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REVIEW

Immune Memory Focus

The architectural design of CD8⁺ T cell responses in acute and chronic infection: Parallel structures with divergent fates

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In response to infection, T cells adopt a range of differentiation states, creating numerous heterogeneous subsets that exhibit different phenotypes, functions, and migration patterns. This T cell heterogeneity is a universal feature of T cell immunity, needed to effectively control pathogens in a context-dependent manner and generate long-lived immunity to those pathogens. Here, we review new insights into differentiation state dynamics and population heterogeneity of CD8⁺ T cells in acute and chronic viral infections and cancer and highlight the parallels and distinctions between acute and chronic antigen stimulation settings. We focus on transcriptional and epigenetic networks that modulate the plasticity and terminal differentiation of antigen-specific CD8⁺ T cells and generate functionally diverse T cell subsets with different roles to combat infection and cancer.

Heterogeneity is a universal feature of T cell differentiation, providing context-specific immunity

A T cell response to infection conceptually has two primary goals. The first is an immediate goal of generating large numbers of effector T cells to help eliminate the present infection. The second is a long-term goal of developing immunologic memory by endowing a portion of the antigen-experienced cells with enhanced longevity and regenerative capacity to protect against future encounters by the same pathogen. To accomplish these goals, T cells have evolved to differentiate into remarkably heterogeneous cellular subsets that differ based on phenotype, function, proliferative capacity, longevity, and anatomic location. Indeed, the ability of a single T cell, especially naive T cells, to differentiate into multiple types of T cells based on the environmental conditions experienced has made "plasticity" a signature T cell trademark (Fig. 1). "Cell plasticity" describes the ability of cells to readily transition from one differentiation state to another in response to environmental fluctuations, as opposed to "terminal differentiation," which describes a cell in a more stable or fixed differentiation state that does not easily transition (see text box). The plasticity of CD4⁺ T cells to adopt distinct effector states was the first to be noted and has been a major focus in the field for >30 yr, fueled by the original discovery of the polarization of CD4⁺ T cells into T helper 1 (Th1) and Th2 subsets in 1986 (Mosmann et al., 1986). CD8⁺ T cells could also be functionally polarized in vitro into cells with more or less cytotoxic activity based on the types of cytokines present (Curtsinger et al., 1999). Subsequently, T cell plasticity was highlighted through in vivo studies showing that, upon infection, a single virus-specific CD4⁺ or CD8⁺ T cell could give rise to heterogeneous subsets of effector and memory cells (Gerlach et al., 2013; Buchholz et al., 2013; Plumlee et al., 2013; Tubo et al., 2013; Fig. 1 and Fig. 2 A). Multiple distinct memory T cell subsets that vary in their migratory and effector properties have been characterized: effector memory (T_{EM}), central memory (T_{CM}), stem-cell memory, tissue-resident memory (T_{RM}), and peripheral memory (T_{PM}) T cells (Schluns et al., 2000; Joshi et al., 2007; Kaech et al., 2002, 2003; Masopust et al., 2001; Wherry et al., 2003b; Gerlach et al., 2013; Buchholz et al., 2013; Sallusto et al., 1999; Gattinoni et al., 2011).

Comparative analyses of the effector and memory CD8⁺ T cell populations over the course of immune response have revealed the complex diversity of cell types (or, as we prefer to call them, cell states; see text box) that arise in different settings of infection and disease. For example, there are notable distinctions in the quality of T cell response, the subtypes of T cells produced

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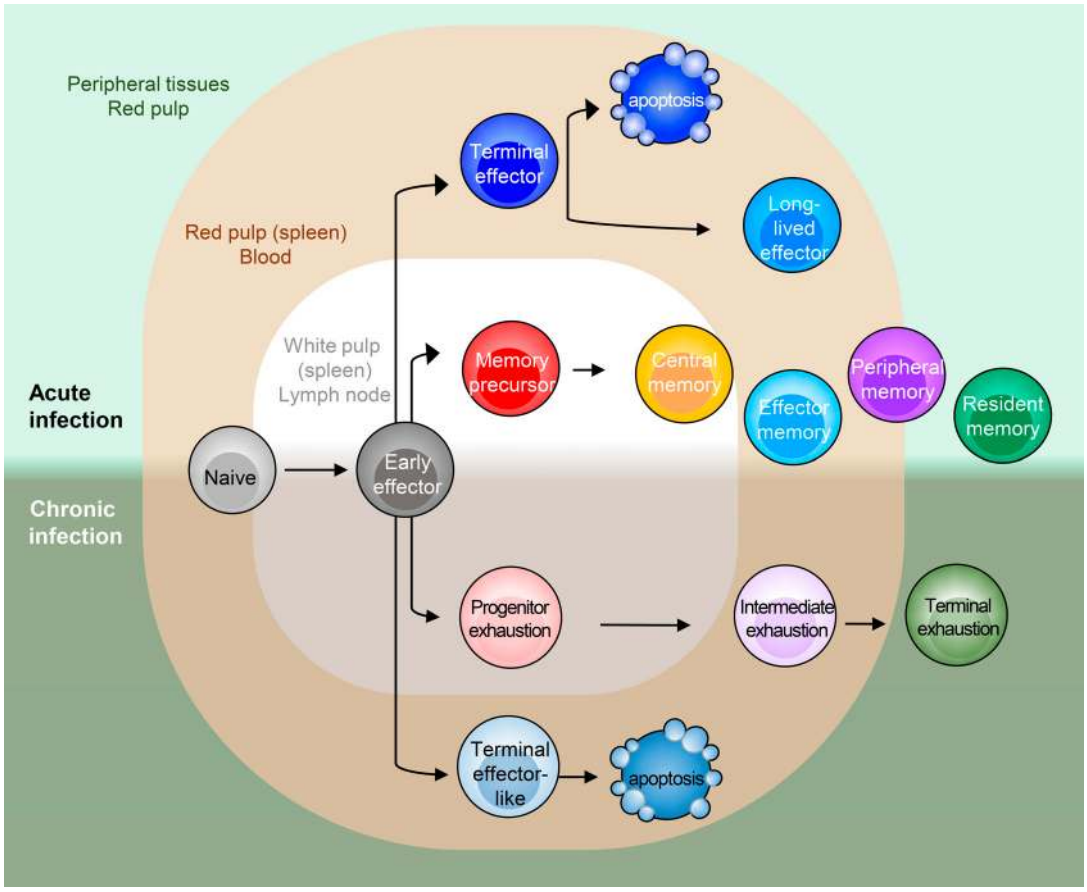

Figure 1. **Heterogeneous immune populations in virus infection model.** In both acute and chronic infection, early effector (EE) cells differentiate in a parallel manner into various CD8⁺ T cell subsets. Notable distinctions in trafficking patterns are signified by residence in lymphoid organs (gray), blood (red), or peripheral tissues (green). In acute infection, several effector and memory states are found. TE cells are typically found in the red pulp of spleen or blood, whereas MP cells are primarily found in white pulp or lymphoid structures, but they are also capable of recirculation. T_{CM} and T_{EM} cells both circulate in the blood, but T_{CM} cells predominate in lymphoid organs, whereas T_{EM} cells are also found in tissues. T_{PM} cells are proposed to circulate throughout lymph, blood, and tissues. T_{RM} cells do not circulate much and reside long-term in tissues. As in acute infection, heterogeneous states and distinct localization of CD8⁺ T cells are found in chronic infection. Tex_{prog} and MP cells are often observed in lymphoid structures, yet their circulation tendency might not be equivalent. Tex_{int} and Tex_{term} cells are predominantly found in blood and peripheral tissue, respectively.

