Journal of Immunology
Research and Therapy
JIRT, 1(1): 49-62
www.scitcentral.com

Original Review: Open Access

# Transcriptional Regulation of T Cell Heterogeneity and Tumor Immunity

**Janaki Purushe[1], Hongxing Sun[2], Shan He[2] and Yi Zhang[1,2*]**

[1]*Department of Microbiology & Immunology, Temple University, USA.*

[2] *Fels Institute for Cancer Research and Molecular Biology, Temple University, USA.*

*Received October 25, 2015; Accepted January 2, 2016; Published April 30, 2016*

## ABSTRACT

Upon antigen recognition, naïve T cells have the capacity to differentiate into a multitude of lineages with distinct effector and memory functions. T cell receptor stimulation, costimulation, and cytokines induce transcriptional program changes that critically regulate T cell proliferation, differentiation and survival. While effector T cells mediate an efficient adaptive immune response to primary antigen encounter, long-lived memory T cells are responsible for rapid response to subsequent antigen counters. Both CD4 and CD8 T cells have the capacity to form memory, however CD8 T memory cells are critical mediators of sustained anti-tumor immunity. Memory CD8 T cells can be classified into several subtypes based on their tissue-homing capacity, self-renewal capability and effector recall responsiveness. Better understanding of the transcriptional programs that regulate the generation and maintenance of T cell subsets, particularly T memory subsets, may have significant implications in the development of cellular therapies that achieve long-lasting anti-tumor effector function.

## INTRODUCTION

During immune response, naïve T cells possess a stunning capability to produce distinct subsets of effector cells and memory T cells [1-7]. Although effector T cells are armed to efficiently eliminate targets such as pathogens and tumor cells, they are short-lived cells that undergo massive apoptotic contraction during late stages of the effector phase [8-10]. Unlike effector T cells, memory T cells are long-lived cells that undergo homeostatic survival in the absence of a specific antigen. Upon re-encounter with the specific antigen, memory T cells rapidly acquire effector functions and undergo clonal expansion to produce large numbers of effector T cells, thereby providing protection against secondary infections [1-3,5,11-15]. Thus, effective protective immunity against infection and tumors requires the collective effort of heterogeneous lineages of T cells.

Targeted antigen specificity is a fundamental characteristic of the T cell response. Both the initial activation of naïve T cells and the effector phases of T cell-mediated elimination are triggered by recognition of the antigen by T cell receptors (TCRs) presented on the surface of T cells [3,16]. Upon activation by antigen-presenting cells (APCs), naïve T cells are triggered through TCR signaling that induces cell-intrinsic transcriptional program changes. Costimulatory signaling amplifies these programs to facilitate T cell proliferation and expansion, while cytokines and notch ligands induce differentiation of these activated T cells into distinct lineages of effector cells [17-24]. Changes in these transcriptional programs are characterized by the amount, location, and interaction of transcription factors that are critical for T cell proliferation, differentiation, and survival. Differentiation of T cells into effector subsets is regulated by master transcription factors such as T-bet, Eomes, GATA3, RORγt, and Foxp3. This regulation is complex and involves feedback mechanisms as well as overlapping contributions from other transcription factors [17,18]. Transcriptional programs can be further modified over time by stimuli present in the environments where T cells execute their function. Transcriptional regulation by Id3, Foxo1, T-bet and Eomes also significantly contributes to memory formation and maintenance [25-27]. This review will discuss the impact of transcription factors in regulating T cell heterogeneity and highlight exciting findings from recent studies of transcription factors in regulating memory T cells.

**Corresponding author**: Yi Zhang, MD, PhD, Fels Institute for Cancer Research and Molecular Biology, Department of Microbiology and Immunology, Temple University School of Medicine, Philadelphia, PA 19140, USA, Tel: 215-707-8901; Email: yi.zhang@temple.edu

**Citation:** Purushe J, Sun H, He S, Zhang Y.(2016) Transcriptional Regulation of T Cell Heterogeneity and Tumor Immunity. J Immunol Res Ther, 1(1): 49-62.

### T Cell Heterogeneity and Nomenclatures

The discovery and dissection of the functional differences between effector and memory T cell subsets have significantly advanced our understanding of the mechanisms controlling the development of T cell heterogeneity. Prior to activation by APCs, both CD4 T cells and CD8 T cells are designated as naïve and are maintained in a quiescent state. Following activation, T cells undergo programmed proliferation and differentiation, producing multiple lineages of effector T cells based on the production of distinct effector molecules [18,28]. Activated CD4 T cells can differentiate into distinct lineages of effector cells (**Figure 1**), such as T helper-1 (Th1), Th2 and Th17 and regulatory T cells (Tregs). Th1 CD4T cells are characterized by production of interferon-γ (IFN-γ), whereas Th2 CD4 T cells secrete interleukin-4 (IL-4), IL-5 and IL-13 [18,20,29]. Th17 CD4 T cells are characterized by their capacity to produce high amounts of IL-17 and IL-21 [18,28]. CD4 T cells can also differentiate into Tregs, which can repress inflammatory T cells through the production of IL-10 and TGF-β1 [30]. CD4 T cells may also differentiate into other subsets such as T follicular helper cells (Tfh) [31] and Th9 cells [32,33]. Tfh primarily reside in B-cell follicles and contribute to humoral immunity [31]. Th9 cells, which display an interesting plasticity, may act with Th2 in inflammatory responses or display immunosuppressive function through production of IL-10 [32.34,35]. Activation of naïve CD8 T cells mainly induces the generation of cytotoxic lymphocytes (CTLs) that produce IFN-γ and cytotoxic molecules such as granzyme B (GZMB), perforin (PRF1), and Fas ligand (FASL). CD8 CTLs are capable of direct cell-mediated killing of target cells [5,6].

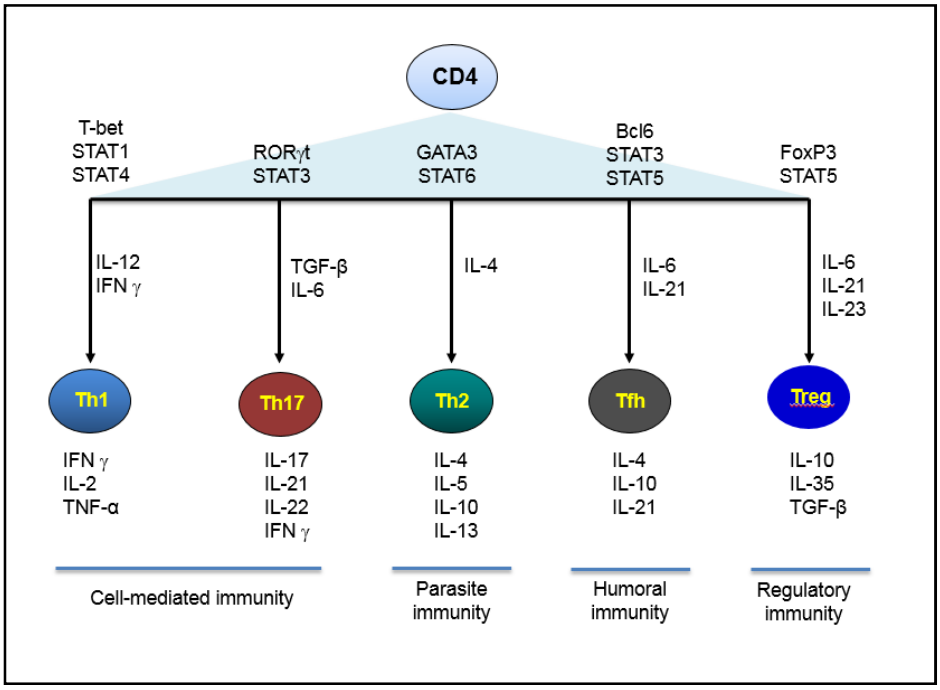


**Figure 1. Naïve CD4 T Cells Differentiate into Distinct Effector Subsets**. Following recognition of a specific antigen presented on an APC, naïve CD4 T cells become activated to differentiate and rapidly expand into distinct T effector subsets. Transcription factors, along with soluble factors present in the extracellular environment are key mediators of changes in T cell transcriptional programs that trigger T effector polarization. T effector subsets are classified by transcription factors that dominantly drive their phenotype as well as the cytokines they express.

CD4 and CD8 T cells both possess the ability to form immunological memory through differentiation into a population of antigen-specific memory T cells that persist throughout the lifetime of an individual after resolution of inflammation [17, 36, 37]. Following re-encounter with a specific antigen, memory T cells can quickly expand and elaborate effector function, thus providing the immune system with long-term protection against secondary antigen encounters. Memory CD8 T cells are heterogeneous populations and have distinct capabilities in the context of providing long-term protection against tumor formation. They can be broadly classified into four subsets based on their tissue homing capacity, self-renewal capability, effector recall responsiveness and surface phenotype (**Figure 2**): effector memory T cells ($T_{EM}$), central memory T cells ($T_{CM}$), resident memory T cells ($T_{RM}$), and stem cell-like memory T cells ($T_{SCM}$) [3,8,38-44]. $T_{EM}$ express low

levels of CD62L and CCR7, allowing them to circulate and preferentially home to non-lymphoid tissues. $T_{CM}$ express CD62L and CCR7, restraining their homing to lymphoid tissues. $T_{RM}$ predominantly reside in the local non-lymphoid tissues, such as the brain, mucosa, lung and skin [7,39]. $T_{RM}$ express CD69 and CD10, surface markers, which distinguish them from $T_{EM}$ [7,45-48]. Finally, $T_{SCM}$ are a memory cell subset expressing a naïve cell-like phenotype of $CD44^{low}CD62L^{high}Sca\text{-}1^{high}CD122^{high}Bcl2^{high}$. They possess the ability to differentiate into all subsets of memory CD8 T cells and effector cells, while maintaining self-renewal capabilities [41,42]. Immunological memory mediated by CD8 and CD4 T cells is critical for prolonged protection against antigen reencounter and tumor formation **(Figure 3)**. The functional complexity of effector and memory subsets characterize T cell heterogeneity. Transcription factors, which critically regulate differentiation into these subsets, play a fundamental role in programming the diverse functions of T cells, which collectively contribute to a comprehensive immune response.

