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Programming CD8+ T cells for effective immunotherapy

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Abstract

The differentiation state of $CD8^+$ T cells has emerged as a crucial determinant of their ability to respond to tumor and infection. Signals from T-cell receptors, co-stimulatory molecules and cytokine receptors direct the differentiation process. These signals 'program' sustained and heritable gene expression patterns that govern progressive differentiation and lineage commitment. The epigenetic mechanisms by which T cells are programmed are just beginning to be elucidated. Understanding the mechanisms that control $CD8^+$ T-cell differentiation is important in the development of novel immunotherapy strategies.

Introduction

Antigen-presenting cells (APCs) and other immune and non-immune cells direct $CD8^+$ T-cell differentiation by engaging receptors for a host of membrane-bound and soluble molecules [1••,2,3]. Signals from these receptors induce epigenetic changes that 'program' sustained but mutable gene expression patterns that govern progressive differentiation and lineage commitment decisions [4,5]. Emerging evidence indicates that the differentiation state crucially determines $CD8^+$ T-cell effectiveness in responding to infection and tumor [6,7••,8, 9••,10,11]. What are the differentiation states that characterize effective $CD8^+$ T cells? How can $CD8^+$ T cells be programmed to differentiate into optimal effector cells? Here we discuss the concepts of T-cell programming and differentiation and their implications for the development of potent new $CD8^+$ T cell-based immunotherapy.

CD8+ T cells exist in dynamic states of progressive differentiation

Dynamic $CD8^+$ T cell–APC interactions drive $CD8^+$ T-cell proliferation, differentiation and lineage commitment. This process results in the generation of cells that have diverse phenotypic and functional characteristics [3,12,13]. The nomenclature, definitions, characteristics and differentiation pathways for $CD8^+$ T cells are controversial, but two broad categories are generally acknowledged and have been named for their apparent function [12,14,15]. 'Effector' cells (T_{EFF}) are highly cytolytic *in vitro* and express high levels of molecules required for cell killing, such as perforin, granzymes, interferon (IFN)-γ, tumor necrosis factor (TNF) and FAS ligand (FASL) [13,14]. 'Memory' cells are less cytolytic in *in vitro* assays, but exhibit increased survival, show the capacity for antigen-independent self-renewal, and respond vigorously to secondary antigen challenge [8,12,14,16].

Two subsets of memory cells, 'effector memory' (T_{EM}) and 'central memory' (T_{CM}), were originally identified on the basis of tissue homing molecules and effector function [17]. T_{CM} were described as $CD62L^+CCR7^+$ cells that home to lymph nodes and have relatively low immediate effector function. T_{EM} were defined as $CD62L^-CCR7^-$ cells that preferentially home to peripheral tissues and inflammatory sites and possess relatively high immediate

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effector function. More recently, the distinction between T_{EM} and T_{CM} based on effector function has been questioned [18,19]. Generalizations about these two memory subsets have been further confused by the discovery of $CCR7^{-}$ antigen-experienced T cells in lymph nodes [20,21]. Another subset, 'memory stem cells', was recently identified in a murine model of graft-versus-host disease. These post-mitotic $CD44^{lo}CD62L^{hi}CD8^{+}$ T cells were characterized by the expression of Sca-1, CD122 and BCL-2 and were capable of self-renewal and could generate all subsets of memory and effector $CD8^{+}$ T cells [22••].

Memory CD8+ T cells are intermediates in the progressive differentiation pathway

Conflicting models of $CD8^{+}$ T-cell differentiation have been proposed to explain the generation of memory subsets. A model suggesting that T_{CM} and T_{EM} arise from distinct lineages was based on the finding that T-cell receptor (TCR) repertoires of T_{CM} and T_{EM} in the peripheral blood of healthy individuals are largely distinct [23]. However, subsequent studies showing that T_{CM} and T_{EM} clones can derive from a common naïve precursor have cast doubt on the separate lineage hypothesis [24].

A linear pathway of differentiation has instead gained acceptance; however, contention remains about whether memory cells arise from T_{EFF} or *vice versa*. A progressive sequence of naïve $\rightarrow T_{EFF} \rightarrow T_{EM} \rightarrow T_{CM}$ differentiation has been proposed based on the finding that $CD62L^{+}$ cells emerged after the adoptive transfer of $CD62L^{-}$ enriched memory cells [6,25•]. Whether these results reflect a true $T_{EM} \rightarrow T_{CM}$ conversion or a selective survival and/or proliferation advantage of T_{CM} over T_{EM} has not been convincingly demonstrated.