among different types of pathogens, and whether they cause acute infection (such as lymphocytic choriomeningitis virus [LCMV] Armstrong strain, *Listeria monocytogenes*, influenza virus, hepatitis A virus, vaccinia virus, and yellow fever vaccine), persistent chronic infection (HIV, hepatitis C virus [HCV], or hepatitis B virus), or latent infection with periods of virus reactivation (EBV or CMV; Boutboul et al., 2005; van Leeuwen et al., 2005; Wherry et al., 2006; Schulte et al., 2011; DeWitt et al., 2015). Likewise, cancer, autoimmunity, and transplantation can also be considered settings of chronic T cell activation. However, as we learn more about how T cells adapt to these settings of chronic stimulation, we also see a great deal of symmetry or parallelism in the overall "design" of the CD8⁺ T cell response between acute and chronic settings. This is the focus of this review.

Furthermore, with the advent of single-cell technologies to characterize the transcriptomes, epigenomes, and clonotypes (TCR repertoire) of T cells, we are operating in an unprecedented manner with exceptional speed to probe and deconvolute T cell diversity and heterogeneity and define T cell subsets or states (Pauken et al., 2016; Sen et al., 2016; Scott-Browne et al., 2016; Philip et al., 2017; Mognol et al., 2017; Scharer et al., 2017; Beltra et al., 2020; Brummelman et al., 2018; Bengsch et al., 2018; Yao et al., 2019; Chen et al., 2019b). As the determination of cell subsets or states using single-cell RNA-sequencing is based on the unbiased clustering of cells according to the genes expressed (of which the upper limit is 30,000, the number of genes in a cell), one may predict that this analysis would reveal infinite numbers of possible T cell differentiation states. However, as more data emerges from various contexts and disease settings, we are identifying a reasonably finite number of T cell subsets, on the order of dozens, not thousands (Tirosh et al., 2016; Zheng et al., 2017; Azizi et al., 2018; Guo et al., 2018; Sade-Feldman et al., 2018; Zhang et al., 2018, 2019; Li et al., 2019; Miller et al., 2019; Yost et al., 2019; Sandu et al., 2020). Thus, although each individual CD8⁺ T cell has the potential to adopt multiple cell fates, there is likely an upper limit to the number of possible cell states acquired, with some states being more stable than others (see text box). Needless to say, the sheer amount of transcriptional and epigenetic information being generated will

