately 80 to 1200 per mouse (5). These small numbers of antigen-specific CD8⁺ T cells need to undergo significant expansion in order to eradicate virulent pathogens effectively that can otherwise rapidly evade and kill the host. In response to some virulent pathogens such as lymphocytic choriomeningitis virus (LCMV) and *Listeria monocytogenes* (LM), naive antigen-specific CD8⁺ T cells can divide >15 times (up to 50 000-fold expansion) to become a large army of effector cytotoxic T lymphocytes (CTLs) within a week (6-8). Contraction of the antigen-specific effector CD8⁺ T-cell population due to programmed cell death follows, and the remaining cells (5–10% of the peak) establish gradually memory populations that can be maintained for life.

The nature of the multitude of signals involved in T-cell expansion and differentiation has begun to be revealed and is an ongoing area of interest. In this review, we primarily focus on the signals that naive CD8⁺ T cells receive by interacting with APCs, which leads to induction of cellular programs that dictates the magnitude and differentiation of the responding CD8⁺ T cells and the ensuing progressive development of T-cell memory.

Advancement by technology

Technological advances during the past 10–15 years have been instrumental for improving the quantification, phenotypic analysis, and behavior of antigen-specific T cells. The development of intracellular cytokine staining (9) and enzyme-linked immunospot (ELISPOT) assay (10) allowed accurate quantification of endogenous antigen-specific T cells, but phenotypic analysis was hampered since these techniques are based on peptide re-stimulations that alter most cell-surface markers (e.g. re-stimulation downmodulates CD62L). With the development of another technology, based on the generation of soluble MHC-peptide tetramer complexes that are able to bind TCRs, T cells could be directly stained (11). These reagents can also be used to isolate unmanipulated T cells for use in adoptive transfer experiments. In addition, TCR transgenic T cells (representing a monoclonal T-cell pool) can be used in adoptive transfer experiments to study and track T cells directly ex vivo (6). These techniques in concert with the advancement of epitope mapping (12) by peptide libraries and epitope prediction algorithms, the ongoing development of multi-parameter flow cytometric analysis, novel cell labeling markers and techniques that permit assessment of cellular viability and cell division (e.g. CFSE), and the generation of immune-related gene-targeted knockout mice and cytokine reporter mice boosted the research in T-cell immunology and led to new insights that could not be generated otherwise. In addition, recent technological advances in intravital imaging allow visualization of immune cell behavior in living animals at cellular and subcellular resolution, leading to new immunological concepts that could not have been established through conventional approaches (13-15).

Events and signals preceding T-cell activation

It is well established that following pathogen challenge, APCs drive the activation and expansion of naive T cells to become effector cells that exhibit potent cytolytic function (16, 17). Only a small fraction, perhaps 5–10%, of these activated T cells will go on to become long-lived memory cells that display long-term survival and can rapidly respond to re-infection (18). Dendritic cells (DCs) are an exceptional population of APCs that are superior among this

class in their ability to stimulate naive T cells (19). DCs are strategically located in both lymphoid and non-lymphoid sites where they constitutively sample the microenvironment and phagocytose microbial and apoptotic cells. Cell surface pattern recognition receptors, such as the Toll-like receptor (TLR) family, and intracellular pathogen-sensing receptors, including the nucleotide oligomerization domain (NOD)-like receptors, play a direct role in determining the activation state of DCs. These signals together with inflammatory cytokines such as TNF and type I interferons (IFNs) are thought to switch DCs from a resting state in which they induce steady-state T-cell tolerance to an activated state in which the outcome of presentation is priming (16,20,21). The activation of DCs by pathogen-derived products belonging to the TLR ligand family induces for example a transient increase in their phagocytic capacity followed by its downregulation, coincident with a substantial increase in the half-life of surface MHC molecules, thereby allowing DCs to durably present specifically the antigens that were captured at the time of pathogenic infection (22). During the maturation process, DCs not only efficiently endocytose and present endogenous and exogenous antigens to T cells using specialized antigen-processing machineries but also acquire the ability to migrate from the tissue to secondary lymphoid organs. These properties together with the upregulation of surface expression of MHC class I and II molecules, T-cell costimulatory molecules, and secretion of cytokines and chemokines provides them with the signals (discussed henceforth) for effective T-cell activation (Fig. 1).

DCs represent a heterogeneous population that can be functionally delineated by the expression of surface antigens (16,17,23). These multiple DC subsets appear to have differential roles with respect to maintaining tolerance, presenting viral antigens, and driving the amplification and differentiation of naive and memory CD8⁺ T cells within the inflammatory milieu of lymphoid tissues. For example, CD8⁺33D1⁺ DCs excel in presentation on MHC class II, CD8⁺DEC205⁺ DCs are efficient in taking up apoptotic cells and cross-presentation, and plasmacytoid DCs rapidly produce large amounts of type I IFNs, thereby influencing directly viral clearance. During local infections (e.g. influenza infection of the lung), tissue antigen-bearing DCs acquire transient migratory properties and travel from peripheral sites of pathogen invasion into the draining lymph node, which is a key step in the initiation of the adaptive response (16,24,25). In some systemic infections (e.g. vaccinia virus and LCMV), not only the resident DCs (14) in secondary lymphoid organs initiate the immune response but other cell types (e.g. parenchymal cells) can also effectively present antigen to CD8⁺ T cells.

Given the important role of DCs in promoting adaptive immune responses, it is not surprising that many pathogenic microorganisms either exert immunomodulatory effects on DCs that impair the ability of infected DCs to initiate T-cell responses or intend to avoid the engagement of the adaptive immune system with more aggressive strategies that overwhelm the innate immune system. Persistent viruses such as cytomegalovirus (CMV) encode for proteins that directly interfere with antigen-presentation pathways and costimulation (26) while influenza virus induces cytopathogenesis (27). Despite these viral inhibitory effects on DCs that hamper direct priming by infected DCs, usually a robust virus-specific T-cell response is initiated which is contributed by complementary immune mechanisms such as cross-presentation (also known as cross-priming), in which extracellular antigens are endocytosed by DCs and presented on MHC class I molecules to ensure that initiation of the immune response occurs (28). Cross-priming is besides important for viral immune responses also essential for immunity against tumor antigens that are not endogenously expressed by DCs (28).

Signals for effector and memory CD8⁺ T-cell development

Interactions between naive T cells and DCs are transient (i.e. lasting seconds to minutes). However, after antigenic stimulation so-called immunological synapses (ISs) or supramolecular activation clusters (SMACs) are formed at the interface between T cells and

DCs that are relatively stable (i.e. hours) and involve adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1) (29-31). This period of more durable interactions, lasting approximately 24 h, coincides with the onset of proliferation and cytokine secretion and is key for commitment of naive T cells to further proliferation and differentiation (31-34).

The CD8⁺ T-cell response is initiated in the IS and leads to antigenic stimulation upon engagement of the TCR and CD8 as a coreceptor, by binding to cognate peptide-MHC (pMHC) complexes presented by APCs (signal 1). TCR-mediated signaling induces phosphorylation of several residues in the CD3 coreceptor chain and activation of ζ -associated protein of 70 kDa (ZAP-70) and the src-family kinases Lck and Fyn, thereby initiating downstream signaling pathways that lead to proliferation and differentiation (35). Costimulatory signals (signal 2) augment TCR signals and prevent induction of anergy or deletion by TCR signaling alone (36,37). The main costimulatory receptor for T cells is the immunoglobulin (Ig) superfamily member CD28, which is constitutively expressed on all naive T cells. The two ligands for CD28, B7-1 (CD80) and B7-2 (CD86) are low to undetectable on the majority of immature APCs but are rapidly upregulated (within hours) after activation (38,39). The CD28 homolog inducible costimulator (ICOS) provides costimulatory signals to T cells as well, albeit less potently than CD28 (38,39). Another important group of costimulatory molecules are members of the TNF receptor family, which includes CD27, 4-1BB, and OX-40 (40,41). After engaging their respective ligands, which are largely expressed on activated APCs, these receptors promote cell cycle progression, survival, and acquisition of effector functions. Recent advances in the field of costimulation focus on their use as therapeutic targets to either dampen T-cell responses in case of autoreactive T cells or to boost the immune system by augmenting the formation of antigen-specific effector and memory T-cell populations to improve vaccinations and anti-tumor responses (40). Caution is required in the latter case because uncontrolled ligation or upregulation of costimulatory molecules can lead to over-activation of the immune system causing severe detrimental effects (42,43).

Several studies have shown that besides antigenic stimulation (signal 1) and costimulation (signal 2), a third signal is important for driving effector T-cell expansion and function, which can be provided by the inflammatory cytokines IL-12 and type I IFNs (44-47). These immunogenic cytokines are directionally secreted by DCs (and other APCs) within the IS, which is likely enhancing the activity of these cytokines by increasing the potency and duration (48,49). Other cytokines (such as TNF and IL-4) are not secreted in a directional preference and thus can potentially also act on bystander cells.

IL-2 was initially characterized as a potent T-cell growth factor in vitro, but the function during primary expansion of CD8⁺ T cells in vivo is dispensable in lymphoid organs and to some extent required in non-lymphoid tissues (50-54). IL-2 signals during priming nonetheless do contribute to secondary clonal expansion of memory CD8⁺ T cells, but it is thus far unknown whether CD4- or DC-derived IL-2 is essential for this phenomenon or that autocrine IL-2 production is sufficient (53,54).

A number of other cytokines such as IL-18, IFN- γ , and IL-21 (55), although not regarded as signal-3 cytokines, do also influence CD8⁺ T-cell expansion and effector and memory cell formation. It is also interesting to note that CD8⁺ T cells can receive cosignals from some but not all TLRs directly (56). Thus, the whole realm of the inflammatory mediators than can act on CD8⁺ T cells is rather complex and likely specific for each individual pathogen.

CD4⁺ T cells can effectively support the antigen-specific CD8⁺ T-cell responses when both the CD4⁺ helper and CD8⁺ T-cells recognize antigen on the same DCs (57,58). This cognate nature of CD4 help operates primarily via activation of CD40 on DCs by primed CD4⁺ T cells that upregulated CD40L and thereby 'license' DCs to initiate CD8⁺ T-cell responses (59-61).

CD40L-expressing DCs might also contribute to this event (62). Licensed DCs enhance the expression of costimulatory molecules (such as CD70, B7-1, and B7-2), and cytokines (e.g. IL-12 and IL-15) (63-68), which in turn leads to enhanced CD8⁺ T-cell proliferation.