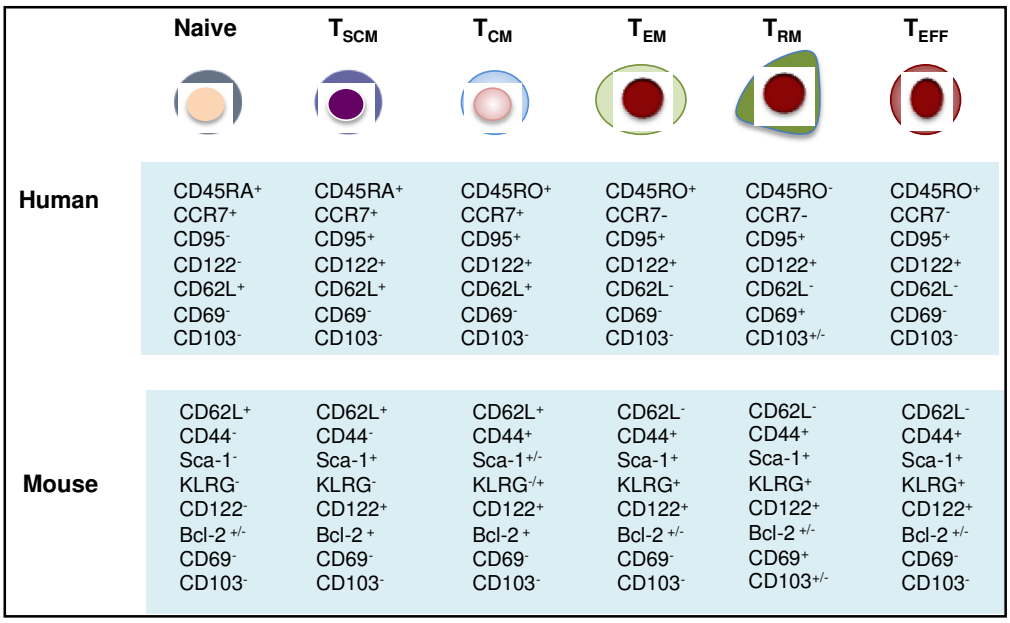


**Figure 2. Surface markers of Distinct Effector and Memory Subsets.**
Upon APC activation, naïve CD8 T cells become activated and differentiate into effector cells. During clonal expansion, effector cells polarize toward various memory subsets that are classified based on their tissue homing capacity, self-renewal capability and effector recall responsiveness. Each subset expresses distinct surface phenotype that facilitates their separation and characterization of biological properties.

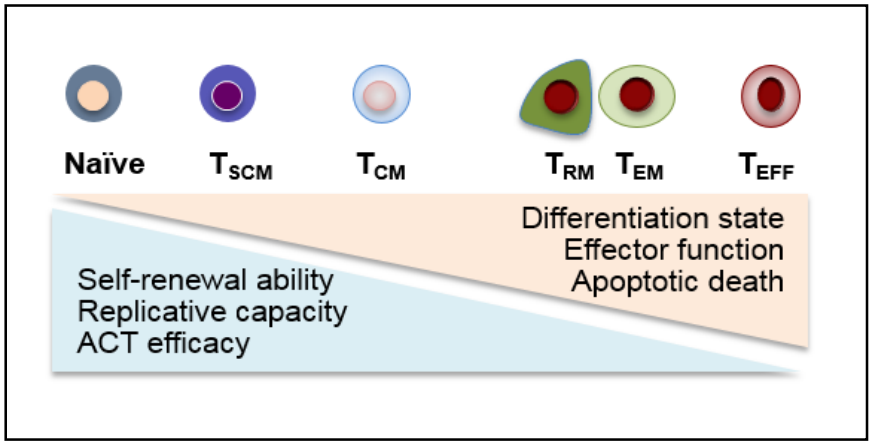


**Figure 3. Heterogeneity of Memory CD8 T Cell Subsets Frames a Comprehensive Immune Response.** Memory CD8 T cell subsets possess varying degrees of effector function and stemness as a consequence of their differentiation state. Stem-cell like memory CD8 T cells retain properties of naïve T cells, allowing them to differentiate into all other CD8 T effector and memory subsets. Life-long homeostatic proliferation of these memory CD8 T cells confers long-lasting protection against secondary antigen encounters.

**Transcription Factors and Distinct Lineages of CD4 T Cells**

Dozens of transcription factors critical for the generation of distinct lineages of effector and memory T cells have now been identified [18,49]. Seminal studies have demonstrated that these transcription factors are important for maintaining the plasticity and stability of effector CD4 T cells [18,28,29,50,51].

**Th1 cells**. Th1 CD4 T cells are important in mediating protection against pathogens and tumor cells. Importantly, Th1 cells also play a critical role in mediating various types of inflammation, such as type I diabetes, graft-rejection of transplanted organs, and graft-versus-host disease (GVHD), a complication of allogeneic hematopoietic stem cell transplantation [18,52-54]. Several transcription factors have been found to regulate CD4 Th1 cell differentiation, including T-bet, Eomes, Runx3, activator of transcription (Stat) 1 and Stat4 [18,28]. These factors cooperate to direct Th1 differentiation and to maintain the stability of differentiated Th1 cells.

T-bet is a master regulator of Th1 differentiation, with loss of T-bet leading to dramatically impaired production of Th1 cells during immune response. T-bet expression was found to be strongly dependent on signal transducer and Stat1, rather than on IL-12–dependent Stat4. Stat1 is activated by IFN-γ, and T-bet expression further induces IFN-γ production by differentiating cells, thereby amplifying T-bet expression and upregulating the expression of IL12Rβ2 [17,18]. CD4 T cells expressing high levels of IL12Rβ2 respond to IL12 produced by APCs, thus ensuring selective expansion of T cells differentiating towards Th1 effector function [17,18]. Stat4, which is induced by IL-12, is also positively regulated by IFN-γ [55]. Activated Stat4 supports Th1 differentiation by further inducing the expression of IFN-γ, IL12Rβ, and T-bet [56,57]. The transcription factor Runx3 is upregulated upon CD4 T cell stimulation and also functions to amplify T-bet and IFN-γ expression [58]. Furthermore, overexpression of Runx3 *in vitro* has been shown to promote and accelerate Th1 differentiation [59].

Recent studies have demonstrated that several other transcription factors, such as Zbtb7b (also called Th-POK) and the Notch effector RBP-j/CSL, may also contribute to the development of distinct lineages of effector $CD4^+$ T cells [20,21,28,60,61]. Eomesodermin (Eomes), another member of the T-box protein family, is dispensable for antigen-induced Th1 cell development and function, but may induce IFN-γ production in CD4 T cells under non-polarizing conditions when T-bet is not upregulated [62]. Thus, T-bet and Eomes cooperate with each other to promote IFN-γ production under different conditions.

Th1 cell differentiation occurs in parallel with the repressed production of inappropriate cytokines such as IL-4 and IL-17 [18]. It is through this mechanism that T-bet suppresses the development of both Th2 and Th17 cells. T-bet prevents Th2 cell differentiation by inhibiting transcription of IL-4, a signature Th2 cytokine, and by inhibiting the function of Gata3, a master regulator for Th2 cell differentiation [63]. T-bet can also interact with the promoter of RORC (which encodes RORγt, a master regulator of Th17) to inhibit Th17 cell differentiation [64,65].

**Th2 cells.** Th2 cells primarily mediate the adaptive immune response to parasitic protozoa and helminths [18,66,67]. Th2 cells are also able to drive B cells to produce several subclasses of IgG and IgE antibodies. Furthermore, cytokines produced by Th2 cells activate eosinophils and mast cells, causing inflammatory damage to tissues including the lung and airway [68-70]. Gata3 and Stat6 are transcription factors critical for the induction of Th2-associated cytokines (i.e., IL-4, IL-5 and IL-13) [63]. GATA3 conditional knockout studies showed that GATA3 expression is required for Th2 differentiation [71]. In differentiated Th2 cells, continuous GATA3 expression is essential for maintaining production of IL-5 and IL-13, but not IL-4. Furthermore, Gata3 has a dual function in the repression of Th1 differentiation by antagonizing T-bet expression in proliferating CD4 T cells [63,71]. Stat6 is the major signal transducer in IL-4-mediated Th2 cell differentiation and is critical for the production of IL-4 in CD4 T cells, as demonstrated by the failure of STAT6-deficient CD4 T cells to develop into IL-4-producing cells *in vitro*. Stat6 activation is also necessary and sufficient for inducing high expression levels of GATA3 [18,28,72-74].