Instead, mounting data, including *ex vivo* phenotypic analyses of virus-specific $CD8^{+}$ T cells in acute and chronic viral disease, measures of telomere length, and *in vitro* differentiation studies, support a linear sequential progression (naïve $\rightarrow T_{CM} \rightarrow T_{EM} \rightarrow T_{EFF}$) model [7••, 9••,12,26]. The recent finding that the gene expression signature of T_{CM} lies between that of naïve cells and T_{EM} further supports the naïve $\rightarrow T_{CM} \rightarrow T_{EM} \rightarrow T_{EFF}$ sequence [27].

Expanding on this model, memory stem cells might represent the earliest antigen-experienced cell population to emerge in the $CD8^{+}$ T-cell differentiation pathway [22••]. Paralleling B-cell differentiation, memory $CD8^{+}$ T cells might represent effector cells in an arrested differentiation state [15,28]. Thus, $CD8^{+}$ T-cell differentiation could be regarded in terms of a continuum from early to late effectors rather than the movement of a T cell between subsets descriptively named memory stem cell, T_{CM}, T_{EM} and T_{EFF} subsets [14].

Early effectors provide the most potent immune response

Where on this differentiation continuum are $CD8^{+}$ T cells most capable of eradicating infection or tumor? Late effectors, owing to their high *in vitro* cytotoxicity, were initially thought to be the most effective cells. However, increasing evidence indicates that progressive differentiation leads to decreased ability to eliminate infection or tumor. For example, impaired function of late effector cells has been observed in patients that have progressive human immunodeficiency virus infection. $CD8^{+}$ T cells in these patients displayed a late effector ($CD27^{-}CD28^{-}$) phenotype and decreased proliferative capacity, deemed 'replicative senescence' [9••]. Furthermore, in the absence of early effectors, late effectors were unable to control cytomegalovirus replication in patients co-infected with human immunodeficiency virus [29]. In clinical trials of adoptive cell transfer therapy for cancer, clonal populations of $CD8^{+}$ T cells that had been multiply stimulated proved ineffective [30,31]. These cells appear to represent late effectors that have poor survival capability as they have a late effector phenotype and do not engraft or persist after adoptive transfer [30,31]. In contrast, treatment

with less-expanded tumor infiltrating lymphocytes caused objective responses in about 50% of treated patients in a recent clinical trial [32]. In patients treated with tumor infiltrating lymphocytes, tumor regression and T-cell persistence correlate with increased telomere length [10,33], and cells that persist express an early effector ($CD27^+CD28^+$) phenotype [34]. These data suggest that an early differentiation state is important for T-cell efficacy.

Studies in murine models have confirmed the superior function of less-differentiated $CD8^+$ T cells and have offered insight into the mechanisms that confer a functional advantage to these cells *in vivo*. In models of viral and intracellular bacterial infection, early effectors provide greater protective immunity, eliminate virus more efficiently, and display greater replicative capacity than late effectors [6,11,13,35]. Similarly, when adoptively transferred, early effectors induce better tumor regression [7••,8], display higher engraftment efficiency, and mediate more severe graft-versus-host disease than more highly differentiated cells [36,22••]. The mechanisms that underlie the loss of function associated with progressive differentiation are complex and include decreased survival and proliferation capacity, reduced responsiveness to homeostatic cytokines, decreased capacity for self-renewal, inability to differentiate into diverse cell types, and impairment of lymphoid tissue homing (Figure 1) [6,7••,14,15].

$CD8^+$ T-cell differentiation is epigenetically programmed

How can $CD8^+$ T-cell differentiation be directed to generate early effectors that are programmed for optimal immune function? Stimulation of $CD8^+$ T cells through TCRs, co-stimulatory molecules and cytokines program changes in gene expression that can be heritable owing to epigenetic modifications in gene transcription [3–5,37]. Although the precise mechanisms are largely unknown, it is likely that these changes include DNA methylation, methyl-CpG-binding proteins, and histone modifications that affect the accessibility to regulatory regions of transcription factors that serve as ‘master regulators’ [4,5].