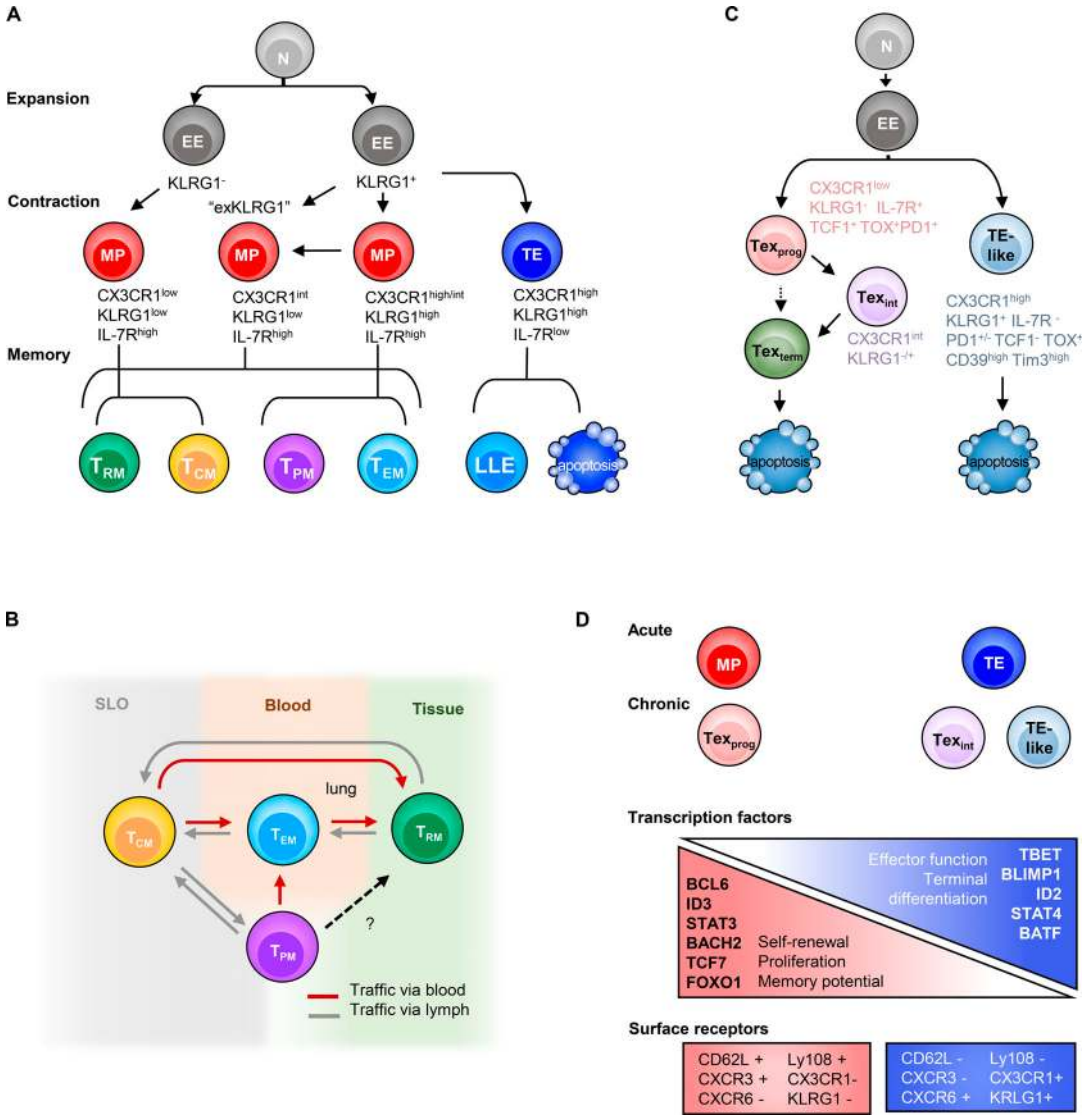

Figure 2. **Heterogeneous T cell subsets in both acute and chronic infection are possibly driven by a similar mechanism. (A)** In acute infection, depending on T cell activation and inflammation intensity, heterogeneous MP cells are formed with various levels of CX3CR1, which give rise to diverse memory subsets. KRLG1^{hi} IL-7Ra^{hi} or exKLRG1 MP cells develop memory cells of higher cytotoxicity (Herndler-Brandstetter et al., 2018; Olson et al., 2013). **(B)** Several memory subsets maintain plasticity, which allows homeostatic (T_{RM} to T_{CM}; T_{EM} or T_{PM} to T_{CM}) or antigen rechallenge–induced (T_{CM} to T_{EM}, T_{PM}, or T_{RM}) cell state conversion (Marzo et al., 2005, 2007; Beura et al., 2018b; Park et al., 2018; Osborn et al., 2019; Fonseca et al., 2020; Frizzell et al., 2020; Wherry et al., 2003b; Wherry and Ahmed, 2004; Bouneaud et al., 2005; Gattinoni et al., 2011; Gerlach et al., 2016). **(C)** Parallel differentiation ontology in chronic infection. Tex_{prog} cells diverge from EE due to TOX and *Tcf7* activation, which suppresses TE-like cell formation. Tex_{prog} further differentiate in a linear manner, or also perhaps in a bifurcated manner, to Tex_{int} or Tex_{term} cells. **(D)** Transcription gradient model for both acute and chronic infection. Shared TF gradients and surface receptors are found that drive the proliferative, stem cell–like cell states to more terminally differentiated cell states with effector function. KRLG1 expression is found in TE in acute and TE-like cells in chronic LCMV clone 13 and exhausted cells in HIV-infected patients. N, naive; SLO, secondary lymphoid organs.

undoubtedly lend critical insight into the vast heterogeneity of functional states, in addition to the identification of key regulators governing T cell differentiation and plasticity.

CD8 T cell responses to acute and chronic infection

The heterogeneity and plasticity of T cells have been revealed at every phase of the immune response: expansion and effector cell differentiation; resolution and effector cell contraction; and memory formation. Decades ago, it was noted in murine viral infections that several proteins are dynamically expressed by virus-specific CD8$^+$ T cells over time, with peak expression of proteins that define highly cytotoxic CD8$^+$ T cells during the first phase: granzymes (GZMB^{hi}), perforin^{hi}, IFNγ^{hi}, CD43 (IB11)^{hi}, CD62L^{lo}, and CD27^{lo} (Tripp et al., 1995; Hamann et al., 1997; Harrington et al., 2000; Kaech et al., 2003). Similar properties distinguishing highly cytotoxic CD8$^+$ T cells (CD45RA^{hi}, CD62L^{lo}, CD27^{lo}, and CD28^{lo}) could also be found in human virus-specific CD8$^+$ T cells (Hamann et al., 1997; Akondy et al., 2017). Gradually, cells with this phenotype mostly

Defining cell types, cell states, and cell fates

A great number of phenotypic marker discoveries and advances in multiomics approaches have led to the identification of an extensive (but not infinite) number of T cell subsets that arise from naive CD4⁺ or CD8⁺ T cells. This subset classification is useful to understand T cell functionality based on the activity of distinct groups of cells. However, this classification raises the following questions: (a) Should different T cell subsets be considered distinct "cell types" or "cell fates"—or rather, "cell states"—based on their differentiation states? (b) How does the stability of a differentiation state (stable vs. quasi-stable vs. transient) influence these definitions? (c) How should differences in signature genes, epigenetic states, functions, and migratory patterns be applied to these definitions?

The term cell type has historically been used to define cells based on certain attributes, such as specialized cellular function, unique morphology, and cytoplasmic architecture. CD4⁺ and CD8⁺ T cells, for example, are well-recognized cell types, yet molecular dissection has revealed that they comprise numerous subtypes. Cellular properties can vary dramatically between subtypes. Alternatively, the difference is subtle and gradual rather than categorical, which makes the classification of cell subsets along developmental trajectories challenging. For example, CD4⁺ effector Th2 cells, Th1 cells, and Th17 cells have a clear difference in the pattern of gene expression, epigenetic states, and cytokine production, but the population of each effector type is inherently heterogeneous, containing multiple subsets that often display a continuum of differentiation states (Marshall et al., 2011; Pepper et al., 2011; Pepper and Jenkins, 2011; Cano-Gamez et al., 2020; Hale et al., 2013).

Cell subsets can be defined as epigenetically and transcriptionally similar groups of cells with a certain level of plasticity. In response to various stimuli, such as cell–cell interactions, signal transduction, and metabolic and mechanosensory cues, one subset could give rise to transcriptionally and epigenetically distinct states and to a new subset, but it is also possible that a subset could transcriptionally respond to stimuli without major epigenetic rewiring, giving rise to a transient cell state (Zheng et al., 2017; Guo et al., 2018; Sade-Feldman et al., 2018; Savas et al., 2018; Zhang et al., 2018, 2019; Satpathy et al., 2019; Yost et al., 2019; Low et al., 2020). Therefore, a cell subset may take on various cell states depending on the stimuli received. Thus, we propose the notion of referring to T cell subsets as cell states, knowing that each state has a variable degree of temporal stability and cell fate potential. For example, there are longer-lived and more stable states such as resting T_{CM} or T_{RM} cells, but there are more transient or conditional states such as transitional T_{EM} cells that convert to T_{CM} cells, "transitory" Tex_{int} cells, or EE cells that convert into KLRG1⁺ TE or IL-7R⁺ MP cells. Unless fate-mapping approaches are used, individual cells are interrogated at just a single point in time, making it difficult to assess the spatiotemporal changes in differentiation states experienced by a cell. Moreover, as we learn more about the epigenetic states of cells on a single-cell level, we will be able to better define cell subsets and their relative cell states.

The term cell fate is more restricted and describes the longer-term developmental destination of a cell as it relates to its natural environment. Cell fate is driven by a sequence of instructions: its genetic program, coupled with how the cell responds or adapts to its environment. The sequences of multiple cell states can end with a specified cell fate. However, as cell states can be plastic, one cell state is not necessarily destined to have a particular cell fate. Understanding of the heterogeneity and stability of T cell states and how they connect to cell fates is improving in T cells, but still faces challenges.

decayed as memory cells formed throughout the contraction phase. However, for a long time, it was not possible to distinguish the cytotoxic effector cells that died during the contraction phase from those that simply down-regulated their protein expression as they matured into resting memory T cells. As flow cytometry expanded the number of proteins and parameters one could analyze simultaneously, it was then revealed that even greater heterogeneity existed within the pool of pathogen-specific CD8⁺ T cells, with numerous subsets developing, spanning a range of differentiation states, and displaying different long-term fates and degrees of plasticity (Kaech et al., 2003; Wherry et al., 2003b; Huster et al., 2004; Gerlach et al., 2013; Herndler-Brandstetter et al., 2018; Chang et al., 2014; Wherry et al., 2007; Sarkar et al., 2008). In this section, we compare how CD8⁺ T cell differentiation varies across time and within different contexts of acute and chronic infection.