**Th17 cells.** The Th17 subset is characterized by production of IL-17 and is important in mediating responses to pathogens. Th17 cells have also been implicated as potent effectors of autoimmune diseases such as Crohn's disease, ulcerative colitis, rheumatoid arthritis and psoriasis [18,50,65,75]. Th17 cell differentiation requires two key transcriptional regulators: RORγt and Stat3. Deficiency of RORγt leads to profound interruption of Th17 cytokine expression, whereas forced expression of RORγt induces the production of IL-17A and IL-17F, both of which mediate pro-inflammatory responses, but differ in the type and site of inflammation [76,77]. Stat3 plays an important role in Th17 cell differentiation by inducing RORγt and by directly binding to IL-17A and IL-17F promoters [50,65,75]. In addition to positive regulation of Th17 differentiation by RORγt and Stat, transactivation of RORγt by Runx1 is also critical for induction of the Th17 subset [63,78,79]. In contrast, the Runx1/FOXP3 interaction or Runx1/T-bet collaboration leads to the interruption of Runx1-mediated transactivation of RORC, thereby repressing Th17 differentiation [63,78,79].

**Treg.** There are two major classes of CD4 Treg cells, including natural Treg (nTreg) and inducible Treg (iTreg), both of which sustain immune system homeostasis by mediating self-tolerance and modulating inflammation. nTregs develop in the thymus during thymopoiesis, and are therefore termed thymic Tregs, whereas iTregs can be induced in peripheral tissues during immune responses [80,81]. Both subsets require the expression of the transcription factor Foxp3, which may be used to characterize these subsets [80]. Mutations of the FOXP3 gene can prevent Treg development, causing the fatal autoimmune disease IPEX [82]. iTreg3, a novel subset recently identified in mice and humans, is noteworthy because unlike previously identified subsets, it does not express Foxp3. Furthermore, this subset mediates immunosuppressive effects via IL-35 rather than the canonical cytokines IL-10 and TGF-β [83,84]. Several elegant papers have recently reviewed Treg biology and it will therefore not be discussed here [85,86].

## Transcriptional Regulation of Effector and Memory CD8 T Cells

**Effector differentiation and expansion.** Upon APC activation, antigen-specific CD8 T cells undergo a highly reproducible pattern of clonal expansion and differentiation. TCR and costimulatory signaling together with cytokines activate transcription programs important for regulating effector differentiation and expansion. T-bet and Eomes have been shown to function as master regulators for promoting CD8 effector T cell differentiation and function [26,87,88]. CD8 T cells lacking both T-bet and Eomes lose CTL identity and abnormally differentiate into IL-17-producing CD8 T cells that cause excessive neutrophil infiltration and a lethal inflammatory syndrome during LCMV infection. During acute response, T-bet and Eomes have cooperative and partially redundant effects on promoting CTL formation by inducing the expression of the cytotoxic molecules perforin and GZMB in activated CD8 T cells [87,88]. Importantly, effector CD8 T cells expressing high levels of T-bet are prone to terminal differentiation and become KLRG1$^{hi}$ short-lived effector cells (SLECs) [9]. During chronic infections, effector CD8 T cells expressing high levels of Eomes are susceptible to exhaustion and ultimately lose their ability to control chronic infection [89]. Interestingly, this demonstrates that the phenotype, function, and long-term fate of effector CD8 T cells are acutely sensitive to the relative ratio of T-bet and Eomes [89], yet the regulation of this ratio in activated T cells remains largely unknown.

Blimp-1 contributes to a transcriptional program that enhances CTL functions, such as migration to sites of inflammation and production of IFN-γ and GZMB [90-93]. Animals with a CD8 T cell-specific deficiency in Blimp-1 have an impaired ability to clear influenza virus due to poor recruitment of virus-specific CD8 T cells to the lungs [93-95]. However, high expression of Blimp-1 promotes terminal differentiation of CD8 SLECs and induces exhaustion of chronically activated CD8 T cells [91-94]. Thus, Blimp-1 has multiple roles in regulating effector T cell responses.

IFN regulatory factor 4 (Irf4) regulates CD8 T cell differentiation and expansion during acute infection [96,97]. While Irf4 is dispensable for early activation of CD8 T cells, it is important for effector differentiation and expansion [96,97]. Irf4 simultaneously promotes the expression and function of Blimp-1 and T-bet along with repressed genes that mediate cell cycle arrest and apoptosis. Selective deletion of IRF4 in peripheral CD8 T cells impairs antiviral CD8 T cell responses [96]. Irf4 also influences the expansion of SLECs at the peak time of infection, but has no effect on the rate of T cell contraction. This effect of Irf4 is associated with increased expression of Eomes and Tcf1 in CD8 T cells [96].

Several other transcription factors regulate the expansion of effector CD8 T cells. Inhibitor of DNA binding 2 (Id2), which is a member of the inhibitor of DNA-binding family, is required for the survival of effector CD8$^+$ T cells during early expansion phase [27,98]. More recent studies suggest that Id2 is especially important for the formation of terminal KLRG-1$^{hi}$T$_{EFF}$ [99]. As compared to Id2, Id3 promotes the survival of T$_{EFF}$ later during effector expansion, in particular when effector cells develop into memory cells [27]. Enforced expression of Id3 has been shown to be sufficient to restore SLEC survival and enhanced recall responses [100]. These data suggest that while both Id2 and Id3 are critical to the survival KLRG-1$^{hi}$SLECs, their effects occur at different stages of effector expansion. Although the precise mechanisms by which Id2 and Id3 regulate the survival and expansion of effector cells remain largely unknown, available data show that their pro-survival effects are likely associated with their regulation of anti-apoptotic genes (e.g., Bcl, Serpinb9 and Bcl2l11) and genomic stability, respectively [27,98-100].

Recent studies have demonstrated the importance of the transcription factor Bcl11b in antigen-dependent clonal expansion and cytolytic activity of CD8 T cells [101]. BCL11b deficiency was shown to have no impact on effector differentiation, but caused significantly decreased proliferation of antigen-activated T cells later during clonal expansion phase. BCL11b deficiency in CD8 T cells also leads to deregulation of CD8 co-receptor and Plcγ, both of which contribute to the impaired responsiveness of activated T cells [101]. It will be interesting to investigate how these transcription factors are coordinated to regulate the survival and expansion of effector CD8 T cells in the environment where effector cells reside and execute function.

**Memory formation and maintenance.** Memory CD8 T cells are derived from proliferating T cells during the clonal expansion phase and may be classified into four different

subsets (**Figure 2**): $T_{CM}$, $T_{EM}$, $T_{RM}$, and $T_{SCM}$ [3,8,38-44]. Identifying the differentiation pathways for heterogeneous memory T cell subset development following naïve T cell activation has been an area of active investigation [7]. In mice, these cells can be classified based on surface phenotype (e.g., CD62L, CD4, CD127 and KLGR-1) [3,5,7]. Genome-wide studies reveal that $T_{SCM}$ express gene programs that resemble, but are distinguishable from naïve T cells, thus being considered less differentiated than other subsets of memory cells [102]. As compared to $T_{CM}$, $T_{EM}$ express more genes associated with effector function, proapoptotic signaling, and certain chemokines [103-105]. This correlates with the difference in effector function between $T_{CM}$ and $T_{EM}$; the former lack immediate effector function and are less differentiated, while the latter have immediate effector function and are further differentiated. A progressive differentiation pathway based on signal strength and/or extent of activation has been proposed, with naïve T cells as the least differentiated cells, followed by $T_{SCM}$, $T_{CM}$ and $T_{EM}$ cells in a differentiation hierarchy (**Figure 3**) [42,43106]. Together, these memory T cell subsets function as precursors for $T_{EFF}$.

Some studies indicate that arresting effector differentiation of antigen-specific CD8 T cells enables them to differentiate into memory T cells. For example, antagonizing IL-2 with IL-21 has been shown to increase the generation of $T_{CM}$ [107,108] and induction of Wnt/β-catenin signaling using inhibitors of glycogen-synthase-kinase (GSK)-3β or Wnt3a protein induces the generation of $T_{SCM}$ [42]. GSK-3β inhibition mimics Wnt signaling by promoting accumulation of β-catenin, the molecule that forms complex with Tcf1 and Lef transcription factors for regulating gene expression [42]. Tcf1 mediates signaling downstream of the Wnt pathway and promotes the development of memory T cells [42]. Mice lacking Tcf7 gene, which encodes Tcf, have a more differentiated effector/effector memory cell phenotype (i.e., $CD44^{high}CD62L^{low}$) [109,110].

The forkhead-box O (Foxo) family of transcription factors is a well-defined target of Akt. Akt phosphorylation at conserved sites of Foxo proteins triggers their nuclear exclusion and inactivation. Foxo1 and Foxo3 are the predominant Foxo members expressed within immune cells [111]. Foxo1, in particular, controls $T_{CM}$ responses to infection [25] and is highly expressed in memory-precursor T cells. Foxo1 binds to and regulates expression of Tcf7 and Ccr7, which have critical functions in $T_{CM}$ formation and trafficking. Deletion of Foxo1 causes defective secondary, but not primary, CD8 T cell responses to *Listeria monocytogenes* in mice [25]. Thus far, Foxo3 has no established role in mediating recall response of CD8+ T cells, as demonstrated by an antigen-specific *in vivo* study [112].