Advances have been made in identifying the master regulator transcription factors that govern T-cell differentiation [4,5,38–40]. GATA-binding protein 3 (GATA-3) induces the uncommitted $CD4^+$ T cells that emerge from the thymus to differentiate into IL-4-, IL-5- and IL-13-releasing T helper (Th)2 cells, whereas T-bet (encoded by $Tbx21^{-/-}$) promotes differentiation into IFN-γ -releasing Th1 cells. Expression of these master regulators, and therefore lineage commitment, is stabilized by amplification loops and by repression of alternative pathway genes [37]. Nevertheless, the functionality of cells polarized to the Th1 lineage by IFN-γ or the Th2 lineage by IL-4 can be partially reversed by switching to the opposite polarizing cytokine; however, this plasticity decreases with progressive differentiation [38,41].

Less is known about lineage commitment in $CD8^+$ T cells. Commitment to the $CD8^+$ effector cell linage is redundantly determined by T-bet and the T-bet paralog eomesodermin (Eomes). Expression of these transcription factors is also important for maintenance of committed cells. $Eomes^{+/-}Tbx21^{-/-}$ mice are deficient in IL-15-dependent lymphocyte lineages including $CD8^+$ memory cells [42••]. Paralleling memory B-cell development, maintenance of T-cell memory might also require transcriptional repressors that arrest the differentiation process. The transcriptional repressor B-cell CLL/lymphoma 6 (Bcl-6) represses B lymphocyte-induced maturation protein-1 (Blimp-1), a transcriptional activator responsible for plasma-cell differentiation. This repression arrests the differentiation of germinal center B cells, thus enabling the generation and maintenance of B-cell memory [28]. Recent studies in $Bcl6^{-/-}$ and *Bcl6* transgenic mice have also revealed a role for Bcl-6 in $CD8^+$ T-cell memory formation [43,44]. Furthermore, a Bcl-6 homologue, Bcl-6b, was recently reported to be important in maintaining memory $CD8^+$ T-cell replication potential [45]. Transcription-repressing isoforms of lymphoid enhancer-binding factor 1 (Lef1) and transcription factor 7 (Tcf7), which maintain

hematopoietic stem cells in an undifferentiated pluripotent state, might also be required to arrest differentiation and to maintain memory $CD8^+$ T cells [46]. These molecules are highly expressed in naïve and T_{CM} $CD8^+$ T cells, and their expression decreases with progressive differentiation [47]. Furthermore, T cells from $TCF7^{-/-}$ mice spontaneously differentiate more rapidly than cells from $TCF7^{+/-}$ littermates, supporting the hypothesis that TCF7 is important in preventing differentiation [48]. New data derived from the analysis of global gene expression are consistent with the hypothesis that hematopoietic stem cells and memory T and B cells — the only cells of the hematopoietic system able to undergo self-renewal for the lifetime of the organism — might share a common pattern of gene expression [49•]. We are just beginning to elucidate how transcription factors determine T-cell fate. Understanding how these master regulators guide T-cell differentiation is crucial to our efforts to generate optimal effector cells.

Inputs from T-cell receptors, cytokine receptors and costimulatory receptors program $CD8^+$ T cells

Membrane-bound and soluble factors direct programmed changes in $CD8^+$ T-cell development. Increases in duration, magnitude and frequency of the TCR stimulus drive progressive differentiation [2,3,50,51]. The TCR signal is integrated with signals from diverse costimulatory, inhibitory and cytokine receptors. The program imparted by the TCR is sustained by demethylation of the IL-2 promoter, and, depending on the strength of the antigen stimulus, one or both IL-2 alleles can be activated [52,53]. IL-2 expression is also regulated by the costimulatory molecule CD28, which induces histone acetylation and loss of cytosine methylation at the IL-2 promoter/enhancer [54]. Costimulation through CD28 also induces preferential differentiation of T cells into T_{CM}, revealing some of the complexity of the differentiation process [36].

The activities of diverse soluble factors help to shape the complex differentiation process of T-cell differentiation. The impact of distinct cytokines on lineage commitment decisions is better established in $CD4^+$ than $CD8^+$ T cells. IFN-γ, acting through T-bet, induces naïve $CD4^+$ T cells to become IFN-γ-releasing Th1 cells, whereas IL-4, acting through GATA-3, programs differentiation into IL-4-, IL-5- and IL-13-releasing Th2 cells. Evidence for additional $CD4^+$ lineages generated under the influence of diverse cytokines is now emerging: in the absence of IFN-γ and IL-4, IL-23 can induce naïve $CD4^+$ T cells to differentiate into IL-17-releasing Th-17 cells [55]. Alternatively, Th-17 cells might be induced by the exposure to TGF-β and IL-6. Transcriptional factors responsible for the generation of Th-17 cells have not been yet identified, but it appears that neither GATA-3 nor T-bet play a role [56]. Furthermore, TGF-β and IL-10 might influence $CD4^+$ T cells to acquire the regulatory attributes of Th3 and Tr1 cells, respectively [57,58].