Acute infection

Upon virus infection, CD8⁺ T cells differentiate into diverse T cell states. One way to explain the rise of various states is the asymmetric division of daughter cells as T cells proliferate (Chang et al., 2007). The first interaction between a CD8⁺ T cell and an antigen-presenting cell polarizes the T cell at the immunological synapse, the site where the T cell receives all of its most essential instructions from antigen:TCR (signal 1), costimulation (signal 2), and cytokines (signal 3). The coordination of these signals leads to polarized segregation of transcription factors (TFs) and cell fate determinants that influence its differentiation trajectory. Sufficient costimulation and inflammatory signals induce clonal expansion and expression of TFs such as RUNX3, T-bet, and Eomesodermin (*Eomes*) that trigger cytotoxic effector T cell differentiation (Banerjee et al., 2010; Intlekofer et al., 2005, 2007; Joshi et al., 2007). The effector cells start to produce granzymes, perforin, cytokines (such as IL-2, IFNγ, and TNF), chemokines (such as CCL5 and CCL3), and chemokine receptors (such as CXCR3, CX3CR1, CXCR6, and CCR5) to traffic to sites of inflammation. However, the effector molecules expressed by these canonical "killer" CD8⁺ T cells can vary across the pool of antigen-specific T cells according to their extent of differentiation. For instance, during an acute infection (Fig. 1, top half, and Fig. 2 A), a large fraction of effector CD8⁺ T cells terminally differentiate into highly specialized cytotoxic effector cells that coproduce IFNγ, GZMB, and perforin but lose the ability to produce IL-2 and often TNF. These cells express the highest levels of chemokine receptors CX3CR1 and S1PR5 and the inhibitory receptor KLRG1 (Joshi et al., 2007; Rao et al., 2010; Gerlach et al., 2016; Sarkar et al., 2008) and are commonly referred to as terminal effector (TE) cells (a.k.a., short-lived effector cells) because while they are highly functional killer T cells and pertinent to fighting the present infection, most TE cells commit to a terminal endpoint, literally, and die during the contraction phase (Joshi et al., 2007; Sarkar et al., 2008). As TE cells terminally differentiate, they induce effector programs and actively repress promemory genetic programs (including IL-7 receptor α [IL-7Ra] and CD27 expression); this diminishes their multipotency, proliferative capacity, and longevity (Kaech et al., 2003; Joshi et al., 2007; Herndler-Brandstetter et al., 2018). However, a small number of TE-like cells persist into the memory pool with some memory-like features, dwell in the blood, and contribute to secondary responses. Such cells are often referred to as long-lived effector (LLE) or terminal T_{EM} cells (Renkema et al., 2020; Milner et al., 2020a; Olson et al., 2013).

As the activated T cells expand, most of the early effector (EE) cells down-regulate IL-7Ra, but then toward the end of the

proliferative burst, a small portion of the cells begin to reexpress IL-7Ra and display enhanced longevity and stem-like properties. These cells are referred to as memory precursor (MP) cells, because they are multipotent and can develop into many types of memory cells (T_{CM}, T_{EM}, T_{RM}, and T_{PM}) or further differentiate into effector cells upon restimulation (Schluns et al., 2000; Joshi et al., 2007; Kaech et al., 2002, 2003; Masopust et al., 2001; Wherry et al., 2003b; Gerlach et al., 2013; Knell et al., 2013). MP cells are lesser differentiated cells typically defined by increased expression of IL-7Ra as well as Bcl-2, CD27, CD28, and CXCR3 (Badovinac and Harty, 2007; Hikono et al., 2007; Kaech et al., 2003; Wherry and Ahmed, 2004; Wherry et al., 2003b). Compared with TE cells, MP cells are more functionally heterogeneous and display greater proliferative capacity and cytokine polyfunctionality (IL-2$^+$, IFNγ$^+$, and TNF$^+$), but lower GZMB. Some IL-7Ra^{hi} MPs also express high to intermediate amounts of CX3CR1 or KLRG1 concordant with greater amounts of cytotoxic molecules. These MPs are often referred to as double-positive (DP) cells, relating to their dual KLRG1^{hi} IL-7Ra^{hi} expression (Obar et al., 2011; Rubinstein et al., 2008; Goldrath et al., 2002; Plumlee et al., 2013; Fig. 2 A). Indeed, many of the MP cells are quite plastic and can be seen converting from one state to another during the resolution and contraction phase (and even much later at memory stages; Kaech et al., 2003; Joshi et al., 2007; Gerlach et al., 2016; Huster et al., 2004; Böttcher et al., 2015; Wherry et al., 2003b; Marzo et al., 2007; Youngblood et al., 2017). This is especially evident in the effector cells that express intermediate amounts of CX3CR1^{int} (Gerlach et al., 2016) or DP (IL-7R^{hi} KLRG1^{hi}) cells (Herndler-Brandstetter et al., 2018; Olson et al., 2013) that convert to KLRG1^{lo} (ex-KLRG1) cells during the contraction/resolution phase and become T_{RM}, T_{PM}, T_{CM}, or T_{EM} cells (Fig. 2 A).

As memory T cells mature following infection, they develop into subsets that play critical and diverse roles in mediating long-term protective immunity. T_{EM} and T_{RM} cells normally confer first-line defense at portals of pathogen entry in the blood or peripheral tissues, respectively, and can exert immediate effector responses, whereas T_{CM} cells and stem-cell memory T cells, located in secondary lymphoid organs, focus their efforts on proliferation to resupply the host with large bursts of effector cells (Jabbari and Harty, 2006; Masopust et al., 2006; Marzo et al., 2007; Gattinoni et al., 2011). However, it is important to remember that these overly simple classifications do not accurately portray the broad phenotypic and functional heterogeneity that exists in the memory T cell population, as nicely reviewed by Jameson and Masopust (2018), and that the composition of memory CD8 T cells are not static. Rather, the composition of memory T cells is dynamic and changes with age, repeated infection, and environmental fluctuations (such as inflammation). For example, interconversions between T_{EM} or T_{RM} cells → T_{CM} (Beura et al., 2018b; Osborn et al., 2019; Frizzell et al., 2020; Fonseca et al., 2020; Slütter et al., 2017; Van Braeckel-Budimir et al., 2018) or conversely T_{CM} → T_{EM}, have been described at rest (Marzo et al., 2005, 2007; Gattinoni et al., 2011; Wherry et al., 2003b; Bouneaud et al., 2005; Wherry and Ahmed, 2004; Gerlach et al., 2016). During a secondary challenge of skin infection, even circulating memory cells can convert to T_{RM} (Osborn et al., 2019; Park et al., 2018; Kok et al., 2020; Beura et al., 2018a; Enamorado et al., 2017). Further, following reinfection or serial reinfections, the secondary and tertiary memory pools contain larger numbers of long-living TE- and T_{EM}-like cells that have elevated cytotoxic properties (Jabbari and Harty, 2006; Masopust et al., 2006; Marzo et al., 2007; Gattinoni et al., 2011; Nolz and Harty, 2011; Wirth et al., 2010; Cui et al., 2011; Russell et al., 2017; Fraser et al., 2013). However, the properties of these "boosted" memory cells are largely influenced by the frequency of preexisting memory cells (Fraser et al., 2013; Joshi and Kaech, 2008). A larger number of preexisting memory cells encourage the formation of hybrid memory cells that display elevated cytotoxicity, IL-7Ra, and proliferative potential.