Id3 plays an important role in regulating the transition of activated CD8 T cells into effector cells and memory cells [27,100,113]. Studies using mice expressing a reporter for Id3 have shown that $Id3^+$ memory precursors occur before the peak of T cell population expansion or upregulation of cell surface receptors associated with memory potential [27]. It is likely that Id3 is important for preserving proliferating CD8 T cells with memory potential early during priming and expansion phase. Loss of Id3 leads to defective formation of long-lived memory cells [27]. Ectopic expression of Id3 reportedly enhances recall response capability of tumor-reactive CD8 T cells and increases the production of memory precursor cells in mice [100]. High expression of Id3 preferentially guides the transition to memory cells, whereas low expression of Id3 leads to differentiation into effector cells [27].

Reducing the abundance of pro-differentiation transcription factors T-bet and Eomes may potentiate the generation of memory T cells. During acute response, CD8 T cells lacking both T-bet and Eomes lose CTL identity, and generate $KLRG1^{low}$ memory precursor cells, including both $T_{SCM}$ and $T_{CM}$. However, their effector recall response capability is impaired upon reencounter of the antigen [114]. In addition, in memory CD8 T cells, Eomes sustains homeostatic survival and proliferation of memory cells through regulating IL-2Rβ expression [26]. Loss of Eomes leads to decreased IL-2Rβ expression, which is required for IL-15-mediated signaling and homeostatic proliferation of memory cells in the absence of antigen. Mice lacking Eomes reportedly have impaired turnover of long-term memory cells, largely due to reduction of IL-2Rβ [26]. Furthermore, despite promoting the generation of memory T cells, reduction of Eomes and T-bet levels simultaneously leads to diminished effector capability. New approaches are needed to investigate if Eomes and T-bet might play an important role in regulating recall responsibility of memory T cells.

**Recall of effector functions.** It is noteworthy that the mechanisms for effector function recalled in memory cells differ from that of the primary effector response. For example, Id2 is required for the survival and expansion of effector cells generated during primary response, but is dispensable for reactivation of effector function by memory CD8 T cells [99]. Blimp-1-deficient effector CD8 T cells are reportedly generated and showed some reduction in expression of effector molecules [91-93]. Both $T_{EFF}$ and $T_{EM}$ have decreased proliferative capacity when rechallenged by their specific antigen. In contrast, loss of Blimp-1 leads to a faster development of $T_{CM}$ and has no impact on recall response of memory T cells to become effector cells [92]. It is likely that other transcription factors are required for regulating the recall response capability of memory T cells. Alternatively, reactivation of effector function by memory cells may involve a multitude of mechanisms rather than a single transcription factor.

**Interplay between Cytokines Signals and Transcription Factors in Memory Cells**

Emerging evidence indicates that T cell heterogeneity is dictated during the antigenic priming phase and can be further modified in response to environmental stimuli. TCR ligation and inflammatory cytokines such as IL-12 and IFN-γ upregulate T-bet in activated CD4 and CD8 T cells [26,88,115]. Some studies report that APC-derived Notch ligand activation of Notch signaling in T cells upregulates their expression of T-bet and Eomes and results in differentiation of effector T cells [19,21,23]. Notch signaling is also known to be important for induction of Gata3 and RORγt in Th2 and Th17 cells, respectively [21,23,24,116,117]. Thus, both the degree and type of inflammatory stimulation serve to establish higher levels of lineage-specifying transcription factors (e.g., T-bet, Eomes, GATA3, RORγt) and induce distinct lineages of effector cells [9].

Recent studies suggest that inflammatory cytokines regulate expression of Id2 and Id3 in activated CD8 T cells. Using Id2-YFP and Id3-GFP reporter mice, Goldrath and colleagues assessed the effect of cytokines on CD8 T cell expression of Id2 and Id3 during antigen-driven immune response [27]. While *in vitro* treatment with IL-2, IL-12 or IL-21 resulted in increase of Id2, *in vivo* experiments further confirmed the effect of IL-2 signaling on Id2 upregulation [27]. However, inactivation of IL-12 did not affect the expression of Id2. Thus, it is likely that IL-2 is a critical factor upregulating Id2 *in vivo*, whereas IL-12's effect may be redundant *in vivo* when IL-2 is available [27]. In contrast, IL-12 lowers Id3 expression in antigen-activated CD8 T cells in an *in vivo* experimental model, suggesting that IL-12 induction of effector differentiation leads to the down-regulation of Id3 [27]. The observation that IL-12 upregulates T-bet in activated T cells and the increasing effector pool [9] suggests that it may be useful to determine how cytokines and transcription factors act in concert to modulate the expression of Id2 and Id3 in T cells for effector differentiation and memory formation.

**T Cell Heterogeneity And Protective T Cell Immunity**

To achieve efficient protective T cell immunity against infection and tumor cells, antigen-specific T cells are partitioned into subsets of memory T cells with distinct homing, self-renewal and effector recall potential. Adoptive cellular immunotherapy (ACT) is emerging as a potentially curative therapy for patients with advanced cancer. A major caveat of ACT is the observation that antigen-experienced T cells at distinct differentiation states may have different antitumor activity *in vivo* [42,102,118-120]. For example, as compared to $T_{EM}$, $T_{CM}$ are less differentiated [2-4,6,8,121,122], have greater ability to proliferate and produce functional effector T cells [2-4,6,8,121,122], and show increased antitumor activity relative in many experimental studies [42,118-120,123,124]. Our recent studies [41] and others [42] have identified a population of antigen-experienced $T_{SCM}$ in mice [42]. As compared to $T_{CM}$ and $T_{EM}$, $T_{SCM}$ have a greater ability to inhibit tumor progression. $T_{SCM}$ have also been discovered in humans and have superior antitumor immunity in humanized mouse models [43]. Recent studies by a separate group further confirmed the potency of human $T_{SCM}$ against minor histocompatibility antigens (miHAs) in mediating potent antitumor activity in humanized mice [125]. Therefore, both $T_{SCM}$ and $T_{CM}$ serve as source for the total pool of memory cells and effector cells. They both have the high degree of cell plasticity and lowest degree of effector function, with $T_{SCM}$ exhibiting these characteristics more potently [43,106,120]. The development of novel approaches which activate memory cells and generate secondary effector cells may have significant implications in augmenting the efficacy of ACT.

The importance of T cell heterogeneity is reportedly important for T cell immunity against chronic infection [89]. Using both human and mouse chronic infection models, Wherry and colleagues have demonstrated that differential expression of T-bet and Eomes in distinct subsets of virus-specific CD8 T cells cooperatively maintain the pool of antiviral CD8 T cells during chronic viral infection [89]. During chronic infection phase, antiviral CD8 T cells expressing high levels of T-bet are slowly proliferating cells, but undergo rapid proliferation in response to the specific antigen and produce terminal progeny cells expressing high levels of Eomes. The absence of T-bet causes a shift toward Eomes-expressing terminal progeny cells and impedes the control chronic viral infection. Deletion of Eomes results in failure to control chronic infection due to the reduction of terminal effector cells [89]. Thus, both the T-bet-dependent and Eomes-dependent subsets of antiviral CD8 T cells cooperatively contribute to an effective protective immunity against chronic infection.

CD4 T cells also provide effective protection against tumor and chronic infection. Recent studies suggest that CD4 T cells not only promote CD8 T cell function, but also play a direct role in tumor elimination [126-130]. The manner in which CD4 T cells mediate anti-tumor immune response depend on the generation of both IFN-γ-producing progeny and cytolytic effector cells that can destroy tumor cells [127,128]. Notably, recent evidence suggests that CD4 Th17 cells help CD8 T cells to mediate long-term anti-tumor immunity [131,132]. Thus, efficient protection immunity against tumor and pathogen reflects collective efforts of differential subsets of antigen-specific T cells.

**Modifying T Cell Heterogeneity for Tumor Immunotherapy**

One of the main barriers to improving the efficacy of ACT is ensuring the preservation of T cell self-renewal, which ensures the continuous production of progeny capable of eradicating tumor after adoptive transfer into patients [42,43,106,120]. Considerable efforts have been made to improve methods used for *ex vivo* expansion of tumor-

reactive T cells for ACT. An approach under active evaluation involves the growth of cells under conditions that enable *ex vivo* proliferation while limiting differentiation (**Figure 4**). The addition of GSK3-β inhibitors into cultures has been shown to reduce effector differentiation and increase the frequency of both $T_{SCM}$ and $T_{CM}$ [42,43]. This subset of $T_{SCM}$ has greater ability than other subsets of memory T cells to control the growth of established tumors upon adoptive transfer [42,43].

TCR, IL-2 receptor and IL-12 receptor signaling have all been demonstrated to stimulate the PI3K/Akt signal transduction pathway [133-135]. Several studies suggest that PI3K/Akt is critical for proliferation and differentiation of activated CD8 T cells. Increased activation of Akt by IL-12, expression of a constitutively active form of Akt and deletion of Foxo1, have all been shown to promote the formation of KLRG1$^{hi}$effector cells [5,9,136]. A recent study shows that inhibiting the Akt pathway leads to generation of highly potent miHA-specific CD8 T cells *ex vivo* [125]. These Akt-inhibited CD8 T cells showed superior expansion potential upon removal of the Akt inhibitor, which results in a superior antitumor effect in a humanized mouse model [125]. Akt inhibition can also enhance persistence of tumor-infiltrating lymphocytes after adoptive transfer into an immunodeficient animal model and augment antitumor immunity of CD8 T cells [137].