Lineage commitment by $CD8^+$ T cells is also affected by the cytokine milieu. $CD8^+$ T cells can be induced to differentiate into cytotoxic T cells secreting Th1-like (Tc1) or Th2-like (Tc2) cytokines by Th1-and Th2-polarizing cytokines respectively [59,60]. However, just as it is becoming clearer that $CD4^+$ T cells differentiate along manifold lineages, it seems likely that $CD8^+$ T-cell differentiation occurs along multiple lines (Figure 2). Many cytokines, including the common gamma-chain (γ_C) cytokines, are integral to $CD8^+$ T-cell differentiation [61]. For example, IL-15 directs $CD8^+$ T cells to preferentially differentiate into T_{CM} whereas IL-2 promotes them to differentiate into T_{EM}. These phenotypic and functional differences are reflected in the distinct gene expression patterns of IL-15- and IL-2-programmed cells [7••,8, 62,63].

The most recently discovered of the γ_C cytokines, IL-21, also confers particular features to $CD8^+$ T cells. IL-21 generates $CD8^+$ T cells that have a distinct $CD45RO^+CD28^{hi}$ stable phenotype. In contrast to cells grown in IL-2, IL-21-programmed $CD8^+$ T cells retain their

capacity to produce IL-2 after antigen exposure [64•,65•]. The pattern of global gene expression by IL-21-programmed $CD8^+$ T cells is unique compared with IL-2-, IL-7- and IL-15-programmed cells, and is characterized by simultaneously increased transcription of genes that encode lymphoid homing molecules and cytolytic effector molecules (CSH, unpublished). This surprising finding challenges the paradigm of linear differentiation for $CD8^+$ T cells, which is characterized by an inverse relationship between the expression of genes for lymphoid homing and for effector function. Thus, differentiation of T cells appears to be multidimensional and is influenced by a host of soluble and cell-bound factors that we have only begun to explore [66–69].

Conclusions

A deeper understanding of programming and differentiation of $CD8^+$ T cells might be valuable in the development of adoptive cell transfer-based immunotherapies for treatment of cancer and chronic infectious disease. It now seems clear that cells at early stages of differentiation have enhanced therapeutic efficacy, but attempts to generate cells that have desired programs have only just begun.

A significant problem in the translation of these ideas into human immunotherapies is that T-cell populations specific for tumor-associated antigens and antigens expressed by chronic pathogens are generally terminally differentiated (exhausted). Recent work suggests it might be possible to reprogram exhausted cells by blockade of negative regulatory receptors, such as programmed death-1 (PD-1) [70••]. Alternatively, antigen-specific cells that have optimal differentiation potential might be generated through gene transfer technology. Although this technology is still evolving, high-efficiency transfer of genetic sequences for specific TCRs into the genome of naïve or less-differentiated $CD8^+$ T cells might soon be possible. In the future, one might be able to attenuate or even reverse progressive differentiation of T cells through manipulation of transcriptional master regulators. The reprogramming of B cells could now be a reality [71••]. As our understanding of programming and differentiation signals improves we might be able to generate a greater diversity of cells and apply them to the treatment of cancer and chronic infectious diseases.