Persistent infections: Chronic and latent infections

Different types of chronic infections, persistent and latent, vary the duration of viremia and types of viral antigens produced. During a chronic infection with persistent viremia, such as infection with LCMV clone 13 in mice or HIV, HCV, or hepatitis B virus in humans, the virus-specific CD8$^+$ T cells initially expand and contract, quite like during acute infection, albeit with different kinetics and outcomes (Badovinac et al., 2004, 2002; Wherry et al., 2003a; Zajac et al., 1998). In contrast to acute infection, which generates memory cell fates long-term, in persistent chronic infections and cancer, the long-term fate of most effector CD8$^+$ T cells is to become dysfunctional, or what is more commonly referred to as "exhausted" (Fig. 1, bottom half; Schietinger et al., 2016; Philip et al., 2017; Willimsky and Blankenstein, 2005; Spranger et al., 2015; Alfei et al., 2019; Khan et al., 2019; Chen et al., 2019a; Seo et al., 2019; Thommen et al., 2018; Li et al., 2019). CD8$^+$ T cell exhaustion is driven by chronic TCR activation and is characterized by (a) increased expression of multiple inhibitory receptors, such as PD1, TIM3, LAG3, CTLA4, and TIGIT, and (b) progressive loss of IL-2, TNF, and IFNγ secretion (Zajac et al., 1998; Paley et al., 2012; Barber et al., 2006; Khan et al., 2019; Wherry et al., 2003a). While the increase in inhibitory receptor expression represses TCR signaling and proinflammatory effector functions in CD8$^+$ T cells, it also appears to protect the cells from activation-induced cell death, as PD-1–deficient T cells fare poorly and deteriorate quickly (Wei et al., 2019; Odorizzi et al., 2015). More recently, it was discovered that sustained Ca^{2+}/nuclear factor of activated T cells (NFAT) signaling leads to induction of the HMG-box TF TOX, which directs a distinct exhaustion transcriptional and epigenetic developmental program (Khan et al., 2019; Seo et al., 2019; Yao et al., 2019; Scott et al., 2019).

Similar to acute infection, the CD8$^+$ T cell response to chronic infection comprises multiple cell subsets that serve different roles in short- and long-term viral control. Akin to the multipotent MP cells in acute infection, in persistent chronic infection or tumors, a related stem-like exhaustion progenitor T cell type (Tex_{prog}) forms that is distinguished by increased SLAMF6 and CXCR5 and decreased TIM3 expression. Tex_{prog} cells have also been referred to as stem cell–like (Im et al., 2016), precursors of exhausted T cells (Utzschneider et al., 2020), or T memory-like exhausted cells (Utzschneider et al., 2016; Fig. 1, bottom half).

Like MP cells, Tex_{prog} cells also display plasticity and proliferative capacity and can differentiate into transitory-intermediate (Tex_{int}) "effector-like" cells that up-regulate CX3CR1, T-bet, and effector molecules (GZMB, IFNγ, and TNF). Sustained production of the Tex_{int} subset is critical to control chronic virus infection or tumor (Hudson et al., 2019; Zander et al., 2019; Yan et al., 2018). Importantly, PD-1 blockade acts on the Tex_{prog} cells by releasing their proliferative restraints and generating new bursts of Tex_{int} cells (Im et al., 2016; Miller et al., 2019; Hudson et al., 2019; Zander et al., 2019; Utzschneider et al., 2016; Leong et al., 2016; Wu et al., 2016; Beltra et al., 2020; Im et al., 2020). Tex_{int} cells then further develop into terminally differentiated exhausted T cells (Tex_{term}; Figs. 1 and 2 C) that express even higher amounts of PD-1, TIM3, and other inhibitory receptors including CD101, CD39, and CD160. Tex_{term} cells show impaired expression of effector-related proteins (TNF, IFNγ, GZMB, and T-bet), and stemness and proliferation-related proteins (TCF1, MYB, MYC, and Ki-67; Beltra et al., 2020; Hudson et al., 2019). It is possible that some multipotent Tex_{prog} cells directly develop into Tex_{term} cells, bypassing the Tex_{int} state, because the Tex_{term} cell population did not seem greatly diminished several weeks after deletion of $CX3CR1^{hi}$ T cells (Zander et al., 2019; Hudson et al., 2019).

However, it is noteworthy that not all chronic infections drive $CD8^+$ T cell exhaustion phenotypes. For example, during latent viral infections followed by episodes of viral reactivation such as commonly seen by adenovirus or herpes viruses (HSV, EBV, CMV, and murine CMV), a mixed population with a variety of effector and memory subsets is formed. This is likely driven mostly by the sporadic production of low-abundance antigen depots at sites of viral latency. Importantly, a distinctive feature is that the $CD8^+$ T cells recognizing latently produced antigens gradually expand over time and develop into a so-called "inflationary" memory cell pool that contains mostly $CX3CR1^{hi}$ $KLRG1^{hi}$ TE-like cells (similar in many ways to memory cells formed after sequential acute virus immunizations; Gordon et al., 2018; Ouyang et al., 2003; Ibegbu et al., 2005). Even though $CX3CR1^{hi}$ inflationary memory cells show some similarity to Tex_{int} cells, unlike their exhaustion counterpart in LCMV clone 13, inflationary memory cells up-regulate KLRG1 (Gordon et al., 2018; Ouyang et al., 2003; Ibegbu et al., 2005) and express low levels of inhibitory receptors such as PD1, TIM3, and CTLA4 (Hertoghs et al., 2010; Sauce et al., 2007). We outline these series of events in comparison to those that occur in acute infection in the next section.

Comparative analysis of $CD8^+$ T cell differentiation trajectories during acute and chronic infection

Even though T cell exhaustion is a progressive process that occurs over several weeks to months, the effector differentiation programs begin to diverge between acute and chronic infection within the first week of infection (Wherry et al., 2007; Ahmadzadeh et al., 2009; Sen et al., 2016; Chen et al., 2019b, 2021; Yao et al., 2019; Zhang et al., 2019). More specifically, the effector cells in both acute and chronic LCMV infection are transcriptionally similar up to day 4.5 after infection (Yao et al., 2019), but a significant transcriptional and epigenetic divergence begins a couple of days later and then continues for several more weeks (Wherry et al., 2007; Ahmadzadeh et al., 2009; Sen et al., 2016; Philip et al., 2017; Chen et al., 2019b, 2021; Yao et al., 2019; Zhang et al., 2019). This time period coincides exactly with changes in the expression of the TF TOX, as it dwindles in antigen-specific $CD8^+$ T cells in acute infection but becomes amplified in chronic infection and tumors (Page et al., 2018; Yao et al., 2019; Khan et al., 2019). Indeed, TOX is critical for inducing chromatin remodeling and gene expression programs associated with T cell exhaustion and sustaining Tex_{prog} cells (Khan et al., 2019; Page et al., 2018; Yao et al., 2019; Scott et al., 2019). Yet, despite these clear divisions in the outcomes of $CD8^+$ T cell fates during acute versus chronic infection, the underlying structure of the T cell differentiation programs appears quite similar between the two types of infections (Fig. 1; and Fig. 2, A and C).