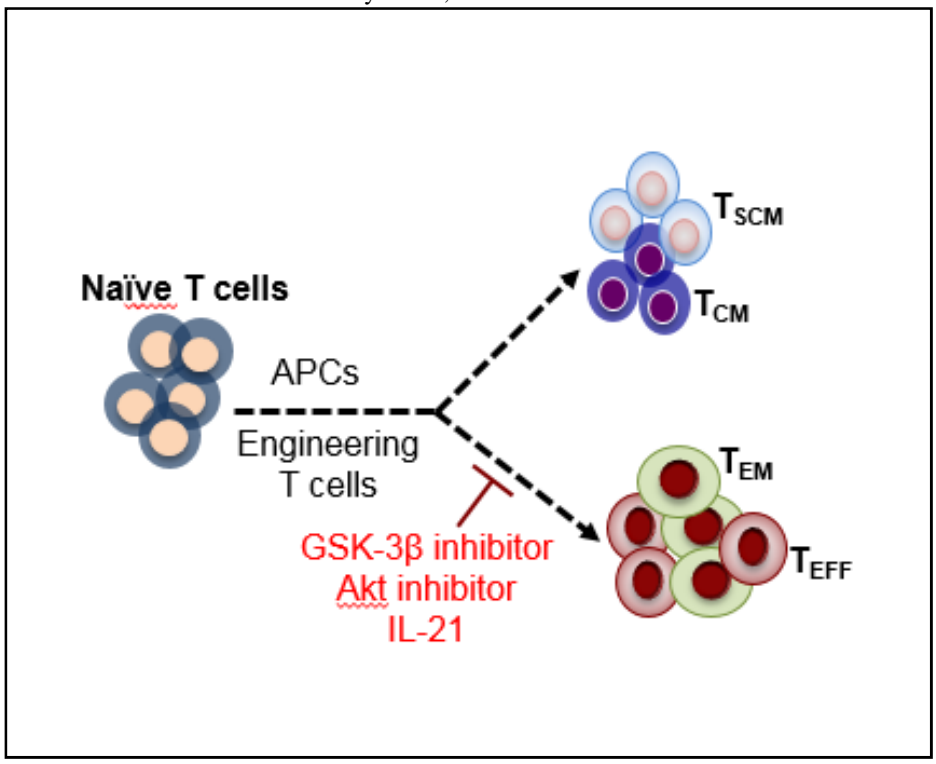


**Figure 4. Arresting CD8 T Cell Differentiation May Improve ACT Efficacy.** ACT is limited by the capacity of transplanted cells to provide continual supply of functional effector cells while retaining self-renewal. An area of active investigation involves the development of an *ex vivo* culture system that promotes cell proliferation while limiting differentiation. GSK3-β inhibitors employed in *ex vivo* cultures have been shown to reduce effector differentiation and increase the frequency of both $T_{SCM}$ and $T_{CM}$ [43], Akt inhibition has been demonstrated to enhance proliferation[125] and persistence[137] of anti-tumor immune cells. Some studies suggest that addition of IL-21 has the potential to arrest differentiation without affecting proliferation [108,140].

Cytokines such as IL-15 and IL-21 can sustain T-cell proliferation while limiting excessive differentiation, exhaustion, and senescence. T cells cultured in IL-15 display a $T_{CM}$-like phenotype and gene expression profile, and have greater anti-tumor function in mice than T cells cultured in IL-2 [138,139]. IL-21 modulates the differentiation of activated T cells and results in development of a population of cells characterized by a $T_{SCM}$ phenotype [108,140]. Human T cells cultured in IL-21 retain the ability to release IL-2 and express markers associated with a minimal differentiated phenotype (e.g., CD45RA, CD28, CD27, IL7Ra and CD62L) [108,140,141]. In a mouse model of melanoma, T cells derived from IL-21 cultures demonstrated markedly enhanced anti-tumor activity compared with cells grown in the presence of other cytokines [108].

The CD27-dependent pathway of T-cell expansion has therapeutic potential to enhance the efficacy of ACT. CD27

is highly expressed on the surface of naïve CD8 T cells [142-145]. Activating CD27 by soluble CD70 promotes cellular expansion of CD8 T cells in the absence of IL-2 without causing significant effector differentiation [142,143,145]. This effect of CD27 signaling resulted in increased cell cycling and survival that was mediated in part by upregulation of IL-7Ra on the T cell surface [142,143,145]. Data from animal experiments also indicate that CD27-null CD8 T cells have impaired primary and secondary expansion in mice challenged by influenza and polymavirus. Finally, CD27 is reported to mediate the generation of antigen-experienced CD8 T cells with memory traits [142,145,146]. Further preclinical studies using ACT models are necessary to evaluate the validity of CD27-dependent expansion of T cells as a feasible approach to improve the efficacy of ACT for patients with advanced cancer.

## CONCLUDING REMARKS

This review has highlighted the significant progress that has been made in understanding how transcription factors regulate the development of T cell heterogeneity. A multitude of transcription factors coordinate their activities to orchestrate distinct transcriptional programs that direct the differentiation and maintenance of a functionally diverse group of T cell subsets. The upstream molecular pathway(s) involved in orchestrating the expression of these subset-specific transcriptional programs remain a critical unresolved question. The continued exploration of transcriptional control of T cell heterogeneity will have broad implications in identifying novel pathways that may be targeted to create therapies for autoimmune diseases, chronic infections and complications involved with transplantation, including graft rejection and GVHD.

In addition, a greater understanding of transcriptional programs controlling terminal differentiation and memory formation will have an immediate impact on T cell-based anti-tumor therapies such as ACT. During ACT, a strong antitumor effect in patients with advanced cancer can be achieved by transfer of large amount of cytolytic effector T cells. Current *in vitro* methods used to expand tumor-reactive T cells are ineffective in maintaining a population of minimally differentiated T cells while generating sufficient cell numbers. The predominant obstacle to retaining this population is the fundamental coupling of clonal expansion and effector differentiation. This coupled expansion and differentiation impairs the generation of memory T cells that are able to persist and replicate to elaborate effector function for eliminating tumor *in vivo* following adoptive transfer. Further exploration of the molecular mechanisms whereby T cells closely link expansion and differentiation will lead to new strategies to improve the efficacy of cancer immunotherapy.

## REFERENCES

1. Fearon DT,Manders P, Wagner SD (2001) Arrested differentiation, the self-renewing memory lymphocyte, and vaccination. Science 293: 248-250.
2. Lanzavecchia A,Sallusto F (2000) Dynamics of T lymphocyte responses: intermediates, effectors, and memory cells. Science 290: 92-97.
3. Lanzavecchia A,Sallusto F (2002) Progressive differentiation and selection of the fittest in the immune response. Nat Rev Immunol 2: 982-987.
4. Kaech SM,Hemby S, Kersh E, Ahmed R (2002) Molecular and functional profiling of memory CD8 T cell differentiation. Cell 111: 837-851.
5. Kaech SM, Cui W (2012) Transcriptional control of effector and memory CD8+ T cell differentiation. Nat Rev Immunol 12: 749-761.
6. Zhang N, Bevan MJ (2011) CD8(+) T cells: foot soldiers of the immune system. Immunity 35: 161-168.
7. Youngblood B, Hale JS, Ahmed R2 (2015) Memory CD8 T cell transcriptional plasticity. F1000Prime Rep 7: 38.
8. Wherry EJ,Teichgräber V, Becker TC, Masopust D, Kaech SM, et al. (2003) Lineage relationship and protective immunity of memory CD8 T cell subsets. Nat Immunol 4: 225-234.
9. Joshi NS, Cui W, Chandele A, Lee HK, Urso DR, et al. (2007) Inflammation directs memory precursor and short-lived effector CD8(+) T cell fates via the graded expression of T-bet transcription factor. Immunity 27: 281-295.
10. Kaech SM, Wherry EJ (2007) Heterogeneity and cell-fate decisions in effector and memory CD8+ T cell differentiation during viral infection. Immunity 27: 393-405.
11. Kaech SM, Wherry EJ, Ahmed R (2002) Effector and memory T-cell differentiation: implications for vaccine development. Nat Rev Immuno l2: 251-262.
12. Wherry EJ, Ahmed R (2004) Memory CD8 T-cell differentiation during viral infection. J Virol 78: 5535-5545.
13. Ahmed R, Bevan MJ, Reiner SL, Fearon DT (2009) The precursors of memory: models and controversies. Nat Rev Immunol 9: 662-668.
14. Fearon DT1 (2007) The expansion and maintenance of antigen-selected CD8(+) T cell clones. AdvImmunol 96: 103-139.
15. Heffner M,Fearon DT (2007) Loss of T cell receptor-induced Bmi-1 in the KLRG1(+) senescent CD8(+) T lymphocyte. Proc Natl AcadSci U S A 104: 13414-13419.
16. Savage PA, Boniface JJ, Davis MM (1999) A kinetic basis for T cell receptor repertoire selection