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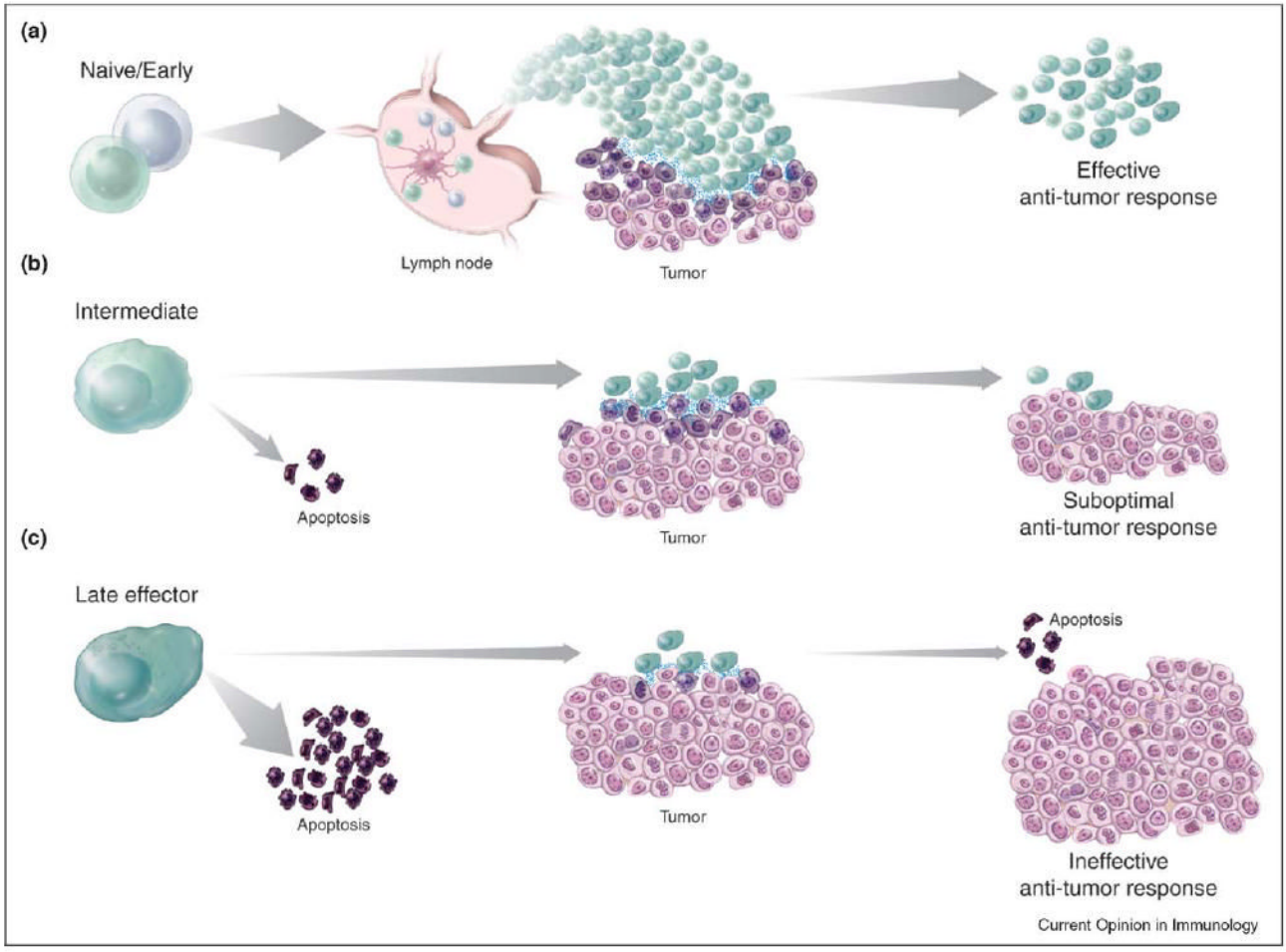

Figure 1.

Progressive differentiation of adoptively transferred $CD8^+$ T cells inversely correlates with *in vivo* anti-tumor efficacy. **(a)** Following adoptive transfer, naïve and early effector $CD8^+$ T cells migrate to lymphoid tissues where they can interact with dendritic cells that are presenting cognate antigens. $CD8^+$ T cells are programmed to proliferate, differentiate and traffic to tumor sites where they can mediate effective anti-tumor responses. After tumor clearance, T cells persist in a variety of differentiation states, providing protective immunity. **(b)** Intermediate effector cells are characterized by down-regulation of lymphoid homing molecules as well as having low proliferative and survival capacity. Following adoptive transfer, these cells become apoptotic or can proliferate moderately and home to tumor sites, where they can exert their cytotoxic potential. Tumor responses are sub-optimal and ultimately result in the exhaustion of T-cell responses. **(c)** Late effectors are characterized by poor survival and proliferative capability. After transfer, the majority of these cells undergo apoptosis. The few surviving late effectors migrate to tumor sites but are insufficient to trigger anti-tumor responses and impact on tumor growth. T cells ultimately are deleted and the tumor inexorably progresses.