Shared features of T cell differentiation in acute and chronic infection

In spite of overt differences in $CD8^+$ T cell phenotypes and effector functions, the core purpose of the developmental trajectories established in acute and chronic infection appears similar: to generate progenitor cells that sustain the pool of virus-specific $CD8^+$ T cells long-term and can regenerate effector cells to battle viral-infected cells when present. Therefore, it is perhaps not surprising that both MP and Tex_{prog} subsets, as well as TE and Tex_{int}, have a great deal in common with regard to trafficking patterns and transcriptional programs (Fig. 1). For example, the precursor cells in both infection settings (Tex_{prog} and MP) prefer to home to lymphoid zones (e.g., white pulp in spleen, lymph nodes, or tertiary lymphoid organs), whereas the CX3CR1-expressing effector-like cells (TE and Tex_{int}) are predominantly found in the blood. Interestingly, Tex_{term} and T_{RM} also seem to have tissue homing in common, and both express CXCR6 and CD69. Regarding the shared transcriptional programs, both Tex_{prog} and MP express high *Tcf7*, *Foxo1*, and *Bach2*; TE and Tex_{int} express *Tbx21* (encoding T-bet) and *Zeb2*; Tex_{term} and T_{RM} express *Prdm1*, *Id2*, *Nr4a2*, *Bhlhe40*, and *Tox* (Fig. 2 D and Fig. 3). Another common element between acute and chronic infection is that terminally differentiated cell types are produced in both acute and chronic infection as they develop into TE ($KLRG1^{hi}$ $CX3CR1^{hi}$ $IL\text{-}7Ra^{lo}$ $CD27^{lo}$) and Tex_{term} ($CD101^{hi}$ $TIM3^{hi}$ $SLAMF6^{lo}$) cells, respectively. This definition of terminal differentiation stems from experiments showing that, when adoptively transferred into naive or infection-matched hosts, these cells maintain their physical and genetic properties.

Distinctive features of T cell differentiation in acute and chronic infection

Before we understood the limits of terminal differentiation of effector cells, it seemed sensible to assume that $CD8^+$ T cell exhaustion was simply an end-stage product of effector differentiation and that TE cells would convert into Tex_{term} cells with prolonged antigenic stimulation. However, this is not the case, because $KLRG1^{hi}$ TE cells do not generate Tex_{term} cells when restimulated (Chen et al., 2019b; Khan et al., 2019; Angelosanto et al., 2012). In fact, there are many similarities between the

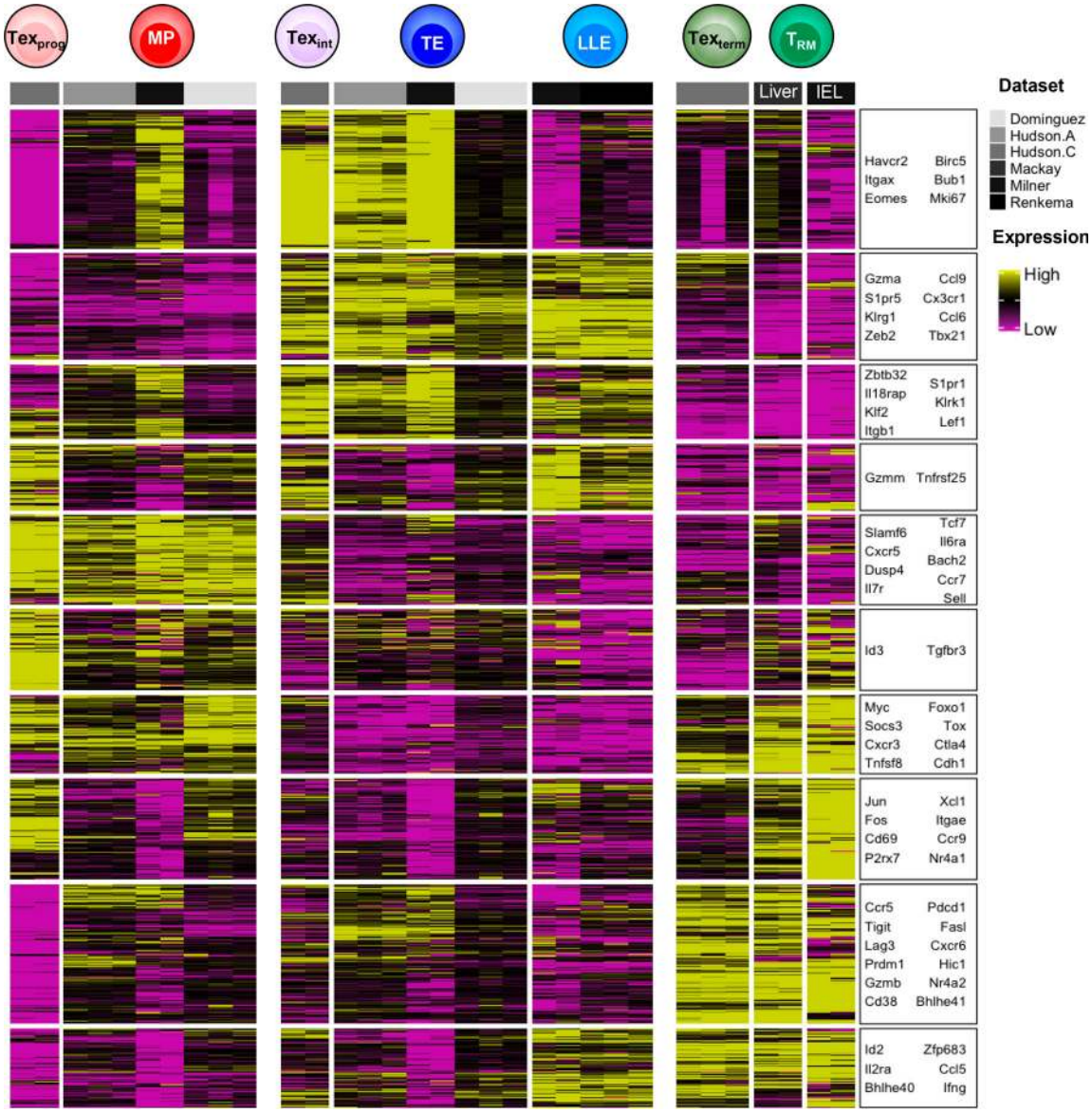

Figure 3. **Transcriptional meta-analysis of T cell subsets in both acute and chronic infection.** Scaled and batch-corrected RNA-sequencing expression from sorted effector and exhausted cell populations (Dominguez et al., 2015; Mackay et al., 2016; Hudson et al., 2019; Milner et al., 2020a; Renkema et al., 2020). T cell states that arise during chronic and acute infection can be characterized by similar transcriptional archetypes, such as up-regulation of sets of stem-like genes (Tex_{prog} and MP cells), cytotoxicity and circulating potential (Tex_{int}, TE, and LLE cells), or inhibitory receptor genes (Tex_{term} and T_{RM} cells).

transcriptional states of TE and Tex_{int} cells found in acute and chronic infection, respectively (Fig. 2 D and Fig. 3). One of the most notable distinctions is that TE cells are terminally differentiated, whereas Tex_{int} cells are intermediary and not yet terminally differentiated. Another notable difference is the expression of KLRG1 itself. KLRG1 is expressed by LCMV-specific TE-like $CD8^+$ T cells during the first week of chronic infection, but these $KLRG1^{hi}$ cells rapidly wane and are highly dependent on $CD4^+$ T cell help (Chen et al., 2019b; Khan et al., 2019; Stelekati et al., 2018). The inability of $KLRG1^{hi}$ TE cells to persist in chronic infection may be because these cells are highly sensitive to activation-induced cell death (Joshi et al., 2007; Chen et al., 2019b) and/or because TOX antagonizes T-bet and impairs maximal T-bet induction and TE cell induction, which circumvents terminal differentiation into TE cells (Beltra et al., 2020; Khan et al., 2019). Even though exhausted HIV- or HCV-specific T cells in humans express KRLG1, they show significant reduction in T-bet expression (Wang et al., 2020; Bengsch et al., 2007). In this way, TOX may help to endow Tex_{int} cells with plasticity to continue differentiating into Tex_{term} cells.