Several other helper mechanisms have previously been proposed besides DC licensing. Based on in vitro experiments, it was proposed that paracrine secretion of IL-2 by CD4⁺ T cells is important for CD8⁺ T cells during primary activation (69), but thus far in vivo data (including from our laboratory) could not support this notion. CD4⁺ T-cell help for CD8⁺ T-cell responses does also not appear to be mediated via increased survival of DCs (70). In lymphoid organs, CD4⁺ helper T cell–DC interactions also induces secretion of the chemokines CCL3 and CCL4, which attracts CD8⁺ T cells and increases the opportunity of DCs to encounter rare antigen-specific CD8⁺ T cells (65,71). In peripheral tissues, activated helper CD4⁺ T cells can induce secretion of the chemokines CXCL9 and CXCL10, which mobilize effector CD8⁺ T cells to the site of action (72). Upon restimulation after priming with a cellular vaccine, apoptotic cells, or LCMV infection, the CD8⁺ T cells that did not receive help from CD4⁺ T cells (termed ‘helpless’ or ‘unhelped’ CD8⁺ T cells) were committed to undergo activation-induced cell death mediated by the TNF apoptosis-inducing ligand (TRAIL) upon subsequent encounter with antigen (73,74). The role of TRAIL during late time points after infection and other (inflammatory) conditions is still controversial (75,76), and therefore the exact parameters under which TRAIL is important requires more in depth studies.

The generation of antigen-specific CD8⁺ T cells to non-inflammatory immunogens, such as cell-associated antigens, depends strongly on the coordinate activation of ‘helper’ CD4⁺ T cells, but primary CD8⁺ T-cell responses to a number of virulent pathogens can be generated without help from CD4⁺ T cells (77). Nevertheless, CD4⁺ T-cell help for CD8⁺ T cells during the primary response and/or thereafter and during recall (depending on the antigenic challenge) is essential for the generation and maintenance of functional memory T cells to both non-inflammatory and inflammatory agents (78-82). When and which CD4 helper-derived signals contribute to optimal secondary expansion is under investigation, but this matter is rather complicated to examine, since most costimulatory and inflammatory signals influence primary CD8⁺ T-cell expansion and therefore hampers the dissection of the role of these signals that are specifically important for secondary expansion. It is also unsettled through which mechanism virulent pathogens can overcome the requirement for CD4 help during primary CD8⁺ T-cell expansion. A potent TLR activation on APCs by the infectious agents that induce inflammatory signals that can surmount CD4 licensing of DCs might be key in this phenomenon.

Negative regulatory signals: keeping CD8⁺ T-cell expansion in check

One can argue that the larger the pool of effector T cells the better clearance of pathogens and immune protection. However, uncontrolled effector T-cell proliferation induces immunopathology. Thus, tuning down T-cell expansion is essential, and several inhibitory mechanisms are operating at different levels. Activated CD8⁺ T cells acquire a self-regulatory role by inhibiting the priming of other CD8⁺ T cells by competing for antigen on DCs (6,83) and by killing directly antigen-loaded DCs in a perforin-dependent manner, thereby limiting continued antigen presentation and preventing continued expansion of antigen-specific T cells (84-86). Direct inhibition of CD8⁺ T-cell proliferation can be induced via signals through either cytotoxic T-lymphocyte antigen-4 (CTLA-4) (CD152) and programmed death-1 (PD-1) (CD279), which are inducibly expressed on activated CD8⁺ T cells. Although both CTLA-4 and PD-1 are members of the Ig domain-containing CD28 family, they transmit distinct inhibitory signals after binding to their respective ligands. CTLA-4 shares its ligands, B7-1 and B7-2, with CD28, and this property was found to be an additional mechanism through which CTLA-4 inhibits T-cell activation, i.e. through competition with CD28 for B7-1 and

B7-2 (39,87). The two PD-1 ligands differ drastically in expression: PDL-1 (B7-H1) is broadly expressed and found on most activated but also resting hematopoietic and non-hematopoietic cells, while PDL-2 (B7-DC) is more restricted and found on stimulated APCs (DCs, macrophages, and B cells)(39).

Regulatory T cells (Tregs), known previously as suppressor T cells, express CD4⁺CD25⁺FOXP3⁺ in the mouse and perhaps in the human and are functionally described as cells inducing peripheral tolerance and maintaining T-cell homeostasis. *In vitro*, Tregs have profound effects on inhibiting proliferation of naive T cells. *In vivo*, their suppressive role for CD8⁺ T cells is important to prevent autoimmunity and maintain homeostasis (88,89). In experimental tumor settings and during chronic pathogen infections, their suppressive role is significant (90-95), but their role during acute microbial challenge remains elusive, ranging from facilitating to inhibiting CD8⁺ T-cell responses (96-98). Tregs have several potential mechanisms of action, including competition for IL-2, secretion of inhibitory cytokines (IL-10, TGF- β), and expression of CTLA-4, but which mechanism(s) under which condition is predominant is still under debate.

Programming of CD8⁺ T-cell responses

Naive CD8⁺ T cells have been shown to require only a brief antigenic stimulation of approximately 24 h to commit to division and differentiate without further stimulus into effector and memory cells (46,99-102). Optimal clonal expansion and memory formation but not the functionality of effector CD8⁺ T cells is however achieved when antigenic stimulation is prolonged to 2-4 days (46,103), and if antigenic encounter is shorter than 20 h, the proliferative response is aborted (46,101). In some types of infection (e.g. herpes simplex virus-1), priming of CD8⁺ T cells continues at least 7 days after infection, which results in optimal expansion (104). If antigen exposure persists, like during high-titer chronic infections (e.g. LCMV clone 13), effector CD8⁺ T cells are being exhausted by the continued presence of antigen (and inflammation) and do not differentiate into competent and long-lasting memory cells (105,106) (Fig. 2).

Besides duration of the antigenic stimulation, the strength of the stimulus (TCR ligation with and without costimulation or cytokines) also directly determines the capacity of primed CD8⁺ T cells to accumulate *In vivo* (107). The onset of antigen-specific CD8⁺ T-cell contraction, however, is not affected by dose, duration of infection, or antigen display, suggesting that early events after infection program the contraction phase (108). Still, some plasticity is in place during contraction, since prolonged triggering of the costimulatory CD27 molecule or enhancing IL-7 and IL-15 signals delays contraction (109-111), likely by inducing survival signals that augment expression of Bcl-2 family members such as Bim (112).

These findings have led to the concept of T-cell programming, which describes that a brief antigen encounter triggers a program leading to autonomous expansion and dictates the differentiation fate of the T cells. We would like to emphasize that programming itself is a dynamic process, which is continuously shaped (especially during the expansion phase) by the unique signals that each individual pathogen instills, leading to differences in strength and duration of antigenic stimulation as well as differences in costimulation, cytokine milieu, and CD4⁺ T-cell help. It remains to be determined in more detail to what extent the initial DC-T-cell interaction are contributing to program naive T-cell clones versus the signals that activated T cells 'accumulate' along their differentiation from multiple (additional) cellular interactions and soluble mediators.

The naive T-cell pool is diverse and contains cells bearing TCRs that differ in their affinity for the same antigen. Not surprisingly, the extent of anti-microbial CD8⁺ T-cell responses with respect to the number of epitopes that elicit detectable responses is large. Even for viruses with

a relative small genome like HIV and LCMV, a large number of epitopes (>25) elicit immune recognition (12)(Immune Epitope Database; <http://www.immuneepitope.org/>). Nevertheless, despite this diversity, the kinetics of CD8⁺ T-cell responses (i.e. expansion, contraction, and memory development) and gene expression patterns are synchronized when comparing different dominant and subdominant epitopes in parallel for the same pathogen (7,8,113). If the affinity between the epitope and TCR is under a certain threshold, these T cells have aborted expansion and leave the lymphoid organs earlier, perhaps mirroring the sequelae of brief stimulation periods modeled in *In vitro* studies (114).

If the TCR affinity for pMHC does not play a role above a certain affinity threshold, what determines the difference between dominant and subdominant T-cell responses? By directly quantifying the precursor frequency, the magnitude of CD8⁺ T-cell but also CD4⁺ T-cell responses correlates well with the precursor frequency of the endogenous repertoire (5,115, 116), indicating that immunodominance is directly determined by the size of clonal T-cell pools in case of sufficient MHC binding affinity. Related to this subject is the question of what determines the actual magnitude of the antigen-specific CD8⁺ T-cell population for a constant epitope, which inflates according to increasing doses of antigen or infectious microbes infection loads (100,108,117,118). Recently, Schumacher and colleagues (119) used a novel kin-ship analysis technology to show that the recruitment of antigen-specific CD8⁺ T cells into clonal expansion is actually extremely efficient during both low and high infectious doses, indicating that the recruitment of precursor cells is a constant parameter and that the difference in magnitude should be primarily attributed to the (inflammatory) signals that program the rate of expansion (e.g. duration and strength of antigenic stimulation, costimulation, cytokines). Optimal CD8⁺ T-cell activation might also be influenced by competition for antigen or the limited number of antigen-loaded APCs, since increasing the numbers of TCR transgenic T cells (reflecting increased TCR precursor frequencies) results in a corresponding reduction of the endogenous primary CD8⁺ T-cell response for the same epitope (6,83). If the precursor frequency of TCR transgenic T cells greatly exceeds that of the endogenous responders, not only is the endogenous response to the same antigen suppressed but this also results in earlier kinetics, altered phenotype, and impaired proliferation and function of the transgenic T cells (120,121). Within the endogenous T-cell pool, competition among T cells during primary responses seems to be non-operative and thus only to be a concern when 'unphysiological' high numbers of antigen-specific T cells are present. In this respect, one could envision that T-cell competition during memory responses, which are characterized by larger pools of antigen-specific T cells, might become relevant (depending on the number of memory cells, antigenic load, and differences in antigen-presentation) (122,123).

Effector cell differentiation and acquisition of effector functions

The majority of naive T cells express low levels of CD44 and high level expression of the homing receptor CD62L (L-selectin) and chemokine receptor CCR7 that facilitate entry into lymph nodes. A proportion of the naive T-cell pool (10–30%) bears markers of memory cells like CD44^{high}LFA1^{high} CD122^{high}Ly6C^{high} and are functionally capable of more rapid initiation of proliferation and IFN- γ production, which might be caused by physiological homeostatic proliferation (124). During the differentiation of naive T cells into effector T cells, both CD62L and CCR7 are downmodulated, while CD44 and S1P1 receptors are upregulated. Thus, antigen-experienced cells have lost the ability to enter lymph nodes and have increased their capability to extravasate into peripheral tissues to rapidly control infection (125). Reactive lymph nodes, however, allow entry of CD62L^{low}CCR7^{low} T cells due to the upregulation of CXCR3 on T cells that bind to the CXCR3 ligand CXCL9 expressed on the lumen (85). Other cell surface molecules that are inducibly upregulated are the activation markers CD43 and CD69, the inhibitory receptor PD-1 and the killer cell lectin-like receptor G1 (KLRG1) while expression of IL-7 receptor α (CD127) is temporarily down-modulated.