during an immune response. Immunity 10: 485-492.
17. Helmstetter C,Flossdorf M,Peine M,Kupz A, Zhu J, et al. (2015) Individual T helper cells have a quantitative cytokine memory. Immunity 42: 108-122.
18. Zhu J, Yamane H, Paul WE (2010) Differentiation of effector CD4 T cell populations (*). Annu Rev Immunol 28: 445-489.
19. Backer RA,Helbig C,Gentek R, Kent A, Laidlaw BJ, et al. (2014) A central role for Notch in effector CD8(+) T cell differentiation. Nat Immunol 15: 1143-1151.
20. Amsen D,Antov A, Flavell RA (2009) The different faces of Notch in T-helper-cell differentiation. Nat Rev Immunol 9: 116-124.
21. Zhang Y, Sandy AR, Wang J, Radojcic V, Shan GT, et al. (2011) Notch signaling is a critical regulator of allogeneic CD4+ T-cell responses mediating graft-versus-host disease. Blood 117: 299-308.
22. Mochizuki K (2013) Delta-like Ligand 4 Identifies a Previously Uncharacterized Population of Inflammatory Dendritic Cells That Plays Important Roles in Eliciting Allogeneic T Cell Responses in Mice. J Immunol 190: 3772-3782.
23. Tran IT, Sandy AR, Carulli AJ, Ebens C, Chung J, et al. (2013) Blockade of individual Notch ligands and receptors controls graft-versus-host disease. J Clin Invest 123: 1590-1604.
24. Bailis W, Yashiro-Ohtani Y, Fang TC, Hatton RD, Weaver CT, et al. (2013) Notch simultaneously orchestrates multiple helper T cell programs independently of cytokine signals. Immunity 39: 148-159.
25. Hess Michelini R,Doedens AL, Goldrath AW, Hedrick SM (2013) Differentiation of CD8 memory T cells depends on Foxo1. J Exp Med 210: 1189-1200.
26. Intlekofer AM, Takemoto N, Wherry EJ, Longworth SA, Northrup JT, et al. (2005) Effector and memory CD8+ T cell fate coupled by T-bet and eomesodermin. Nat Immunol 6: 1236-1244.
27. Yang CY, Best JA, Knell J, Yang E, Sheridan AD, et al. (2011) The transcriptional regulators Id2 and Id3 control the formation of distinct memory CD8+ T cell subsets. Nat Immunol 12: 1221-1229.
28. Wilson CB, Rowell E, Sekimata M (2009) Epigenetic control of T-helper-cell differentiation. Nat Rev Immunol 9: 91-105.
29. Zhou L, Chong MM, Littman DR (2009) Plasticity of CD4+ T cell lineage differentiation. Immunity 30: 646-655.
30. Asseman C,Mauze S, Leach MW, Coffman RL, Powrie F (1999) An essential role for interleukin 10 in the function of regulatory T cells that inhibit intestinal inflammation. J Exp Med 190: 995-1004.
31. Fazilleau N, Mark L, McHeyzer-Williams LJ, McHeyzer-Williams MG (2009) Follicular helper T cells: lineage and location. Immunity 30: 324-335.
32. Temann UA, Ray P, Flavell RA (2002) Pulmonary overexpression of IL-9 induces Th2 cytokine expression, leading to immune pathology. J Clin Invest 109: 29-39.
33. Tan C, Aziz MK, Lovaas JD, Vistica BP, Shi G, et al. (2010) Antigen-specific Th9 cells exhibit uniqueness in their kinetics of cytokine production and short retention at the inflammatory site. J Immunol 185: 6795-6801.
34. Dardalhon V,Awasthi A, Kwon H, Galileos G, Gao W, et al. (2008) IL-4 inhibits TGF-beta-induced Foxp3+ T cells and, together with TGF-beta, generates IL-9+ IL-10+ Foxp3(-) effector T cells. Nat Immunol 9: 1347-1355.
35. Veldhoen M,Uyttenhove C, van Snick J, Helmby H, Westendorf A, et al. (2008) Transforming growth factor-beta 'reprograms' the differentiation of T helper 2 cells and promotes an interleukin 9-producing subset. Nat Immunol 9: 1341-1346.
36. Harrington LE,Janowski KM, Oliver JR, Zajac AJ, Weaver CT (2008) Memory CD4 T cells emerge from effector T-cell progenitors. Nature 452: 356-360.
37. Ndejembi MP,Teijaro JR, Patke DS, Bingaman AW, Chandok MR, et al. (2006) Control of memory CD4 T cell recall by the CD28/B7 costimulatory pathway. J Immunol 177: 7698-7706.
38. Sallusto F, Lenig D, Forster R, Lipp M, Lanzavecchia A (1999) Two subsets of memory T lymphocytes with distinct homing potentials and effector functions. Nature40: 708-712.
39. Wakim LM,Waithman J, van Rooijen N, Heath WR, Carbone FR (2008) Dendritic cell-induced memory T cell activation in nonlymphoid tissues. Science 319: 198-202.
40. Zhang Y, Joe G, Hexner E, Zhu J, Emerson SG (2005) Alloreactive memory T cells are responsible for the persistence of graft-versus-host disease. J Immunol 174: 3051-3058.
41. Zhang Y, Joe G, Hexner E, Zhu J, Emerson SG (2005) Host-reactive CD8+ memory stem cells in graft-versus-host disease. Nat Med 11: 1299-1305.
42. Gattinoni L,Zhong XS, Palmer DC, Ji Y, Hinrichs CS, et al. (2009) Wnt signaling arrests effector T cell differentiation and generates CD8+ memory stem cells. Nat Med 15: 808-813.
43. Gattinoni L,Lugli E, Ji Y, Pos Z, Paulos CM, et al. (2011) A human memory T cell subset with stem cell-like properties. Nat Med 17: 1290-1297.

44. Masopust D,Kaech SM, Wherry EJ, Ahmed R (2004) The role of programming in memory T-cell development. CurrOpinImmunol 16: 217-225.
45. Masopust D,Vezys V, Wherry EJ, Barber DL, Ahmed R (2006) Cutting edge: gut microenvironment promotes differentiation of a unique memory CD8 T cell population. J Immunol 176: 2079-2083.
46. Wakim LM, Woodward-Davis A, Liu R, Hu Y, Villadangos J, et al. (2012) The molecular signature of tissue resident memory CD8 T cells isolated from the brain. J Immunol 189: 3462-3471.
47. Wakim LM, Smith J, Caminschi I, Lahoud MH, Villadangos JA (2015) Antibody-targeted vaccination to lung dendritic cells generates tissue-resident memory CD8 T cells that are highly protective against influenza virus infection. Mucosal Immunol 8: 1060-1071.
48. Wakim LM, Gupta N, Mintern JD, Villadangos JA (2013) Enhanced survival of lung tissue-resident memory CD8â ° T cells during infection with influenza virus due to selective expression of IFITM3. Nat Immunol 14: 238-245.
49. O'Shea JJ, Paul WE (2010) Mechanisms underlying lineage commitment and plasticity of helper CD4+ T cells. Science 327: 1098-1102.
50. Stockinger B,Veldhoen M (2007) Differentiation and function of Th17 T cells. CurrOpinImmunol 19: 281-286.
51. Weaver CT, Hatton RD, Mangan PR, Harrington LE (2007) IL-17 family cytokines and the expanding diversity of effector T cell lineages. Annu Rev Immunol 25: 821-852.
52. Blazar BR, Murphy WJ, Abedi M (2012) Advances in graft-versus-host disease biology and therapy. Nat Rev Immunol 12: 443-458.
53. Zwang NA,Turka LA1 (2014) Transplantation immunology in 2013: New approaches to diagnosis of rejection. Nat Rev Nephrol 10: 72-74.
54. Hancock WW, Turka LA (2011) Immunogenetics and transplantation. CurrOpinImmunol 23: 639-640.
55. Kaplan MH, Sun YL, Hoey T, Grusby MJ (1996) Impaired IL-12 responses and enhanced development of Th2 cells in Stat4-deficient mice. Nature 382: 174-177.
56. Usui T, Preiss JC, Kanno Y, Yao ZJ, Bream JH, et al. (2006) T-bet regulates Th1 responses through essential effects on GATA-3 function rather than on IFNG gene acetylation and transcription. J Exp Med 203: 755-766.
57. Usui T,Nishikomori R, Kitani A, Strober W (2003) GATA-3 suppresses Th1 development by downregulation of Stat4 and not through effects on IL-12Rbeta2 chain or T-bet. Immunity 18: 415-428.
58. Djuretic IM,Levanon D, Negreanu V, Groner Y, Rao A, et al. (2007) Transcription factors T-bet and Runx3 cooperate to activate Ifng and silence Il4 in T helper type 1 cells. Nat Immunol 8: 145-153.
59. Kohu K,Ohmori H, Wong WF, Onda D, Wakoh T, et al. (2009) The Runx3 transcription factor augments Th1 and down-modulates Th2 phenotypes by interacting with and attenuating GATA3. J Immunol 183: 7817-7824.
60. Carpenter AC, Grainger JR, Xiong Y, Kanno Y, Chu HH, et al. (2012) The transcription factors Thpok and LRF are necessary and partly redundant for T helper cell differentiation. Immunity 37: 622-633.
61. Steinke FC, Yu S, Zhou X, He B, Yang W, et al. (2014) TCF-1 and LEF-1 act upstream of Th-POK to promote the CD4(+) T cell fate and interact with Runx3 to silence Cd4 in CD8(+) T cells. Nat Immunol 15: 646-656.
62. Tumes DJ, Onodera A, Suzuki A, Shinoda K, Endo Y, et al. (2013) The polycomb protein Ezh2 regulates differentiation and plasticity of CD4(+) T helper type 1 and type 2 cells. Immunity 39: 819-832.
63. Yagi R, Zhu J, Paul WE (2011) An updated view on transcription factor GATA3-mediated regulation of Th1 and Th2 cell differentiation. IntImmunol 23: 415-420.
64. Cohen CJ,Crome SQ, MacDonald KG, Dai EL, Mager DL, et al. (2011) Human Th1 and Th17 cells exhibit epigenetic stability at signature cytokine and transcription factor loci. J Immunol 187: 5615-5626.
65. Zhou L, Littman DR (2009) Transcriptional regulatory networks in Th17 cell differentiation. CurrOpinImmunol 21: 146-152.
66. Lee DU, Agarwal S, Rao A (2002) Th2 lineage commitment and efficient IL-4 production involves extended demethylation of the IL-4 gene. Immunity 16: 649-660.
67. Fang TC, Yashiro-Ohtani Y, Del Bianco C, Knoblock DM, Blacklow SC, et al. (2007) Notch directly regulates Gata3 expression during T helper 2 cell differentiation. Immunity 27: 100-110.
68. Suzuki A,Iwamura C, Shinoda K, Tumes DJ, Kimura MY, et al. (2010) Polycomb group gene product Ring1B regulates Th2-driven airway inflammation through the inhibition of Bim-mediated apoptosis of effector Th2 cells in the lung. J Immunol 184: 4510-4520.
69. Yamashita M,Hirahara K, Shinnakasu R, Hosokawa H, Norikane S, et al. (2006) Crucial role of MLL for the maintenance of memory T helper type 2 cell responses. Immunity 24: 611-622.
70. Finotto S,Neurath MF, Glickman JN, Qin S, Lehr HA, et al. (2002) Development of spontaneous