Transcriptional and epigenetic regulation of $CD8^+$ T cell heterogeneity during acute and chronic infection

Active maintenance of plasticity and terminally differentiated states in $CD8^+$ T cells

The spectrum of $CD8^+$ T cell differentiation states that arise during infection depend on the coordinated actions of multiple TFs and chromatin remodeling complexes that can generate

positive feed-forward circuits to promote one cell state while simultaneously opposing another. Indeed, some differentiation states seem more stable (e.g., naive, T_{CM}, TEMRA [effector memory T cells re-expressing CD45RA], Tex_{term}, and TE states) than others (e.g., MP, CX3CR1^{int}, or Tex_{int} states). These stable states perhaps represent epigenetic and metabolic equilibrium, since the epigenetic landscape of a cell is directly influenced by its metabolic activities (Franco et al., 2020; Buck et al., 2017). In this section, we outline several transcriptional and epigenetic changes that occur to generate the subsets described above.

TE cell differentiation in acute infection is a stepwise process. In EE cells, TFs such as T-bet, *Zeb2*, *Stat4*, *Rbpj*, *Irf4*, Blimp-1 (*Prdm1*), and *Id2* are induced and set up a counterregulatory network that leads to the transcriptional and epigenetic repression of critical promemory TFs (such as *Tcf7*, *Bach2*, *Bcl6*, *Id3*, *Foxo1*, *Zeb1*, and *Stat3*) active within MP cells to resist terminal differentiation and maintain plasticity, multipotency, and memory cell development (Fig. 2 D; Yang et al., 2011; Joshi et al., 2007; Cui et al., 2011; Ichii et al., 2002; Oestreich et al., 2012; Delpoux et al., 2017; Intlekofer et al., 2005; McLane et al., 2013; Kaech and Cui, 2012; Dominguez et al., 2015; Guan et al., 2018; Omilusik et al., 2015; Kallies et al., 2009; Rutishauser et al., 2009; Knell et al., 2013; Best et al., 2013). The developing TE cells epigenetically repress promemory genes via targeted deposition of H3K27me3 and H3K9me3 and DNA methylation on promemory genes by the enzymes EZH2, SUV39H1, and DNMT3a, respectively (Gray et al., 2017; Pace et al., 2018; Ladle et al., 2016; Youngblood et al., 2017). This epigenetic remodeling likely occurs several days after the initial transcriptional repression of promemory genes that begins as early as the first CD8$^+$ T cell division (Arsenio et al., 2014). In other words, EE cells may begin to commit to TE cell differentiation (e.g., via transcriptional induction of TE genes [*Klrg1* and *Cx3cr1*]), but their determination to a terminally differentiated TE state (as defined by epigenetic silencing of promemory genetic programs) is not achieved until several days later. These findings, nearly 20 yr later, provide a molecular framework for the decreasing-potential model to describe the contraction of effector cells and survival of memory cells originally put forth by Ahmed and Gray (1996). Evidence for a similar process occurring in chronic infection to promote terminally differentiated Tex_{term} by restricting Tex_{int} and Tex_{prog} signature genes also exists but needs to be investigated more closely (Ghoneim et al., 2017; Schietinger et al., 2016; Beltra et al., 2020; Yao et al., 2019; Zander et al., 2019; Hudson et al., 2019).

MP cells, on the other hand, maintain promemory, pro-survival, and many TE-signature gene loci in active or permissive epigenetic states, allowing MP cells to simultaneously promote memory development and remain poised for the future expression of "effector" genes. Similarly, T_{CM} and T_{EM} cells maintain poised and bivalent epigenetic marks, activating H3K4me3 and repressive H3K27me3, on the promoter regions of *Id2*, *Tbx21*, *Eomes*, *Irf4*, *Map3k1*, *Mlk4*, and *Mkx* (Russ et al., 2014; Araki et al., 2009). Bivalency allows multipotent memory cells to epigenetically mark transcriptionally silenced genes that are destined for activation followed by T cell activation, facilitating a rapid transition of memory cells from resting to an activated state. The capacity of the transition can be stably maintained and has been observed in smallpox-specific T cells up to 83 yr after infection, suggesting that this effector inducibility is extremely durable (Hammarlund et al., 2010). The plasticity seen in MP and memory cells depends on the continued expression of promemory genes such as *Foxo1* and exposure to cytokines via TGFβ receptor, because late deletion of these genes in resting memory CD8$^+$ T cells leads to the spontaneous acquisition of TE-like states (Ma and Zhang, 2015; Utzschneider et al., 2018). Possibly, FOXO1 acts in MP cells to insulate promemory genes from (a) EZH2-mediated silencing by locally impairing H3K27me3 deposition at such loci (Gray et al., 2017) or (b) recruiting DNA-demethylase machinery because the selective loss of DNA methylation at promemory-associated loci such as *Sell* (CD62L) and *Tcf7* was observed in MP cells, but not TE cells (Youngblood et al., 2017). These data provide mechanistic insight into how developmental plasticity is epigenetically wired in subsets of effector CD8$^+$ T cells or lost in others as they are terminally differentiated.

Terminally differentiated states are usually associated with long-term stability; therefore, it was surprising to see that maintenance of TE states during the contraction and resolution phase depended on sustained ID2 expression (Omilusik et al., 2015). When ID2 was deleted after day 8 of acute virus infection, the KLRG1^{hi} TE cells rapidly converted into MP-like cells, indicating that terminal differentiation depends on sustained expression of key TFs that prevent dedifferentiation. Coupled with the data above that sustained expression of *Foxo1* is needed to preserve resting memory cell states, these data suggest that maintaining T cell differentiation states, even those that appear to be fairly stable like TE or T_{CM} cells, is an active process and requires sustained genomic surveillance by "inducer" TFs. Natural perturbations in the expression of such inducer TFs and epigenetic modifiers such as demethylases likely account for the dynamic interconversions found between various differentiation states (e.g., $T_{EM} \rightarrow T_{CM}$, DP $\rightarrow T_{RM}$, $T_{RM} \rightarrow T_{CM}$) in CD8$^+$ T cells after infection has resolved.