Following initial antigenic stimulation and clonal expansion of naive T cells, two critical effector functions of T cells are acquired: direct contact-mediated cytotoxicity and secretion of cytokines (126). Contact-mediated cytotoxicity proceeds through release of preformed cytolytic molecules (i.e. perforin and granzymes) into the synaptic cleft between T cells and target cells as well as through triggering of the TNFR family member CD95 (Fas). Both the release of cytotoxic molecules and CD95 ligation will eventually lead to programmed cell death (apoptosis) of the target cell. Secretion of the effector cytokines IFN- γ and TNF has broad modifying immunological consequences for immune cells and can contribute to local inflammatory responses (127). CD8⁺ T cells rapidly produce IFN- γ and TNF when their TCR is engaged by the pMHC complex of the target cell but will immediately cease IFN- γ production when antigenic contact is broken, presumably until they encounter the next target cell (128, 129). TNF production is even more strictly regulated and ceases after a short period even when antigen contact is sustained (129). Effector cytokines produced by antigen-specific CD8⁺ T cells are likely strictly regulated to minimize the damage to the host (127). In contrast with the on/off cycling of cytokines, expression of the pore-forming cytotoxic protein perforin is constitutively maintained (128).

Another important capacity that effector and subsets of memory CD8⁺ T cells acquire is the ability to migrate to virtually any extra-lymphoid tissues after both localized and systemic infections (130). Interestingly, in certain tissues CD8⁺ T cells acquire a distinct phenotype. For example, T cells in the small intestine uniquely express the integrin $\alpha 4\beta 7$ and the chemokine receptor CCR9, while in the skin T cells specifically express CCR4 and E-selectin ligand (131). Several studies showed that the 'imprint' of these tissue-specific phenotypes was caused by the tissue environment and local DCs itself (132-135). During localized infections, selective homing of the imprinted T cells in the skin was found to be a dynamic process, primarily effective during the first days postinfection after which the ability of T cells to indistinguishably traffic to additional peripheral tissues was additionally acquired (134,136). Whether intestinal mucosal imprinted T cells acquire a similar dynamic imprint remains to be determined. Remarkably, memory CD8⁺ T cells that were initially primed in lymphoid organs can only gradually enter the intestinal mucosa (while other organs are more rapidly seeded), since access to this organ involves expression of $\beta 7$ integrin, the same molecule that is specifically induced by this tissue (135,137). Thus, imprinting by tissue specific factors programs unique but still flexible properties to CD8⁺ T cells.

Memory CD8⁺ T-cell formation

The antigen-specific CD8⁺ T-cell response is characterized by clonal expansion, followed by contraction and memory formation. During this process, memory T cells gained several properties that are crucial for their protection to otherwise lethal infections. First of all, memory CD8⁺ T cells have higher frequencies than naive T cells, which, depending on the presence of CD4⁺ T cells and the cytokines IL-7 and IL-15 (2) can be maintained for long periods of time without antigenic stimulation. This increment in size, the ability of memory cells to rapidly reactivate and kill upon antigenic stimulation and their varied tissue distribution makes the memory CD8⁺ T-cell compartment able to protect it's host better and faster to recurrent infections than compared to naive T-cell pools.

Depending on the subtype of memory T cell, most but not all cell-surface markers are gradually reversing to baseline during the ensuing development of effector cells into memory T cells. Commonly, memory T cells are subdivided into two main subsets: effector memory cells (T_{EM}; CD62L⁻CCR7⁻) and central memory cells (T_{CM}; CD62L⁺CCR7⁺) (130,138) but other memory subsets defined by markers like CD27, CD28, CD43 do also exist (139). T_{EM} are preferentially localized in non-lymphoid tissues and mucosal sites and have more rapid cytotoxic potential to confront the invading pathogen, whereas T_{CM} are mainly concentrated

in secondary lymphoid organs and possess superior expansion potential. Both T_{EM} and T_{CM} have protective capacity, but the type of infection determines the relative importance of this. During peripheral vaccinia virus infection the presence of T_{EM} determines the protective capacity, while during systemic LCMV infection T_{CM} were more potent to protect (140,141). Thus protection is basically linked to the anatomical location of memory T-cell subsets and hence the route of infection. In light of this, it is worth mentioning that recent studies show correlates of protection for vaccines based on multifunctional T cells (i.e. simultaneous production of the cytokines IFN- γ , TNF, and IL-2) (142), implying that not only location and quantity of memory $CD8^+$ T cells but also 'fitness' should be considered important for protection.

The three phases toward memory cell formation (expansion, contraction, and memory development) are found in response to many different types of acute infection and for different epitopes within the same pathogen, indicating a common pathway for memory T-cell formation. At the moment, there is no consensus and several models have been suggested for the differentiation path of naive $CD8^+$ T cells into effector and central memory T cells (143). Most convincing data using different techniques and models proposes a model for a linear development of naive T cells into memory cells in which most memory T cells pass through an effector phase. First, adoptively transferring effector T cells results in the generation of memory cells and microarray studies have shown that this transition from naïve to effector to memory phenotype is a gradual process (144-146). Moreover, several labs generated reporter mice that marked specifically effector T cells and their studies showed convincingly that the bulk of the $CD8^+$ T (and CD4) memory cells arise from effector T-cell progenitors (147-149). Thus, at least a large portion of the memory T-cell pools arises from effector cells after infection.

These observations, however, do not exclude that parallel development might occur. Some cells of the effector $CD8^+$ T-cell pool at the peak of the response after virulent infection have already gained the ability to undergo secondary expansion, a key property of memory cells (150). Also, several studies indicate that naive T cells can become (faster) memory T cells without going through the full effector phase under influence of certain (minor inflammatory) stimuli. For example, immunizing with peptide-loaded DCs or truncating LM infection using antibiotics renders $CD8^+$ T cells with proliferative potential as early as day 4 after primary challenge, while it takes >10 days in case of a primary virulent infection (151,152). Also, heat-killed LM generates memory $CD8^+$ T-cell populations, albeit less potently, without overt effector cell formation (153). Apparently, inflammation enhances the formation of effector cells and hence clearance of pathogens and at the same time delays formation of memory properties such as responding to secondary challenge. Thus, strong inflammatory responses that accompany virulent microbial infections are beneficial in clearing the pathogen.

A recent study observed that mice with a point mutation in the TCR β -chain develop impaired memory T cells, while development of effector $CD8^+$ T cells seems normal, which indicates that differential TCR signaling might be involved in programming separate fates. Also, the recent finding that naive T cells can undergo asymmetric cell division (154) indicates that diversification might occur early after antigenic stimulation, but so far it is indecisive whether asymmetry in cell division has actual functional consequences regarding the formation of T-cell subsets found later during an immune response.

Several cell surface markers such as IL-7R α (CD127) and CD8 $\alpha\alpha$ have been proposed to distinguish memory precursors from terminally effector T cells within the effector T-cell pool (155-157), but later studies showed that expression of these molecules were correlative in some but not in all circumstances and showed that these molecules are not essential for memory T-cell formation, which underlines caution in using these phenotypic markers (158-163).

Recently expression of Killer cell-lectin-like receptor G1 (KLRG1) has been found to mark effector CD8⁺ T cells that are predominantly short lived (146,164). Whether KLRG1 is directly involved in the fate of positively marked CD8⁺ T cells is currently unknown.

Another matter of debate has been whether the T_{EM} and T_{CM} subsets are distinct or related lineages and whether they undergo interconversion. Studies by independent laboratories have shown that the initial precursor frequency is influencing this memory lineage commitment. Using physiological precursor frequencies indicated that interconversion is scarcely occurring, while using higher (unphysiological) precursor frequencies implied that T_{EM} converts more easily to T_{CM} (120,141,165). Others have found that T_{CM} can also convert into T_{EM} (166, 167).

By experimentally tracking the fate of a single naive CD8⁺ precursor T cell upon clonal expansion *in vivo*, Busch and colleagues (168) found that similar phenotypic and functional subsets of effector and memory T cells arise, as observed with polyclonal responses (also when cells were transferred 2 days postinfection). This result implies that pre-existing factors within the naive T-cell repertoire can be excluded as a contributor to subset diversification but are induced during priming conditions.

Memory CD8⁺ T-cell differentiation during chronic infections is distinct from acute infection and leads to functional impairment characterized by reduction of proliferative potential, cytotoxic capacity, and gradual loss of effector cytokines (IL-2 > TNF > IFN- γ) (169). Phenotypically, these exhausted CD8⁺ T cells acquire expression of a unique set of cell surface inhibitory molecules including PD-1, CTLA-4, LAG-3, CD160, and CD244 (2B4) (170,171). In contrast with memory cells that are developed after clearance of pathogen or during latent infections (herpes viruses), exhausted memory cells that develop during high-titer chronic infections [LCMV (clone 13) or HIV] are principally maintained by extensive cell division triggered by continuous antigenic stimulation (105,172-174). During CMV infection, a unique so-called inflationary CD8⁺ T-cell response progresses, in parallel with canonical memory responses, that is characterized by negligible contraction and prolonged incremental increase of antigen-specific T-cell numbers driven by continuous activation of low viral titers. These CMV-responsive T cells have acquired a distinctive phenotype (CCR7⁻CD27⁻CD28⁻), which is not found in other viral infections (175). Thus, depending on the nature of the infection and infectious agent, different and unique memory T-cell subsets develop.

We conclude that these studies reflect the plasticity of our immune system. It harbors default pathways for shaping effector and memory cell formation but also instills alternative lanes of differentiation (especially during the expansion phase), thereby allowing adaptation to a wider variety of microbes.

Epigenetic programming of CD8⁺ T cells

The integrated signals (antigenic stimulation, costimulation, cytokines) that CD8⁺ T cells receive induce activation of transcriptional regulators and epigenetic modifications (DNA methylation, histone modifications, chromatin remodeling) (176,177). Recent progress has been made in understanding the contribution of these important factors for CD8⁺ T-cell differentiation. The T-box factors T-bet and eomesodermin (Eomes), inducibly expressed in effector T cells, are singularly moderately important but in concert are crucial to promote effector cell functions like production of IFN- γ , perforin, and granzymes (178-181). Interestingly, the inflammatory cytokine IL-12 enhances T-bet expression but represses Eomes leading to the speculation that inflammation directs the dominance of these T-box family members directly and effector/memory fate decisions indirectly (182). In fact, a study by Kaech and colleagues (164) showed that the higher the degree of inflammation (and especially IL-12) induced correlating higher amounts of T-bet, which promoted effector cell formation. The

precise differential roles of T-bet and Eomes in effector and memory CD8⁺ T-cell differentiation remain to be determined.