airway changes consistent with human asthma in mice lacking T-bet. Science 295: 336-338.

71. Zhu J, Min B, Hu-Li J, Watson CJ, Grinberg A, et al. (2004) Conditional deletion of Gata3 shows its essential function in T(H)1-T(H)2 responses. Nat Immunol 5: 1157-1165.
72. Onodera A, Yamashita M, Endo Y, Kuwahara M, Tofukuji S, et al. (2010) STAT6-mediated displacement of polycomb by trithorax complex establishes long-term maintenance of GATA3 expression in T helper type 2 cells. J Exp Med 207: 2493-2506.
73. Wei J,Duramad O, Perng OA, Reiner SL, Liu YJ, et al. (2007) Antagonistic nature of T helper 1/2 developmental programs in opposing peripheral induction of Foxp3+ regulatory T cells. Proc Natl AcadSci U S A 104: 18169-18174.
74. Yamashita M,Shinnakasu R, Nigo Y, Kimura M, Hasegawa A, et al. (2004) Interleukin (IL)-4-independent maintenance of histone modification of the IL-4 gene loci in memory Th2 cells. J BiolChem 279: 39454-39464.
75. Segal BM1 (2010) Th17 cells in autoimmune demyelinating disease. SeminImmunopathol 32: 71-77.
76. Yang XO, Chang SH, Park H, Nurieva R, Shah B, et al. (2008) Regulation of inflammatory responses by IL-17F. J Exp Med 205: 1063-1075.
77. Ishigame H,Kakuta S, Nagai T, Kadoki M, Nambu A, et al. (2009) Differential roles of interleukin-17A and -17F in host defense against mucoepithelial bacterial infection and allergic responses. Immunity 30: 108-119.
78. Cruz-Guilloty F,Pipkin ME, Djuretic IM, Levanon D, Lotem J, et al. (2009) Runx3 and T-box proteins cooperate to establish the transcriptional program of effector CTLs. J Exp Med 206: 51-59.
79. Lazarevic V, Chen X, Shim JH, Hwang ES, Jang E, et al. (2011) T-bet represses T(H)17 differentiation by preventing Runx1-mediated activation of the gene encoding RORÎ³t. Nat Immunol 12: 96-104.
80. Fontenot JD, Gavin MA, Rudensky AY (2003) Foxp3 programs the development and function of CD4+CD25+ regulatory T cells. Nat Immunol 4: 330-336.
81. Kitagawa Y,Ohkura N, Sakaguchi S (2015) Epigenetic control of thymicTreg-cell development. Eur J Immunol 45: 11-16.
82. Goettel JA, Biswas S,Lexmond WS,Yeste A,Passerini L, et al. (2015) Fatal autoimmunity in mice reconstituted with human hematopoietic stem cells encoding defective FOXP3. Blood 125: 3886-3895.
83. Chaturvedi V, Collison LW, Guy CS, Workman CJ, Vignali DA (2011) Cutting edge: Human regulatory T cells require IL-35 to mediate suppression and infectious tolerance. J Immunol 186: 6661-6666.
84. Collison LW,Chaturvedi V, Henderson AL, Giacomin PR, Guy C, et al. (2010) IL-35-mediated induction of a potent regulatory T cell population. Nat Immunol 11: 1093-1101.
85. Miyara M, Ito Y,Sakaguchi S3 (2014) TREG-cell therapies for autoimmune rheumatic diseases. Nat Rev Rheumatol 10: 543-551.
86. Morikawa H,Sakaguchi S (2014) Genetic and epigenetic basis of Treg cell development and function: from a FoxP3-centered view to an epigenome-defined view of natural Treg cells. Immunol Rev 259: 192-205.
87. Pearce EL, Mullen AC, Martins GA, Krawczyk CM, Hutchins AS, et al. (2003) Control of effector CD8+ T cell function by the transcription factor Eomesodermin. Science 302: 1041-1043.
88. Intlekofer AM, Takemoto N, Kao C, Banerjee A, Schambach F, et al. (2007) Requirement for T-bet in the aberrant differentiation of unhelped memory CD8+ T cells. J Exp Med 204: 2015-2021.
89. Paley MA,Kroy DC, Odorizzi PM, Johnnidis JB, Dolfi DV, et al. (2012) Progenitor and terminal subsets of CD8+ T cells cooperate to contain chronic viral infection. Science 338: 1220-1225.
90. Martins GA,Cimmino L, Shapiro-Shelef M, Szabolcs M, Herron A, et al. (2006) Transcriptional repressor Blimp-1 regulates T cell homeostasis and function. Nat Immunol 7: 457-465.
91. Shin H, Blackburn SD, Intlekofer AM, Kao C, Angelosanto JM, et al. (2009) A role for the transcriptional repressor Blimp-1 in CD8(+) T cell exhaustion during chronic viral infection. Immunity 31: 309-320.
92. Rutishauser RL, Martins GA, Kalachikov S, Chandele A, Parish IA, et al. (2009) Transcriptional repressor Blimp-1 promotes CD8(+) T cell terminal differentiation and represses the acquisition of central memory T cell properties. Immunity 31: 296-308.
93. Kallies A, Xin A, Belz GT, Nutt SL (2009) Blimp-1 transcription factor is required for the differentiation of effector CD8(+) T cells and memory responses. Immunity 31: 283-295.
94. Martins G, Calame K (2008) Regulation and functions of Blimp-1 in T and B lymphocytes. Annu Rev Immunol 26: 133-169.
95. Kallies A1 (2008) Distinct regulation of effector and memory T-cell differentiation. Immunol Cell Biol 86: 325-332.
96. Yao S,Buzo BF, Pham D, Jiang L, Taparowsky EJ, et al. (2013) Interferon regulatory factor 4 sustains CD8(+) T cell expansion and effector differentiation. Immunity 39: 833-845.