Imprinting CD8$^+$ T cell exhaustion

Along the trajectory from $Tex_{prog} \rightarrow Tex_{int} \rightarrow Tex_{term}$, there is extensive remodeling of the transcriptional and epigenetic landscape that occurs from one state to another (Philip et al., 2017; Beltra et al., 2020; Utzschneider et al., 2020; Miller et al., 2019). Chronic TCR signaling is proposed as a core mechanistic driver of functional exhaustion, especially via calcineurin-dependent TF, NFAT, and other NFAT-driven, TCR-responsive TFs such as IRF4, BATF, nuclear receptor subfamily 4 group A (NR4A), and TOX (Zajac et al., 1998; Paley et al., 2012; Barber et al., 2006; Seo et al., 2019; Chen et al., 2019a). The formation and maintenance of Tex_{prog} cells depend on TOX, but also on TFs found in memory cells and their precursors, such as TCF1 (encoded by *Tcf7*) and FOXO1 (Utzschneider et al., 2018). As discussed in the section Comparative analysis of CD8$^+$ T cell differentiation trajectories..., TOX promotes Tex differentiation in part by inhibiting TE differentiation. TOX was found to bind to Kat7, the acetyl transferase component of the HBO1 complex, suggesting it may direct histone H4 and H3 acetylation in

exhausted CD8$^+$ T cells (Khan et al., 2019; Yao et al., 2019; Page et al., 2018). TOX may also have a role in regulating DNA methylation (Khan et al., 2019; Alfei et al., 2019). Now, an important next step is to understand how TOX specifically interacts with and influences the functions of promemory TFs to steer the cells toward T cell exhaustion in chronic infection and tumors.

The transition of Tex_{prog} to Tex_{int} cells is associated with the proliferation and induction of *Tbx21*, *Zeb2*, *Cx3Cr1*, and *S1pr5* (similar to TE cells), but then these cells continue to convert into CD101^{hi} Tex_{term} cells and progressively methylate the DNA and silence *Ifng*, *Tcf7*, and *Tbx21*. In contrast, the promoters of *Pdcd1*, *Lag3*, and *Havcr1* (encoding TIM3) and *Cd101* are demethylated and further up-regulated (Scharer et al., 2013; Ghoneim et al., 2017; Scott-Browne et al., 2016; Sen et al., 2016). De novo methylation of effector loci by DNMT3a is critical for commitment to exhaustion by silencing effector- and memory-related gene loci (Ghoneim et al., 2017). Indeed, the epigenetic stability of the exhaustion program is remarkably robust. Even though greater numbers of functional Tex_{int}-like cells emerge with anti-PD-1 treatment, this therapy is unable to reprogram the epigenetic landscape of Tex cells to that of an effector or memory state (Pauken et al., 2016). Thus, drugs that inhibit epigenetic modifiers will likely be needed to redirect the differentiation of exhausted CD8$^+$ T cells (Chiappinelli et al., 2016; Henning et al., 2018; Utzschneider et al., 2020; Alfei et al., 2019).

How does the same TF specify distinct differentiation states?

While we use differentially expressed genes to distinguish one CD8$^+$ T cell differentiation state from another, there are also shared sets of coordinately expressed genes across multiple T cell subsets. This indicates that certain TFs are "multitaskers" and are used repeatedly to generate multiple types of differentiation states within T cells. For example, as mentioned above, *Tcf7* and *Foxo1* are involved in the generation of both MP cells in acute infection and Tex_{prog} cells in chronic infection (Fig. 2 D and Fig. 3). Similarly, T-bet and *Zeb2* mRNA is common to both TE and Tex_{int} cells, and there is a great deal of overlap between TFs expressed in T_{RM} and Tex_{term} cells (e.g., *Nr4a1*, *Nr4a2*, *Prdm1*, *Irf4*, and *Tox*; Fig. 3; Hudson et al., 2019; Beltra et al., 2020; Mackay et al., 2016; Milner et al., 2017, 2020b). *Eomes* is particularly busy, because it is expressed the most in EE, T_{CM}, and Tex_{term} cells and plays a role in the development of each cell subtype. Interestingly, Eomes demonstrates cooperative behavior with T-bet in EE cells (Pearce et al., 2003) but develops a seemingly antagonistic relationship later in the balance between MP versus TE, T_{CM} versus T_{EM}, or Tex_{int} versus Tex_{term} cell states. Collectively, this raises the all-important question: How does a CD8$^+$ T cell commit to one cell fate over another if similar TFs are being used?

Another way to ask this question is, what are the context-dependent specifiers that differentially control the activity of the same TF in discrete differentiation states? One of the first mechanisms put forth by our laboratory was the use of TF gradients, wherein the expression level of a TF has one type of activity and regulates certain target genes at low amounts, but then regulates other target genes at higher amounts. Strong support for this mechanism has been observed with T-bet controlling the differentiation of MP → TE cells, Tex_{prog} → Tex_{int} cells, and T_{RM} versus circulating T_{CM} and T_{EM} cells (Joshi et al., 2007; Wherry et al., 2003b; Mackay et al., 2016, 2015). Furthermore, T-bet nuclear localization is also used to regulate its function in different CD8 T cell subsets (McLane et al., 2013), but little is known about how T-bet functions to control gene expression at high and low amounts in the nucleus. Likewise, the levels of TOX can also influence Tex states (Khan et al., 2019; Scott et al., 2019; Seo et al., 2019). Other common mechanisms to alter the activity of a single TF in a context-dependent manner is via posttranslational modifications, such as phosphorylation, which allows the same TF to acquire multiple activities or binding partners. Shockingly, we know very little about the differences between TF posttranslational modifications, their genomic binding patterns, and partnerships with other TFs in acute versus chronic infection. This knowledge would go a long way toward understanding how the same TFs can be involved in overtly distinct differentiation programs.

Concluding remarks

T cell immunity has coevolved over millions of years, with pathogens adapting to diverse forms of acute and chronic infections. The result has been the creation of T cells that develop immense plasticity and heterogeneity to enable the generation of (a) expendable pools of effector cells to control the present infection and (b) longer-lived pools of multipotent cells to sustain immune responses to chronic infection or provide secondary "recall" responses at a later time of reinfection. Therefore, while the qualities of the CD8$^+$ T cells between acute and chronic infection are overtly distinct, one can see core similarities in the underlying structure of the T cell responses between these two types of infections.

With recent technological advances, including sequencing at the single-cell level, we are now able to appreciate the heterogeneity and plasticity of antigen-specific T cells even more in various tissues, infections, cancer, autoimmunity, and other diseases. All things considered, we propose referring to T cell subsets as cell states (see text box) to convey the dynamic nature of T cell differentiation with different levels of plasticity. The current comprehensive understanding of T cell differentiation in multiple contexts raises outstanding questions: (a) Given the similarity in the transcriptional networks between Tex_{prog} versus MP, Tex_{int} versus TE or LLE, and Tex_{term} versus T_{RM} cells, will these states be capable of transdifferentiation to one another? What are the signaling and transcriptional pathways that drive the conversion from one cell state to another? Is it possible to reverse epigenetic imprinting of exhaustion and reprogram the state to effector or memory T cells? Can we exploit this plasticity that possibly allows transdifferentiation or reverse imprinting to synthetically create even more diverse states, aiming for better therapy for different diseases? (b) Other than TCR signaling, what are the environmental factors and their signaling pathways that divert the T cell differentiation trajectory from memory to exhaustion? (c) Which T cell states offer the most desirable therapeutic benefit in a tumor or chronic infection? Understanding these similarities and differences in T cell differentiation and

programmability at different stages of the T cell response will provide insight into and new targets for novel therapeutic interventions to both rejuvenate dysfunctional, exhausted cells and to promote memory in virus infection and cancer.

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