The transcription factor Blimp-1, originally reported to be essential for plasma cell differentiation, was recently found to be required for the migratory capacity and cytotoxic activity of CD8⁺ T cells during viral infections (183). In addition, it was found that Blimp-1 promotes terminal differentiation of CD8⁺ T cells into short-lived effector cells rather than long-lived central memory cells (183-185).

Besides these main transcription factors for cytotoxic CD8⁺ T cells, other transcription factors are expected to contribute also to the effector functions of CD8⁺ T cells. The loci of the effector molecules perforin, granzyme B, and Fas ligand, for example, contain DNA binding motifs for several transcription factors that are either shared (NFAT, AP1, ETS1, NFκB, SP1) or unique (Ikaros, RUNX1, MEF, STAT3) (186).

The gene loci of effector cytokines such as IFN-γ and IL-2 are heavily methylated in naive T cells. TCR activation in combination with CD28 signals (and not independently) induce stable histone acetylation, loss of cytosine methylation, and chromatin remodeling of the IL-2 promoter/enhancer (187). Also help from CD4⁺ T cells influences epigenetic remodeling of the IFN-γ and IL-2 loci in CD8⁺ T cells after infection (188,189). Thus, signals that normally occur during activation of naive CD8⁺ T cells induce epigenetic modification, allowing their enhanced transcription of effector cytokines and these epigenetic changes are stably maintained in memory cells. The inflammatory cytokines IL-12 and IFN-α/β also enforce chromatin remodeling, leading to an upregulation of a number of genes that are critical for CD8⁺ T-cell function and memory (190).

Hierarchy, redundancy, and synergy

Several levels of complexity exist between the multitudes of (early) signals that CD8⁺ T cells receive. Signals between and within the different collection of mediators (antigenic stimulation, costimulation, type-3 cytokines, and CD4⁺ T-cell help) exist, which are influenced by the dose and duration of the infection.

Signaling through the TCR is by definition an absolute requirement for antigenic stimulation, and in concert with additional signals, TCR signaling leads to the generation of effector and memory CD8⁺ T cells. Naive and especially memory CD8⁺ T cells, however, can become phenotypically activated (e.g. CD69^{high}) independently of specific TCR stimulation. This type of non-antigen-specific T-cell activation, designated as 'bystander' activation, is mainly caused by inflammatory cytokines (i.e. type I IFNs) secreted by TLR-stimulated DCs (191,192) and does not likely involve costimulation. The physiological relevance of bystander T-cell activation is still unclear but may be limited, since *in vivo* bystander activation occurs only in a small portion of T cells (7,193).

Ligation of the principal costimulatory molecule CD28 is essential for the magnitude of the response for most but not all primary CD8⁺ T-cell responses (194). Memory CD8⁺ T cells require CD28 costimulation to expand optimally upon re-exposure to antigen *in vivo* (195-197). During certain infections like LCMV, the requirement for CD28-mediated signals to enhance CD8⁺ T-cell responses is minute, which might be attributed to high virulence of this virus inducing strong and prolonged TCR activation (194,198), but perhaps the inflammatory environment cytokines and/or that dominance of alternative costimulatory molecules are also involved. In this regard, it is worth mentioning that the costimulatory signals mediated by the costimulatory TNF receptor (TNFR) family members (CD27, OX40, and 4-1BB) complement CD28 costimulation and deliver independent signals for optimal and long-lasting CD8⁺ T-cell differentiation and memory cell development. Remarkably, signaling

through all of the costimulatory TNFR family members leads eventually to activation of NF- κ B and Jun kinase pathways, but these cosignaling molecules still possess non-redundant contributions to effector and memory CD8⁺ T-cell formation during various infections, leading to the concept of spatially and temporally regulated utilization of costimulatory molecules (40,41,199).

The signal-3 cytokines IL-12 and type I IFNs have several overlapping positive actions that operate on APCs and CD8⁺ T cells. For example, IL-12 and type I IFNs promote cross-priming of APCs, likely via an autocrine loop (200,201) and both increase survival of CD8 T cells directly during the antigen-driven expansion phase (47,202,203). During infections, however, IL-12 and type I IFNs have distinctive expression levels (204) and are differentially required for CD8⁺ T-cell expansion and memory formation. For example, direct IL-12 signals are important for CD8⁺ T-cell stimulation during LM infections, while type I IFNs are essential during LCMV infections (47,203,205).

Toward a rational design of vaccines

Peptide immunization or subunit vaccines are poor in eliciting protective CD8⁺ T-cell responses, while virulent acute pathogens and live vaccines generally induce excellent effector and memory cell formation (206). It can thus be anticipated that mimicking the signals that are operational during challenge with virulent microbes, by inducing similar signals in a comparably timed manner, should significantly improve peptide vaccination regimens. Several well-designed experiments have been performed that indeed corroborated this concept. Peptide vaccination alone does not provoke protective CD8⁺ T-cell responses, but when used in combination with booster stimuli that target TLRs, CD8⁺ T-cell-mediated immunity was elicited (207,208). Targeting multiple immune receptors that synergize (i.e. TLRs and CD40 or IL-2) results in even greater expansion of antigen-specific CD8⁺ T cells and protection from infection than with either stimulus alone (209-211). Also, combining CD4⁺ T-cell help with peptide/adjuvant immunizations enhanced effector and memory CD8⁺ T-cell formation (71). Lastly, a study by Johansen (212) showed that exponential incremental antigenic triggering (with peptide and CpG or peptide-pulsed DCs) for several days is superior to a single high dose or multiple equal doses. Together, these studies underline that mirroring the multiple stimulatory signals and the antigen kinetics that occur during virulent infections yields superior CD8⁺ T-cell activation and immunity.

Concluding remarks

The interplay of antigenic stimuli, costimulatory molecules, inflammatory mediators, and help from CD4⁺ T cells all combine to define the specific gene expression patterns that form the developmental program of CD8⁺ T cells after infection or vaccination. We have begun to unravel the mechanisms of these (early) critical signals that induce expansion and progressive yet flexible differentiation of T cells and the acquisition of T-cell memory over several weeks, which is key to protect against recurring infections. Understanding in more detail the hierarchy, synergy, and redundancy of these mediators will be important, and future studies that aim to gain more fundamental insight into dissecting which signals received by CD8⁺ T cells induce phenotypic and qualitative heterogeneity will also lay the foundation for a more rational design of T-cell-based vaccines, which may hold the key to developing protective immunity to not only acute infections but also to tumors and chronic infections such as HIV, HCV, and malaria in humans.

Acknowledgments

The authors thank Shahram Salek-Ardakani and Stefan Nierkens for critical review of the manuscript.

References

1. Palmer E. Negative selection – clearing out the bad apples from the T-cell repertoire. *Nat Rev Immunol* 2003;3:383–391. [PubMed: 12766760]
2. Surh CD, Sprent J. Homeostasis of naive and memory T cells. *Immunity* 2008;29:848–862. [PubMed: 19100699]
3. Blattman JN, et al. Estimating the precursor frequency of naive antigen-specific CD8 T cells. *J Exp Med* 2002;195:657–664. [PubMed: 11877489]
4. Laouini D, Casrouge A, Dalle S, Lemonnier F, Kourilsky P, Kanellopoulos J. V beta T cell repertoire of CD8+ splenocytes selected on nonpolymorphic MHC class I molecules. *J Immunol* 2000;165:6381–6386. [PubMed: 11086076]
5. Obar JJ, Khanna KM, Lefrancois L. Endogenous naive CD8+ T cell precursor frequency regulates primary and memory responses to infection. *Immunity* 2008;28:859–869. [PubMed: 18499487]
6. Butz EA, Bevan MJ. Massive expansion of antigen-specific CD8+ T cells during an acute virus infection. *Immunity* 1998;8:167–175. [PubMed: 9491998]
7. Murali-Krishna K, et al. Counting antigen-specific CD8 T cells: a reevaluation of bystander activation during viral infection. *Immunity* 1998;8:177–187. [PubMed: 9491999]
8. Busch DH, Pilip IM, Vijh S, Pamer EG. Coordinate regulation of complex T cell populations responding to bacterial infection. *Immunity* 1998;8:353–362. [PubMed: 9529152]
9. Prussin C, Metcalfe DD. Detection of intracytoplasmic cytokine using flow cytometry and directly conjugated anti-cytokine antibodies. *J Immunol Methods* 1995;188:117–128. [PubMed: 8551029]
10. Miyahira Y, et al. Quantification of antigen specific CD8+ T cells using an ELISPOT assay. *J Immunol Methods* 1995;181:45–54. [PubMed: 7537312]
11. Altman JD, et al. Phenotypic analysis of antigen-specific T lymphocytes. *Science* 1996;274:94–96. [PubMed: 8810254]
12. Peters B, Sette A. Integrating epitope data into the emerging web of biomedical knowledge resources. *Nat Rev Immunol* 2007;7:485–490. [PubMed: 17479127]
13. Mempel TR, Bauer CA. Intravital imaging of CD8+ T cell function in cancer. *Clin Exp Metastasis* 2009;26:311–327. [PubMed: 18665448]
14. Henrickson SE, von Andrian UH. Single-cell dynamics of T-cell priming. *Curr Opin Immunol* 2007;19:249–258. [PubMed: 17433876]
15. Cahalan MD, Parker I. Choreography of cell motility and interaction dynamics imaged by two-photon microscopy in lymphoid organs. *Annu Rev Immunol* 2008;26:585–626. [PubMed: 18173372]
16. Steinman RM, Hawiger D, Nussenzweig MC. Tolerogenic dendritic cells. *Annu Rev Immunol* 2003;21:685–711. [PubMed: 12615891]
17. Masson F, Mount AM, Wilson NS, Belz GT. Dendritic cells: driving the differentiation programme of T cells in viral infections. *Immunol Cell Biol* 2008;86:333–342. [PubMed: 18347609]
18. Williams MA, Bevan MJ. Effector and memory CTL differentiation. *Annu Rev Immunol* 2007;25:171–192. [PubMed: 17129182]
19. Jung S, et al. In vivo depletion of CD11c(+) dendritic cells abrogates priming of CD8(+) T cells by exogenous cell-associated antigens. *Immunity* 2002;17:211–220. [PubMed: 12196292]
20. Iwasaki A, Medzhitov R. Toll-like receptor control of the adaptive immune responses. *Nat Immunol* 2004;5:987–995. [PubMed: 15454922]
21. Martinon F, Mayor A, Tschopp J. The inflammasomes: guardians of the body. *Annu Rev Immunol* 2009;27:229–265. [PubMed: 19302040]
22. Blander JM, Medzhitov R. Toll-dependent selection of microbial antigens for presentation by dendritic cells. *Nature* 2006;440:808–812. [PubMed: 16489357]
23. Shortman K, Naik SH. Steady-state and inflammatory dendritic-cell development. *Nat Rev Immunol* 2007;7:19–30. [PubMed: 17170756]
24. Legge KL, Braciale TJ. Accelerated migration of respiratory dendritic cells to the regional lymph nodes is limited to the early phase of pulmonary infection. *Immunity* 2003;18:265–277. [PubMed: 12594953]