97. Nowyhed HN, Huynh TR, Thomas GD, Blatchley A, Hedrick CC(2015) Cutting Edge: The Orphan Nuclear Receptor Nr4a1 Regulates CD8+ T Cell Expansion and Effector Function through Direct Repression of Irf4. J Immunol 195: 3515-3519.
98. Cannarile MA, Lind NA, Rivera R, Sheridan AD, Camfield KA, et al. (2006) Transcriptional regulator Id2 mediates CD8+ T cell immunity. Nat Immunol 7: 1317-1325.
99. Masson F,Minnich M, Olshansky M, Bilic I, Mount AM, et al. (2013) Id2-mediated inhibition of E2A represses memory CD8+ T cell differentiation. J Immunol 190: 4585-4594.
100. Ji Y,Pos Z, Rao M, Klebanoff CA, Yu Z, et al. (2011) Repression of the DNA-binding inhibitor Id3 by Blimp-1 limits the formation of memory CD8+ T cells. Nat Immunol 12: 1230-1237.
101. Zhang S,Rozell M, Verma RK, Albu DI, Califano D, et al. (2010) Antigen-specific clonal expansion and cytolytic effector function of CD8+ T lymphocytes depend on the transcription factor Bcl11b. J Exp Med 207: 1687-1699.
102. Hinrichs CS (2009) Adoptively transferred effector cells derived from naive rather than central memory CD8+ T cells mediate superior antitumor immunity. Proc Natl AcadSci USA 106: 17469-17474.
103. Araki Y, Wang Z, Zang C, Wood WH 3rd, Schones D, et al. (2009) Genome-wide analysis of histone methylation reveals chromatin state-based regulation of gene transcription and function of memory CD8+ T cells. Immunity 30: 912-925.
104. Kato K, Cui S, Kuick R, Mineishi S, Hexner E, et al. (2010) Identification of stem cell transcriptional programs normally expressed in embryonic and neural stem cells in alloreactive CD8+ T cells mediating graft-versus-host disease. Biol Blood Marrow Transplant 16: 751-771.
105. Sarkar S,Kalia V, Haining WN, Konieczny BT, Subramaniam S, et al. (2008) Functional and genomic profiling of effector CD8 T cell subsets with distinct memory fates. J Exp Med 205: 625-640.
106. Rosenberg SA,Restifo NP1 (2015) Adoptive cell transfer as personalized immunotherapy for human cancer. Science 348: 62-68.
107. Leonard WJ, Zeng R, Spolski R (2008) Interleukin 21: a cytokine/cytokine receptor system that has come of age. J LeukocBiol 84: 348-356.
108. Hinrichs CS,Spolski R, Paulos CM, Gattinoni L, Kerstann KW, et al. (2008) IL-2 and IL-21 confer opposing differentiation programs to CD8+ T cells for adoptive immunotherapy. Blood 111: 5326-5333.
109. Zhou X, Yu S, Zhao DM, Harty JT, Badovinac VP, et al. (2010) Differentiation and persistence of memory CD8(+) T cells depend on T cell factor 1. Immunity 33: 229-240.
110. Tiemessen MM,Baert MR,Kok L, van Eggermond MC, van den Elsen PJ, et al. (2014) T Cell factor 1 represses CD8+ effector T cell formation and function. J Immunol 193: 5480-5487.
111. Dejean AS, Hedrick SM, Kerdiles YM (2011) Highly specialized role of Forkhead box O transcription factors in the immune system. Antioxid Redox Signal 14: 663-674.
112. Togher S,Larange A,Schoenberger SP,Feau S1 (2015) FoxO3 is a negative regulator of primary CD8+ T-cell expansion but not of memory formation. Immunol Cell Biol 93: 120-125.
113. Guo Z, Li H, Han M, Xu T, Wu X, et al. (2011) Modeling Sjögren's syndrome with Id3 conditional knockout mice. Immunol Lett 135: 34-42.
114. Li G, Yang Q, Zhu Y, Wang HR, Chen X, et al. (2013) T-Bet and Eomes Regulate the Balance between the Effector/Central Memory T Cells versus Memory Stem Like T Cells. PLoS One 8: e67401.
115. Szabo SJn (2000) A novel transcription factor, T-bet, directs Th1 lineage commitment. Cell 100: 655-669.
116. Keerthivasan S, Suleiman R, Lawlor R, Roderick J, Bates T, et al. (2011) Notch signaling regulates mouse and human Th17 differentiation. J Immunol 187: 692-701.
117. Mukherjee S, Schaller MA, Neupane R, Kunkel SL, Lukacs NW (2009) Regulation of T cell activation by Notch ligand, DLL, promotes IL-17 production and Rorc activation. J Immunol 182: 7381-7388.
118. Gattinoni L,Klebanoff CA, Palmer DC, Wrzesinski C, Kerstann K, et al. (2005) Acquisition of full effector function in vitro paradoxically impairs the in vivo antitumor efficacy of adoptively transferred CD8+ T cells. J Clin Invest 115: 1616-1626.
119. Klebanoff CA,Gattinoni L, Torabi-Parizi P, Kerstann K, Cardones AR, et al. (2005) Central memory self/tumor-reactive CD8+ T cells confer superior antitumor immunity compared with effector memory T cells. Proc Natl AcadSci U S A 102: 9571-9576.
120. Restifo NP, Dudley ME, Rosenberg SA (2012) Adoptive immunotherapy for cancer: harnessing the T cell response. Nat Rev Immunol 12: 269-281.
121. Wherry EJ, Ha SJ, Kaech SM, Haining WN, Sarkar S, et al. (2007) Molecular signature of CD8+ T cell exhaustion during chronic viral infection. Immunity 27: 670-684.
122. Williams MA, Bevan MJ (2007) Effector and memory CTL differentiation. Annu Rev Immunol 25: 171-192.

123. Hinrichs CS,Gattinoni L, Restifo NP (2006) Programming CD8+ T cells for effective immunotherapy. CurrOpinImmunol 18: 363-370.
124. Berger C, Jensen MC, Lansdorp PM, Gough M, Elliott C, et al. (2008) Adoptive transfer of effector CD8+ T cells derived from central memory cells establishes persistent T cell memory in primates. J Clin Invest 118: 294-305.
125. van der Waart AB, van de Weem NM, Maas F, Kramer CS, Kester MG, et al. (2014) Inhibition of Akt signaling promotes the generation of superior tumor-reactive T cells for adoptive immunotherapy. Blood 124: 3490-3500.
126. Yuan J,Adamow M, Ginsberg BA, Rasalan TS, Ritter E, et al. (2011) Integrated NY-ESO-1 antibody and CD8+ T-cell responses correlate with clinical benefit in advanced melanoma patients treated with ipilimumab. Proc Natl AcadSci U S A 108: 16723-16728.
127. Xie Y,Akpinarli A, Maris C, Hipkiss EL, Lane M, et al. (2010) Naive tumor-specific CD4(+) T cells differentiated in vivo eradicate established melanoma. J Exp Med 207: 651-667.
128. Quezada SA (2010) Tumor-reactive CD4(+) T cells develop cytotoxic activity and eradicate large established melanoma after transfer into lymphopenic hosts. J Exp Med 207: 637-650.
129. Hunder NN,Wallen H, Cao J, Hendricks DW, Reilly JZ, et al. (2008) Treatment of metastatic melanoma with autologous CD4+ T cells against NY-ESO-1. N Engl J Med 358: 2698-2703.
130. Hung K, Hayashi R, Lafond-Walker A, Lowenstein C, Pardoll D, et al. (1998) The central role of CD4(+) T cells in the antitumor immune response. J Exp Med 188: 2357-2368.
131. Muranski P,Borman ZA, Kerkar SP, Klebanoff CA, Ji Y, et al. (2011) Th17 cells are long lived and retain a stem cell-like molecular signature. Immunity 35: 972-985.
132. Kryczek I, Zhao E, Liu Y, Wang Y, Vatan L, et al. (2011) Human TH17 cells are long-lived effector memory cells. SciTransl Med 3: 104ra100.
133. Juntilla MM,Koretzky GA (2008) Critical roles of the PI3K/Akt signaling pathway in T cell development. Immunol Lett 116: 104-110.
134. Rathmell JC,Elstrom RL, Cinalli RM, Thompson CB (2003) Activated Akt promotes increased resting T cell size, CD28-independent T cell growth, and development of autoimmunity and lymphoma. Eur J Immunol 33: 2223-2232.
135. Kane LP, Weiss A (2003) The PI-3 kinase/Akt pathway and T cell activation: pleiotropic pathways downstream of PIP3. Immunol Rev 192: 7-20.
136. Joshi NS,Kaech SM (2008) Effector CD8 T cell development: a balancing act between memory cell potential and terminal differentiation. J Immunol 180: 1309-1315.
137. Crompton JG,Sukumar M,Roychoudhuri R, Clever D,Gros A, et al. (2015) Akt inhibition enhances expansion of potent tumor-specific lymphocytes with memory cell characteristics. Cancer Res 75: 296-305.
138. Weng NP, Liu K, Catalfamo M, Li Y, Henkart PA (2002) IL-15 is a growth factor and an activator of CD8 memory T cells. Ann N Y AcadSci 975: 46-56.
139. Berard M, Brandt K, Bulfone-Paus S, Tough DF (2003) IL-15 promotes the survival of naive and memory phenotype CD8+ T cells. J Immunol 170: 5018-5026.
140. Zeng R,Spolski R, Finkelstein SE, Oh S, Kovanen PE, et al. (2005) Synergy of IL-21 and IL-15 in regulating CD8+ T cell expansion and function. J Exp Med 201: 139-148.
141. Zeng R,Spolski R, Casas E, Zhu W, Levy DE, et al. (2007) The molecular basis of IL-21-mediated proliferation. Blood 109: 4135-4142.
142. Carr JM, Carrasco MJ, Thaventhiran JE, Bambrough PJ, Kraman M, et al. (2006) CD27 mediates interleukin-2-independent clonal expansion of the CD8+ T cell without effector differentiation. Proc Natl AcadSci U S A 103: 19454-19459.
143. Nolte MA, van Olffen RW, van Gisbergen KP, van Lier RA (2009) Timing and tuning of CD27-CD70 interactions: the impact of signal strength in setting the balance between adaptive responses and immunopathology. Immunol Rev 229: 216-231.
144. Roberts DJ, Franklin NA, Kingeter LM, Yagita H, Tutt AL, et al. (2010) Control of established melanoma by CD27 stimulation is associated with enhanced effector function and persistence, and reduced PD-1 expression of tumor infiltrating CD8(+) T cells. J Immunother 33: 769-779.
145. Libregts S, van Olffen RW, van der Sluijs KF, van Lier RA, Nolte MA (2011) Function of CD27 in helper T cell differentiation. Immunol Lett 136: 177-186.
146. Ochsenbein AF, Riddell SR, Brown M, Corey L, Baerlocher GM, et al. (2004) CD27 expression promotes long-term survival of functional effector-memory CD8+ cytotoxic T lymphocytes in HIV-infected patients. J Exp Med 200: 1407-1417.