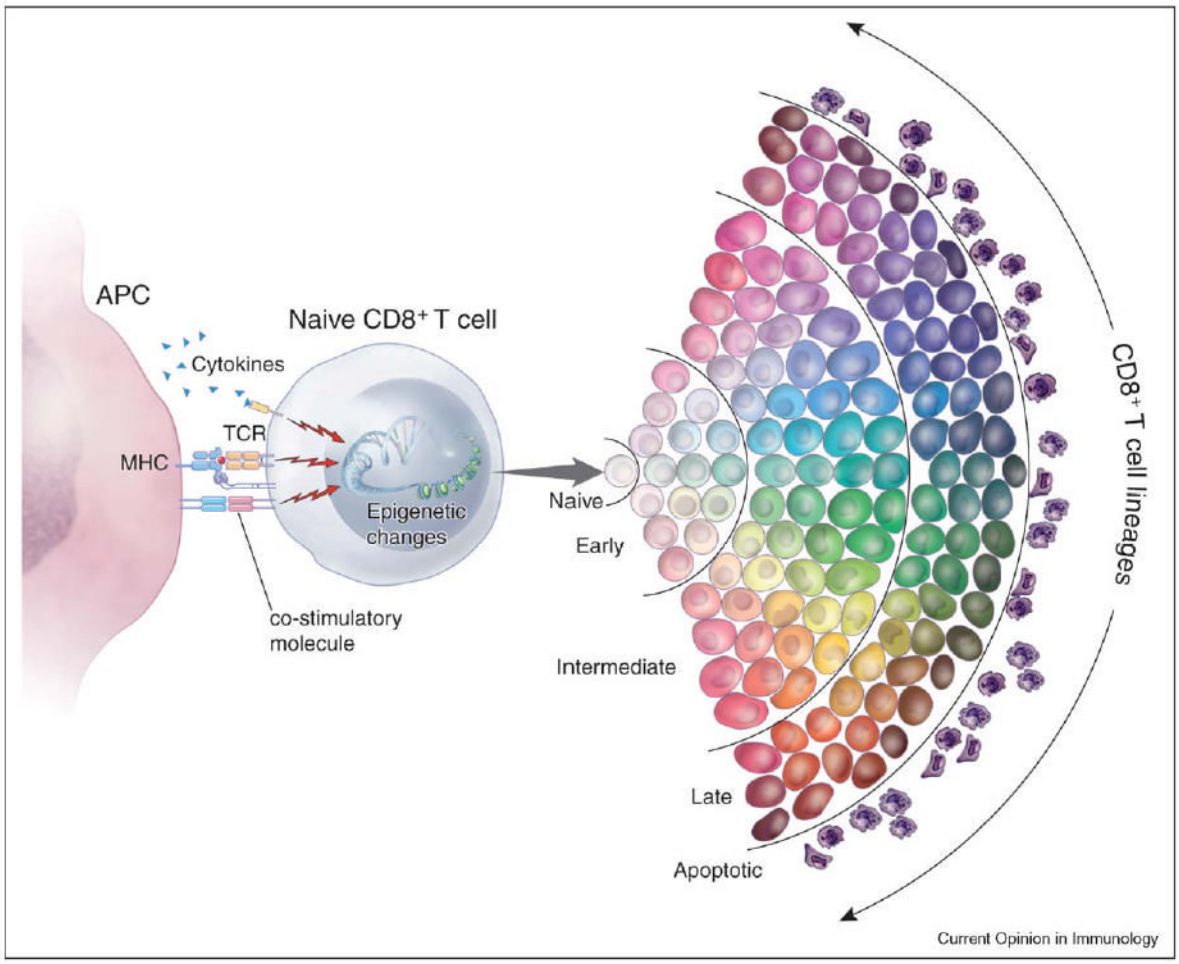

Figure 2.

A new hypothetical multidimensional model of $CD8^+$ T-cell programming and differentiation. $CD8^+$ T-cell differentiation is programmed by a variety of stimulatory and inhibitory signals from T-cell receptors (TCRs), co-stimulatory molecules and cytokine receptors. These signals induce distinct patterns of gene expression that can, through epigenetic mechanisms, be sustained and heritably transmitted. The quality of the integrated signals influences T-cell lineage commitment. Phenotypic and functional attributes are symbolized by different color hues. The strength of the integrated signals drives the T cell toward progressive senescence. T-cell differentiation through early, intermediate and late stages is represented by progressive darkening shades of cell colors. In this model, de-differentiation is not possible under physiologic conditions, although lineage commitment can be mutable. Plasticity of phenotype and function is progressively reduced as the cells approach senescence.