25. Dudziak D, et al. Differential antigen processing by dendritic cell subsets in vivo. *Science* 2007;315:107–111. [PubMed: 17204652]
26. Lilley BN, Ploegh HL. Viral modulation of antigen presentation: manipulation of cellular targets in the ER and beyond. *Immunol Rev* 2005;207:126–144. [PubMed: 16181332]
27. Lyles DS. Cytopathogenesis and inhibition of host gene expression by RNA viruses. *Microbiol Mol Biol Rev* 2000;64:709–724. [PubMed: 11104816]
28. Heath WR, et al. Cross-presentation, dendritic cell subsets, and the generation of immunity to cellular antigens. *Immunol Rev* 2004;199:9–26. [PubMed: 15233723]
29. Grakoui A, et al. The immunological synapse: a molecular machine controlling T cell activation. *Science* 1999;285:221–227. [PubMed: 10398592]
30. Monks CR, Freiberg BA, Kupfer H, Sciaky N, Kupfer A. Three-dimensional segregation of supramolecular activation clusters in T cells. *Nature* 1998;395:82–86. [PubMed: 9738502]
31. Scholer A, Hugues S, Boissonnas A, Fetler L, Amigorena S. Intercellular adhesion molecule-1-dependent stable interactions between T cells and dendritic cells determine CD8+ T cell memory. *Immunity* 2008;28:258–270. [PubMed: 18275834]
32. Mempel TR, Henrickson SE, Von Andrian UH. T-cell priming by dendritic cells in lymph nodes occurs in three distinct phases. *Nature* 2004;427:154–159. [PubMed: 14712275]
33. Iezzi G, Karjalainen K, Lanzavecchia A. The duration of antigenic stimulation determines the fate of naive and effector T cells. *Immunity* 1998;8:89–95. [PubMed: 9462514]
34. Hugues S, Fetler L, Bonifaz L, Helft J, Amb-Iard F, Amigorena S. Distinct T cell dynamics in lymph nodes during the induction of tolerance and immunity. *Nat Immunol* 2004;5:1235–1242. [PubMed: 15516925]
35. Salmond RJ, Filby A, Qureshi I, Caserta S, Zamoyska R. T-cell receptor proximal signaling via the Src-family kinases, Lck and Fyn, influences T-cell activation, differentiation, and tolerance. *Immunol Rev* 2009;228:9–22. [PubMed: 19290918]
36. Viola A, Lanzavecchia A. T cell activation determined by T cell receptor number and tunable thresholds. *Science* 1996;273:104–106. [PubMed: 8658175]
37. Bandyopadhyay S, Soto-Nieves N, Macian F. Transcriptional regulation of T cell tolerance. *Semin Immunol* 2007;19:180–187. [PubMed: 17387022]
38. Sharpe AH, Freeman GJ. The B7-CD28 superfamily. *Nat Rev Immunol* 2002;2:116–126. [PubMed: 11910893]
39. Greenwald RJ, Freeman GJ, Sharpe AH. The B7 family revisited. *Annu Rev Immunol* 2005;23:515–548. [PubMed: 15771580]
40. Croft M. The role of TNF superfamily members in T-cell function and diseases. *Nat Rev Immunol* 2009;9:271–285. [PubMed: 19319144]
41. Watts TH. TNF/TNFR family members in costimulation of T cell responses. *Annu Rev Immunol* 2005;23:23–68. [PubMed: 15771565]
42. Tesselaar K, et al. Lethal T cell immunodeficiency induced by chronic costimulation via CD27-CD70 interactions. *Nat Immunol* 2003;4:49–54. [PubMed: 12469117]
43. Suntharalingam G, et al. Cytokine storm in a phase 1 trial of the anti-CD28 monoclonal antibody TGN1412. *N Engl J Med* 2006;355:1018–1028. [PubMed: 16908486]
44. Curtsinger JM, et al. Inflammatory cytokines provide a third signal for activation of naive CD4+ and CD8+ T cells. *J Immunol* 1999;162:3256–3262. [PubMed: 10092777]
45. Curtsinger JM, Lins DC, Mescher MF. Signal 3 determines tolerance versus full activation of naive CD8 T cells: dissociating proliferation and development of effector function. *J Exp Med* 2003;197:1141–1151. [PubMed: 12732656]
46. Curtsinger JM, Johnson CM, Mescher MF. CD8 T cell clonal expansion and development of effector function require prolonged exposure to antigen, costimulation, and signal 3 cytokine. *J Immunol* 2003;171:5165–5171. [PubMed: 14607916]
47. Kolumam GA, Thomas S, Thompson LJ, Sprent J, Murali-Krishna K. Type I interferons act directly on CD8 T cells to allow clonal expansion and memory formation in response to viral infection. *J Exp Med* 2005;202:637–650. [PubMed: 16129706]

48. Huse M, Lillemeier BF, Kuhns MS, Chen DS, Davis MM. T cells use two directionally distinct pathways for cytokine secretion. *Nat Immunol* 2006;7:247–255. [PubMed: 16444260]
49. Chen DS, et al. Marked differences in human melanoma antigen-specific T cell responsiveness after vaccination using a functional microarray. *PLoS Med* 2005;2:e265. [PubMed: 16162034]
50. Wong P, Pamer EG. Disparate in vitro and in vivo requirements for IL-2 during antigen-independent CD8 T cell expansion. *J Immunol* 2004;172:2171–2176. [PubMed: 14764683]
51. D'Souza WN, Schluns KS, Masopust D, Lefrancois L. Essential role for IL-2 in the regulation of antiviral extralymphoid CD8 T cell responses. *J Immunol* 2002;168:5566–5572. [PubMed: 12023352]
52. D'Souza WN, Lefrancois L. IL-2 is not required for the initiation of CD8 T cell cycling but sustains expansion. *J Immunol* 2003;171:5727–5735. [PubMed: 14634080]
53. Williams MA, Tyznik AJ, Bevan MJ. Inter-leukin-2 signals during priming are required for secondary expansion of CD8+ memory T cells. *Nature* 2006;441:890–893. [PubMed: 16778891]
54. Bachmann MF, Wolint P, Walton S, Schwarz K, Oxenius A. Differential role of IL-2R signaling for CD8+ T cell responses in acute and chronic viral infections. *Eur J Immunol* 2007;37:1502–1512. [PubMed: 17492805]
55. Zeng R, et al. Synergy of IL-21 and IL-15 in regulating CD8+ T cell expansion and function. *J Exp Med* 2005;201:139–148. [PubMed: 15630141]
56. Cotalorda A, et al. TLR2 engagement on CD8 T cells lowers the threshold for optimal antigen-induced T cell activation. *Eur J Immunol* 2006;36:1684–1693. [PubMed: 16761317]
57. Bennett SR, Carbone FR, Karamalis F, Miller JF, Heath WR. Induction of a CD8+ cytotoxic T lymphocyte response by cross-priming requires cognate CD4+ T cell help. *J Exp Med* 1997;186:65–70. [PubMed: 9206998]
58. Smith CM, et al. Cognate CD4(+) T cell licensing of dendritic cells in CD8(+) T cell immunity. *Nat Immunol* 2004;5:1143–1148. [PubMed: 15475958]
59. Schoenberger SP, Toes RE, van der Voort EI, Offringa R, Melief CJ. T-cell help for cytotoxic T lymphocytes is mediated by CD40–CD40L interactions. *Nature* 1998;393:480–483. [PubMed: 9624005]
60. Bennett SR, Carbone FR, Karamalis F, Flavell RA, Miller JF, Heath WR. Help for cytotoxic-T-cell responses is mediated by CD40 signalling. *Nature* 1998;393:478–480. [PubMed: 9624004]
61. Ridge JP, Di Rosa F, Matzinger P. A conditioned dendritic cell can be a temporal bridge between a CD4+ T-helper and a T-killer cell. *Nature* 1998;393:474–478. [PubMed: 9624003]
62. Johnson S, et al. Selected Toll-like receptor ligands and viruses promote helper-independent cytotoxic T cell priming by upregulating CD40L on dendritic cells. *Immunity* 2009;30:218–227. [PubMed: 19200758]
63. Bullock TN, Yagita H. Induction of CD70 on dendritic cells through CD40 or TLR stimulation contributes to the development of CD8+ T cell responses in the absence of CD4+ T cells. *J Immunol* 2005;174:710–717. [PubMed: 15634890]
64. Prilliman KR, et al. Cutting edge: a crucial role for B7-CD28 in transmitting T help from APC to CTL. *J Immunol* 2002;169:4094–4097. [PubMed: 12370335]
65. Castellino F, Huang AY, Altan-Bonnet G, Stoll S, Scheinecker C, Germain RN. Chemokines enhance immunity by guiding naive CD8+ T cells to sites of CD4+ T cell-dendritic cell interaction. *Nature* 2006;440:890–895. [PubMed: 16612374]
66. Taraban VY, Rowley TF, Al-Shamkhani A. Cutting edge: a critical role for CD70 in CD8 T cell priming by CD40-licensed APCs. *J Immunol* 2004;173:6542–6546. [PubMed: 15557144]
67. Filatenkov AA, Jacovetty EL, Fischer UB, Curtsinger JM, Mescher MF, Ingulli E. CD4 T cell-dependent conditioning of dendritic cells to produce IL-12 results in CD8-mediated graft rejection and avoidance of tolerance. *J Immunol* 2005;174:6909–6917. [PubMed: 15905533]
68. Oh S, Perera LP, Terabe M, Ni L, Waldmann TA, Berzofsky JA. IL-15 as a mediator of CD4+ help for CD8+ T cell longevity and avoidance of TRAIL-mediated apoptosis. *Proc Natl Acad Sci USA* 2008;105:5201–5206. [PubMed: 18362335]
69. Keene JA, Forman J. Helper activity is required for the in vivo generation of cytotoxic T lymphocytes. *J Exp Med* 1982;155:768–782. [PubMed: 6801178]

70. Matthews KE, et al. Increasing the survival of dendritic cells in vivo does not replace the requirement for CD4+ T cell help during primary CD8+ T cell responses. *J Immunol* 2007;179:5738–5747. [PubMed: 17947646]
71. Castellino F, Germain RN. Chemokine-guided CD4+ T cell help enhances generation of IL-6RalphahighIL-7Ralphahigh prememory CD8+ T cells. *J Immunol* 2007;178:778–787. [PubMed: 17202339]
72. Nakanishi Y, Lu B, Gerard C, Iwasaki A. CD8(+) T lymphocyte mobilization to virus-infected tissue requires CD4(+) T-cell help. *Nature* 2009;462:510–513. [PubMed: 19898495]
73. Janssen EM, et al. CD4+ T-cell help controls CD8+ T-cell memory via TRAIL-mediated activation-induced cell death. *Nature* 2005;434:88–93. [PubMed: 15744305]
74. Griffith TS, et al. Apoptotic cells induce tolerance by generating helpless CD8+ T cells that produce TRAIL. *J Immunol* 2007;178:2679–2687. [PubMed: 17312109]
75. Sacks JA, Bevan MJ. TRAIL deficiency does not rescue impaired CD8+ T cell memory generated in the absence of CD4+ T cell help. *J Immunol* 2008;180:4570–4576. [PubMed: 18354179]
76. Badovinac VP, Messingham KA, Griffith TS, Harty JT. TRAIL deficiency delays, but does not prevent, erosion in the quality of “helpless” memory CD8 T cells. *J Immunol* 2006;177:999–1006. [PubMed: 16818756]
77. Bevan MJ. Helping the CD8(+) T-cell response. *Nat Rev Immunol* 2004;4:595–602. [PubMed: 15286726]
78. Janssen EM, Lemmens EE, Wolfe T, Christen U, von Herrath MG, Schoenberger SP. CD4+ T cells are required for secondary expansion and memory in CD8+ T lymphocytes. *Nature* 2003;421:852–856. [PubMed: 12594515]
79. Sun JC, Bevan MJ. Defective CD8 T cell memory following acute infection without CD4 T cell help. *Science* 2003;300:339–342. [PubMed: 12690202]
80. Shedlock DJ, Shen H. Requirement for CD4 T cell help in generating functional CD8 T cell memory. *Science* 2003;300:337–339. [PubMed: 12690201]
81. Sun JC, Williams MA, Bevan MJ. CD4+ T cells are required for the maintenance, not programming, of memory CD8+ T cells after acute infection. *Nat Immunol* 2004;5:927–933. [PubMed: 15300249]
82. Novy P, Quigley M, Huang X, Yang Y. CD4 T cells are required for CD8 T cell survival during both primary and memory recall responses. *J Immunol* 2007;179:8243–8251. [PubMed: 18056368]
83. Kedl RM, et al. T cells compete for access to antigen-bearing antigen-presenting cells. *J Exp Med* 2000;192:1105–1113. [PubMed: 11034600]
84. Wong P, Pamer EG. Feedback regulation of pathogen-specific T cell priming. *Immunity* 2003;18:499–511. [PubMed: 12705853]
85. Guarda G, et al. L-selectin-negative CCR7-effector and memory CD8+ T cells enter reactive lymph nodes and kill dendritic cells. *Nat Immunol* 2007;8:743–752. [PubMed: 17529983]
86. Yang J, Huck SP, McHugh RS, Hermans IF, Ronchese F. Perforin-dependent elimination of dendritic cells regulates the expansion of antigen-specific CD8+ T cells in vivo. *Proc Natl Acad Sci USA* 2006;103:147–152. [PubMed: 16373503]
87. Pentcheva-Hoang T, Corse E, Allison JP. Negative regulators of T-cell activation: potential targets for therapeutic intervention in cancer, autoimmune disease, and persistent infections. *Immunol Rev* 2009;229:67–87. [PubMed: 19426215]
88. Shevach EM. CD4+ CD25+ suppressor T cells: more questions than answers. *Nat Rev Immunol* 2002;2:389–400. [PubMed: 12093005]
89. Sakaguchi S. Naturally arising CD4+ regulatory t cells for immunologic self-tolerance and negative control of immune responses. *Annu Rev Immunol* 2004;22:531–562. [PubMed: 15032588]
90. Belkaid Y, Piccirillo CA, Mendez S, Shevach EM, Sacks DL. CD4+CD25+ regulatory T cells control *Leishmania* major persistence and immunity. *Nature* 2002;420:502–507. [PubMed: 12466842]
91. Dittmer U, et al. Functional impairment of CD8(+) T cells by regulatory T cells during persistent retroviral infection. *Immunity* 2004;20:293–303. [PubMed: 15030773]
92. Chen ML, et al. Regulatory T cells suppress tumor-specific CD8 T cell cytotoxicity through TGF-beta signals in vivo. *Proc Natl Acad Sci USA* 2005;102:419–424. [PubMed: 15623559]

93. Mempel TR, et al. Regulatory T cells reversibly suppress cytotoxic T cell function independent of effector differentiation. *Immunity* 2006;25:129–141. [PubMed: 16860762]
94. Rouse BT, Sarangi PP, Suvas S. Regulatory T cells in virus infections. *Immunol Rev* 2006;212:272–286. [PubMed: 16903920]
95. Li S, Gowans EJ, Chougnet C, Plebanski M, Dittmer U. Natural regulatory T cells and persistent viral infection. *J Virol* 2008;82:21–30. [PubMed: 17855537]
96. Lund JM, Hsing L, Pham TT, Rudensky AY. Coordination of early protective immunity to viral infection by regulatory T cells. *Science* 2008;320:1220–1224. [PubMed: 18436744]
97. Lanteri MC, et al. Tregs control the development of symptomatic West Nile virus infection in humans and mice. *J Clin Invest* 2009;119:3266–3277. [PubMed: 19855131]
98. Zelinskyy G, Dietze K, Sparwasser T, Dittmer U. Regulatory T cells suppress antiviral immune responses and increase viral loads during acute infection with a lymphotropic retrovirus. *PLoS Pathog* 2009;5:e1000406. [PubMed: 19714239]
99. van Stipdonk MJ, Lemmens EE, Schoenberger SP. Naive CTLs require a single brief period of antigenic stimulation for clonal expansion and differentiation. *Nat Immunol* 2001;2:423–429. [PubMed: 11323696]
100. Kaech SM, Ahmed R. Memory CD8+ T cell differentiation: initial antigen encounter triggers a developmental program in naive cells. *Nat Immunol* 2001;2:415–422. [PubMed: 11323695]
101. van Stipdonk MJ, et al. Dynamic programming of CD8+ T lymphocyte responses. *Nat Immunol* 2003;4:361–365. [PubMed: 12640451]
102. Mercado R, Vijh S, Allen SE, Kerksiek K, Pilip IM, Pamer EG. Early programming of T cell populations responding to bacterial infection. *J Immunol* 2000;165:6833–6839. [PubMed: 11120806]
103. Prlic M, Hernandez-Hoyos G, Bevan MJ. Duration of the initial TCR stimulus controls the magnitude but not functionality of the CD8+ T cell response. *J Exp Med* 2006;203:2135–2143. [PubMed: 16908626]
104. Stock AT, Mueller SN, van Lint AL, Heath WR, Carbone FR. Cutting edge: prolonged antigen presentation after herpes simplex virus-1 skin infection. *J Immunol* 2004;173:2241–2244. [PubMed: 15294935]
105. Wherry EJ, Barber DL, Kaech SM, Blattman JN, Ahmed R. Antigen-independent memory CD8 T cells do not develop during chronic viral infection. *Proc Natl Acad Sci USA* 2004;101:16004–16009. [PubMed: 15505208]
106. Fuller MJ, et al. Cutting edge: emergence of CD127^{high} functionally competent memory T cells is compromised by high viral loads and inadequate T cell help. *J Immunol* 2005;174:5926–5930. [PubMed: 15879083]
107. Gett AV, Sallusto F, Lanzavecchia A, Geginat J. T cell fitness determined by signal strength. *Nat Immunol* 2003;4:355–360. [PubMed: 12640450]
108. Badovinac VP, Porter BB, Harty JT. Programmed contraction of CD8(+) T cells after infection. *Nat Immunol* 2002;3:619–626. [PubMed: 12055624]
109. Arens R, et al. Tumor rejection induced by CD70-mediated quantitative and qualitative effects on effector CD8+ T cell formation. *J Exp Med* 2004;199:1595–1605. [PubMed: 15184507]
110. Yajima T, et al. IL-15 regulates CD8+ T cell contraction during primary infection. *J Immunol* 2006;176:507–515. [PubMed: 16365444]
111. Nanjappa SG, Walent JH, Morre M, Suresh M. Effects of IL-7 on memory CD8 T cell homeostasis are influenced by the timing of therapy in mice. *J Clin Invest* 2008;118:1027–1039. [PubMed: 18246202]
112. Prlic M, Bevan MJ. Exploring regulatory mechanisms of CD8+ T cell contraction. *Proc Natl Acad Sci USA* 2008;105:16689–16694. [PubMed: 18946035]
113. Munitic I, et al. Epitope specificity and relative clonal abundance do not affect CD8 differentiation patterns during lymphocytic choriomeningitis virus infection. *J Virol* 2009;83:11795–11807. [PubMed: 19726518]
114. Zehn D, Lee SY, Bevan MJ. Complete but curtailed T-cell response to very low-affinity antigen. *Nature* 2009;458:211–214. [PubMed: 19182777]

115. Moon JJ, et al. Naive CD4(+) T cell frequency varies for different epitopes and predicts repertoire diversity and response magnitude. *Immunity* 2007;27:203–213. [PubMed: 17707129]
116. Kotturi MF, et al. Naive precursor frequencies and MHC binding rather than the degree of epitope diversity shape CD8+ T cell immunodominance. *J Immunol* 2008;181:2124–2133. [PubMed: 18641351]
117. Schiemann M, Busch V, Linkemann K, Huster KM, Busch DH. Differences in maintenance of CD8+ and CD4+ bacteria-specific effector-memory T cell populations. *Eur J Immunol* 2003;33:2875–2885. [PubMed: 14515271]
118. Wherry EJ, McElhaugh MJ, Eisenlohr LC. Generation of CD8(+) T cell memory in response to low, high, and excessive levels of epitope. *J Immunol* 2002;168:4455–4461. [PubMed: 11970989]
119. van Heijst JW, et al. Recruitment of antigen-specific CD8+ T cells in response to infection is markedly efficient. *Science* 2009;325:1265–1269. [PubMed: 19729659]
120. Marzo AL, Klonowski KD, Le Bon A, Borrow P, Tough DF, Lefrancois L. Initial T cell frequency dictates memory CD8+ T cell lineage commitment. *Nat Immunol* 2005;6:793–799. [PubMed: 16025119]
121. Badovinac VP, Haring JS, Harty JT. Initial T cell receptor transgenic cell precursor frequency dictates critical aspects of the CD8(+) T cell response to infection. *Immunity* 2007;26:827–841. [PubMed: 17555991]
122. Badovinac VP, Messingham KA, Hamilton SE, Harty JT. Regulation of CD8+ T cells undergoing primary and secondary responses to infection in the same host. *J Immunol* 2003;170:4933–4942. [PubMed: 12734336]
123. Kastenmuller W, et al. Cross-competition of CD8+ T cells shapes the immunodominance hierarchy during boost vaccination. *J Exp Med* 2007;204:2187–2198. [PubMed: 17709425]
124. Haluszczak C, et al. The antigen-specific CD8+ T cell repertoire in unimmunized mice includes memory phenotype cells bearing markers of homeostatic expansion. *J Exp Med* 2009;206:435–448. [PubMed: 19188498]
125. Bromley SK, Thomas SY, Luster AD. Chemokine receptor CCR7 guides T cell exit from peripheral tissues and entry into afferent lymphatics. *Nat Immunol* 2005;6:895–901. [PubMed: 16116469]
126. Chowdhury D, Lieberman J. Death by a thousand cuts: granzyme pathways of programmed cell death. *Annu Rev Immunol* 2008;26:389–420. [PubMed: 18304003]
127. Slifka MK, Whitton JL. Antigen-specific regulation of T cell-mediated cytokine production. *Immunity* 2000;12:451–457. [PubMed: 10843378]
128. Slifka MK, Rodriguez F, Whitton JL. Rapid on/off cycling of cytokine production by virus-specific CD8+ T cells. *Nature* 1999;401:76–79. [PubMed: 10485708]
129. Badovinac VP, Corbin GA, Harty JT. Cutting edge: OFF cycling of TNF production by antigen-specific CD8+ T cells is antigen independent. *J Immunol* 2000;165:5387–5391. [PubMed: 11067887]
130. Masopust D, Vezys V, Marzo AL, Lefrancois L. Preferential localization of effector memory cells in nonlymphoid tissue. *Science* 2001;291:2413–2417. [PubMed: 11264538]
131. Campbell DJ, Kim CH, Butcher EC. Chemokines in the systemic organization of immunity. *Immunol Rev* 2003;195:58–71. [PubMed: 12969310]
132. Mora JR, Cheng G, Picarella D, Briskin M, Buchanan N, von Andrian UH. Reciprocal and dynamic control of CD8 T cell homing by dendritic cells from skin- and gut-associated lymphoid tissues. *J Exp Med* 2005;201:303–316. [PubMed: 15642741]
133. Dudda JC, Simon JC, Martin S. Dendritic cell immunization route determines CD8+ T cell trafficking to inflamed skin: role for tissue microenvironment and dendritic cells in establishment of T cell-homing subsets. *J Immunol* 2004;172:857–863. [PubMed: 14707056]
134. Liu L, Fuhlbrigge RC, Karibian K, Tian T, Kupper TS. Dynamic programming of CD8+ T cell trafficking after live viral immunization. *Immunity* 2006;25:511–520. [PubMed: 16973385]
135. Masopust D, Vezys V, Wherry EJ, Barber DL, Ahmed R. Cutting edge: gut microenvironment promotes differentiation of a unique memory CD8 T cell population. *J Immunol* 2006;176:2079–2083. [PubMed: 16455963]
136. Schepers K, et al. Dissecting T cell lineage relationships by cellular barcoding. *J Exp Med* 2008;205:2309–2318. [PubMed: 18809713]

137. Klonowski KD, Williams KJ, Marzo AL, Blair DA, Lingenheld EG, Lefrancois L. Dynamics of blood-borne CD8 memory T cell migration in vivo. *Immunity* 2004;20:551–562. [PubMed: 15142524]
138. Sallusto F, Lenig D, Forster R, Lipp M, Lanzavecchia A. Two subsets of memory T lymphocytes with distinct homing potentials and effector functions. *Nature* 1999;401:708–712. [PubMed: 10537110]
139. Hikono H, Kohlmeier JE, Takamura S, Wittmer ST, Roberts AD, Woodland DL. Activation phenotype, rather than central-or effector-memory phenotype, predicts the recall efficacy of memory CD8+ T cells. *J Exp Med* 2007;204:1625–1636. [PubMed: 17606632]
140. Bachmann MF, Wolint P, Schwarz K, Oxenius A. Recall proliferation potential of memory CD8+ T cells and antiviral protection. *J Immunol* 2005;175:4677–4685. [PubMed: 16177115]
141. Wherry EJ, et al. Lineage relationship and protective immunity of memory CD8 T cell subsets. *Nat Immunol* 2003;4:225–234. [PubMed: 12563257]
142. Seder RA, Darrah PA, Roederer M. T-cell quality in memory and protection: implications for vaccine design. *Nat Rev Immunol* 2008;8:247–258. [PubMed: 18323851]
143. Kaech SM, Wherry EJ. Heterogeneity and cell-fate decisions in effector and memory CD8+ T cell differentiation during viral infection. *Immunity* 2007;27:393–405. [PubMed: 17892848]
144. Opferman JT, Ober BT, Ashton-Rickardt PG. Linear differentiation of cytotoxic effectors into memory T lymphocytes. *Science* 1999;283:1745–1748. [PubMed: 10073942]
145. Kaech SM, Hemby S, Kersh E, Ahmed R. Molecular and functional profiling of memory CD8 T cell differentiation. *Cell* 2002;111:837–851. [PubMed: 12526810]
146. Sarkar S, Kalia V, Haining WN, Konieczny BT, Subramaniam S, Ahmed R. Functional and genomic profiling of effector CD8 T cell subsets with distinct memory fates. *J Exp Med* 2008;205:625–640. [PubMed: 18316415]
147. Jacob J, Baltimore D. Modelling T-cell memory by genetic marking of memory T cells in vivo. *Nature* 1999;399:593–597. [PubMed: 10376601]
148. Harrington LE, Janowski KM, Oliver JR, Zajac AJ, Weaver CT. Memory CD4 T cells emerge from effector T-cell progenitors. *Nature* 2008;452:356–360. [PubMed: 18322463]
149. Bannard O, Kraman M, Fearon DT. Secondary replicative function of CD8+ T cells that had developed an effector phenotype. *Science* 2009;323:505–509. [PubMed: 19164749]
150. Wong P, Lara-Tejero M, Ploss A, Leiner I, Pamer EG. Rapid development of T cell memory. *J Immunol* 2004;172:7239–7245. [PubMed: 15187098]
151. Badovinac VP, Harty JT. Manipulating the rate of memory CD8+ T cell generation after acute infection. *J Immunol* 2007;179:53–63. [PubMed: 17579021]
152. Badovinac VP, Messingham KA, Jabbari A, Haring JS, Harty JT. Accelerated CD8+ T-cell memory and prime-boost response after dendritic-cell vaccination. *Nat Med* 2005;11:748–756. [PubMed: 15951824]
153. Lauvau G, et al. Priming of memory but not effector CD8 T cells by a killed bacterial vaccine. *Science* 2001;294:1735–1739. [PubMed: 11721060]
154. Chang JT, et al. Asymmetric T lymphocyte division in the initiation of adaptive immune responses. *Science* 2007;315:1687–1691. [PubMed: 17332376]
155. Huster KM, et al. Selective expression of IL-7 receptor on memory T cells identifies early CD40L-dependent generation of distinct CD8+ memory T cell subsets. *Proc Natl Acad Sci USA* 2004;101:5610–5615. [PubMed: 15044705]
156. Kaech SM, Tan JT, Wherry EJ, Konieczny BT, Surh CD, Ahmed R. Selective expression of the interleukin 7 receptor identifies effector CD8 T cells that give rise to long-lived memory cells. *Nat Immunol* 2003;4:1191–1198. [PubMed: 14625547]
157. Madakamutil LT, et al. CD8alphaalpha-mediated survival and differentiation of CD8 memory T cell precursors. *Science* 2004;304:590–593. [PubMed: 15105501]
158. Bachmann MF, Beerli RR, Agnellini P, Wolint P, Schwarz K, Oxenius A. Long-lived memory CD8+ T cells are programmed by prolonged antigen exposure and low levels of cellular activation. *Eur J Immunol* 2006;36:842–854. [PubMed: 16552716]

159. Haring JS, Jing X, Bollenbacher-Reilley J, Xue HH, Leonard WJ, Harty JT. Constitutive expression of IL-7 receptor alpha does not support increased expansion or prevent contraction of antigen-specific CD4 or CD8 T cells following *Listeria monocytogenes* infection. *J Immunol* 2008;180:2855–2862. [PubMed: 18292507]
160. Chande A, Kaech SM. Cutting edge: memory CD8 T cell maturation occurs independently of CD8alpha. *J Immunol* 2005;175:5619–5623. [PubMed: 16237050]
161. Lacombe MH, Hardy MP, Rooney J, Labrecque N. IL-7 receptor expression levels do not identify CD8+ memory T lymphocyte precursors following peptide immunization. *J Immunol* 2005;175:4400–4407. [PubMed: 16177081]
162. Klonowski KD, Williams KJ, Marzo AL, Lefrancois L. Cutting edge: IL-7-independent regulation of IL-7 receptor alpha expression and memory CD8 T cell development. *J Immunol* 2006;177:4247–4251. [PubMed: 16982855]
163. Carrio R, Rolle CE, Malek TR. Non-redundant role for IL-7R signaling for the survival of CD8+ memory T cells. *Eur J Immunol* 2007;37:3078–3088. [PubMed: 17935075]
164. Joshi NS, et al. Inflammation directs memory precursor and short-lived effector CD8(+) T cell fates via the graded expression of T-bet transcription factor. *Immunity* 2007;27:281–295. [PubMed: 17723218]
165. Sarkar S, et al. Strength of stimulus and clonal competition impact the rate of memory CD8 T cell differentiation. *J Immunol* 2007;179:6704–6714. [PubMed: 17982060]
166. Huster KM, Koffler M, Stemberger C, Schiemann M, Wagner H, Busch DH. Unidirectional development of CD8+ central memory T cells into protective *Listeria*-specific effector memory T cells. *Eur J Immunol* 2006;36:1453–1464. [PubMed: 16637009]
167. Marzo AL, Yagita H, Lefrancois L. Cutting edge: migration to nonlymphoid tissues results in functional conversion of central to effector memory CD8 T cells. *J Immunol* 2007;179:36–40. [PubMed: 17579018]
168. Stemberger C, et al. A single naive CD8+ T cell precursor can develop into diverse effector and memory subsets. *Immunity* 2007;27:985–997. [PubMed: 18082432]
169. Shin H, Wherry EJ. CD8 T cell dysfunction during chronic viral infection. *Curr Opin Immunol* 2007;19:408–415. [PubMed: 17656078]
170. Barber DL, et al. Restoring function in exhausted CD8 T cells during chronic viral infection. *Nature* 2006;439:682–687. [PubMed: 16382236]
171. Blackburn SD, et al. Coregulation of CD8+ T cell exhaustion by multiple inhibitory receptors during chronic viral infection. *Nat Immunol* 2009;10:29–37. [PubMed: 19043418]
172. Shin H, Blackburn SD, Blattman JN, Wherry EJ. Viral antigen and extensive division maintain virus-specific CD8 T cells during chronic infection. *J Exp Med* 2007;204:941–949. [PubMed: 17420267]
173. Cush SS, Anderson KM, Ravneberg DH, Weslow-Schmidt JL, Flano E. Memory generation and maintenance of CD8+ T cell function during viral persistence. *J Immunol* 2007;179:141–153. [PubMed: 17579032]
174. Trautmann L, et al. Upregulation of PD-1 expression on HIV-specific CD8+ T cells leads to reversible immune dysfunction. *Nat Med* 2006;12:1198–1202. [PubMed: 16917489]
175. van Lier RA, ten Berge IJ, Gamadia LE. Human CD8(+) T-cell differentiation in response to viruses. *Nat Rev Immunol* 2003;3:931–939. [PubMed: 14647475]
176. Pearce EL, Shen H. Making sense of inflammation, epigenetics, and memory CD8+ T-cell differentiation in the context of infection. *Immunol Rev* 2006;211:197–202. [PubMed: 16824128]
177. Reiner SL. Epigenetic control in the immune response. *Hum Mol Genet* 2005;14:R41–R46. [PubMed: 15809272]
178. Pearce EL, et al. Control of effector CD8+ T cell function by the transcription factor Eomesodermin. *Science* 2003;302:1041–1043. [PubMed: 14605368]
179. Sullivan BM, Juedes A, Szabo SJ, von Herrath M, Glimcher LH. Antigen-driven effector CD8 T cell function regulated by T-bet. *Proc Natl Acad Sci USA* 2003;100:15818–15823. [PubMed: 14673093]
180. Intlekofer AM, et al. Effector and memory CD8+ T cell fate coupled by T-bet and eomesodermin. *Nat Immunol* 2005;6:1236–1244. [PubMed: 16273099]

181. Intlekofer AM, et al. Anomalous type 17 response to viral infection by CD8+ T cells lacking T-bet and eomesodermin. *Science* 2008;321:408–411. [PubMed: 18635804]
182. Takemoto N, Intlekofer AM, Northrup JT, Wherry EJ, Reiner SL. Cutting edge: IL-12 inversely regulates T-bet and eomesodermin expression during pathogen-induced CD8+ T cell differentiation. *J Immunol* 2006;177:7515–7519. [PubMed: 17114419]
183. Kallies A, Xin A, Belz GT, Nutt SL. Blimp-1 transcription factor is required for the differentiation of effector CD8(+) T cells and memory responses. *Immunity* 2009;31:283–295. [PubMed: 19664942]
184. Shin H, et al. A role for the transcriptional repressor Blimp-1 in CD8(+) T cell exhaustion during chronic viral infection. *Immunity* 2009;31:309–320. [PubMed: 19664943]
185. Rutishauser RL, et al. Transcriptional repressor Blimp-1 promotes CD8(+) T cell terminal differentiation and represses the acquisition of central memory T cell properties. *Immunity* 2009;31:296–308. [PubMed: 19664941]
186. Glimcher LH, Townsend MJ, Sullivan BM, Lord GM. Recent developments in the transcriptional regulation of cytolytic effector cells. *Nat Rev Immunol* 2004;4:900–911. [PubMed: 15516969]
187. Thomas RM, Gao L, Wells AD. Signals from CD28 induce stable epigenetic modification of the IL-2 promoter. *J Immunol* 2005;174:4639–4646. [PubMed: 15814687]
188. Northrop JK, Thomas RM, Wells AD, Shen H. Epigenetic remodeling of the IL-2 and IFN-gamma loci in memory CD8 T cells is influenced by CD4 T cells. *J Immunol* 2006;177:1062–1069. [PubMed: 16818762]
189. Northrop JK, Wells AD, Shen H. Cutting edge: chromatin remodeling as a molecular basis for the enhanced functionality of memory CD8 T cells. *J Immunol* 2008;181:865–868. [PubMed: 18606637]
190. Agarwal P, et al. Gene regulation and chromatin remodeling by IL-12 and type I IFN in programming for CD8 T cell effector function and memory. *J Immunol* 2009;183:1695–1704. [PubMed: 19592655]
191. McNally JM, Zarozinski CC, Lin MY, Brehm MA, Chen HD, Welsh RM. Attrition of bystander CD8 T cells during virus-induced T-cell and interferon responses. *J Virol* 2001;75:5965–5976. [PubMed: 11390598]
192. Kamath AT, Sheasby CE, Tough DF. Dendritic cells and NK cells stimulate bystander T cell activation in response to TLR agonists through secretion of IFN-alpha beta and IFN-gamma. *J Immunol* 2005;174:767–776. [PubMed: 15634897]
193. Ehl S, Hombach J, Aichele P, Hengartner H, Zinkernagel RM. Bystander activation of cytotoxic T cells: studies on the mechanism and evaluation of in vivo significance in a transgenic mouse model. *J Exp Med* 1997;185:1241–1251. [PubMed: 9104811]
194. Bertram EM, Dawicki W, Watts TH. Role of T cell costimulation in anti-viral immunity. *Semin Immunol* 2004;16:185–196. [PubMed: 15130503]
195. Fuse S, Zhang W, Usherwood EJ. Control of memory CD8+ T cell differentiation by CD80/CD86-CD28 costimulation and restoration by IL-2 during the recall response. *J Immunol* 2008;180:1148–1157. [PubMed: 18178855]
196. Borowski AB, et al. Memory CD8+ T cells require CD28 costimulation. *J Immunol* 2007;179:6494–6503. [PubMed: 17982038]
197. Farber DL. Biochemical signaling pathways for memory T cell recall. *Semin Immunol* 2009;21:84–91. [PubMed: 19298946]
198. Kundig TM, et al. Duration of TCR stimulation determines costimulatory requirement of T cells. *Immunity* 1996;5:41–52. [PubMed: 8758893]
199. Hendriks J, et al. During viral infection of the respiratory tract, CD27, 4-1BB, and OX40 collectively determine formation of CD8+ memory T cells and their capacity for secondary expansion. *J Immunol* 2005;175:1665–1676. [PubMed: 16034107]
200. Le Bon A, et al. Cross-priming of CD8+ T cells stimulated by virus-induced type I interferon. *Nat Immunol* 2003;4:1009–1015. [PubMed: 14502286]
201. Leibundgut-Landmann S, Osorio F, Brown GD, Reis e Sousa C. Stimulation of dendritic cells via the dectin-1/Syk pathway allows priming of cytotoxic T-cell responses. *Blood* 2008;112:4971–4980. [PubMed: 18818389]

202. Marrack P, Kappler J, Mitchell T. Type I interferons keep activated T cells alive. *J Exp Med* 1999;189:521–530. [PubMed: 9927514]
203. Xiao Z, Casey KA, Jameson SC, Curtsinger JM, Mescher MF. Programming for CD8 T cell memory development requires IL-12 or type I IFN. *J Immunol* 2009;182:2786–2794. [PubMed: 19234173]
204. Dalod M, et al. Interferon alpha/beta and interleukin 12 responses to viral infections: pathways regulating dendritic cell cytokine expression in vivo. *J Exp Med* 2002;195:517–528. [PubMed: 11854364]
205. Keppeler SJ, Theil K, Vucikuj S, Aichele P. Effector T-cell differentiation during viral and bacterial infections: role of direct IL-12 signals for cell fate decision of CD8(+) T cells. *Eur J Immunol* 2009;39:1774–1783. [PubMed: 19548244]
206. Zinkernagel RM. On natural and artificial vaccinations. *Annu Rev Immunol* 2003;21:515–546. [PubMed: 12500980]
207. Krieg AM. CpG motifs in bacterial DNA and their immune effects. *Annu Rev Immunol* 2002;20:709–760. [PubMed: 11861616]
208. Datta SK, Cho HJ, Takabayashi K, Horner AA, Raz E. Antigen-immunostimulatory oligonucleotide conjugates: mechanisms and applications. *Immunol Rev* 2004;199:217–226. [PubMed: 15233737]
209. Verdeil G, Marquardt K, Surh CD, Sherman LA. Adjuvants targeting innate and adaptive immunity synergize to enhance tumor immunotherapy. *Proc Natl Acad Sci USA* 2008;105:16683–16688. [PubMed: 18936481]
210. Ahonen CL, et al. Combined TLR and CD40 triggering induces potent CD8+ T cell expansion with variable dependence on type I IFN. *J Exp Med* 2004;199:775–784. [PubMed: 15007094]
211. Ahonen CL, et al. Enhanced efficacy and reduced toxicity of multifactorial adjuvants compared with unitary adjuvants as cancer vaccines. *Blood* 2008;111:3116–3125. [PubMed: 18202224]
212. Johansen P, et al. Antigen kinetics determines immune reactivity. *Proc Natl Acad Sci USA* 2008;105:5189–5194. [PubMed: 18362362]

Fig. 1. Activation of antigen-specific CD8⁺ T cells by APCs

During infection, microbes are detected by pattern recognition receptors such as intra-cellular and extracellular TLRs and intracellular NLRs, which are expressed by APCs. Activation of these receptors initiates signaling pathways that converge at the activation of transcription factors like NF-κB and IRFs, which in turn leads to upregulation of costimulatory molecules and expression of inflammatory cytokines. Activated helper CD4⁺ T cells additionally stimulate APCs by triggering of CD40 expressed on the cell surface. Receptive CD8⁺ T cells that bind with their exclusive TCRs to MHC complexes that are loaded with microbial peptides receive in that case not only TCR signals but also costimulatory signals by ligation of CD28 (signal 2) and TNFR family members such as CD27 (and OX40 and 4-1BB) (signal 2). In addition, the released IL-12 and type I IFNs (signal 3) but also other cytokines and certain chemokines provide supplementary important signals for expansion, differentiation, and migration. Inhibitory molecules like CTLA-4 and PD-1 are upregulated and expressed on the cell surface of CD8⁺ T cells after activation and serve as inhibitory molecules to prevent immunopathology.

Fig. 2. Expansion and differentiation of naive antigen-specific CD8⁺ T cells into effector and memory cells after interaction with pathogen-activated APCs

Early clearance of antigen (but not earlier than a day) allows formation of effective memory cells. Further antigenic stimulation and inflammation leads to enhanced development of effector cells in magnitude and function. After antigen clearance, the majority of these effector CD8⁺ T cells undergo apoptosis, and the remaining forms the effector (T_{EM}) and central memory T-cell (T_{CM}) pools. If high loads of antigen persist, the responsive CD8⁺ T cells become eventually functionally exhausted. Antigen clearance of these exhausted cells compromises their maintenance severely.