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Not-so-opposite ends of the spectrum: CD8+ T cell dysfunction across chronic infection, cancer, and autoimmunity

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Abstract

CD8+ T cells are critical mediators of cytotoxic effector function in infection, cancer, and autoimmunity. In cancer and chronic viral infection, CD8+ T cells undergo a progressive loss of cytokine production and cytotoxicity, a state termed T cell exhaustion. In autoimmunity, autoreactive CD8+ T cells retain the capacity to effectively mediate the destruction of host tissues. Although the clinical outcome differs in each context, CD8+ T cells are chronically exposed to antigen in all three. These chronically stimulated CD8+ T cells share some common phenotypic features, as well as transcriptional and epigenetic programming, across disease contexts. A better understanding of these CD8+ T cell states may reveal novel strategies to augment clearance of chronic viral infection and cancer, and mitigate self-reactivity leading to tissue damage in autoimmunity.

Introduction

CD8+ T cells are often described as existing at opposite ends of a spectrum of functionality in the context of chronic viral infection and cancer versus autoimmunity (Figure 1). In autoimmunity, CD8+ T cells overcome numerous tolerance mechanisms, including thymic selection and T cell activation requirements, to exert inappropriate effector function and cause damage to self tissue. In contrast, CD8+ T cells in chronic infection are exposed to

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Author contributions

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Competing interests

A.H.S. has patents/pending royalties on the PD-1 pathway from Roche and Novartis. A.H.S. is on advisory boards for Surface Oncology, Elstar, SQZ Biotechnologies, Elpiscience, Selecta, Bicara and Monopteros, GlaxoSmithKline and Janssen, and consults for Novartis. A.H.S. has received research funding from Novartis, Roche, UCB, Ipsen, Quark, Merck and AbbVie unrelated to this project.

appropriate activating cues (signal 1: antigen/T cell receptor (TCR), signal 2: costimulation, and signal 3: inflammatory cytokines). While CD8+ T cells exhibit similar profiles of transcriptional activation at the early phases of both acute and chronic infection at an early timepoint,[1] responding CD8+ T cells in chronic infection quickly begin to exhibit insufficient effector function and are unable to clear the infection. This state of relative dysfunction, termed “exhaustion,” is also observed in the tumor microenvironment (TME). Blockade or deficiency of immune checkpoints like PD-1 ameliorates aspects of T cell exhaustion; these same perturbations can precipitate or exacerbate autoimmunity.

Yet, the settings of chronic infection, cancer, and autoimmunity share a key defining feature: chronic antigen exposure. New research has begun to define phenotypic similarities between CD8+ T cells in exhausted and autoimmune contexts, including functional, transcriptional, and epigenetic features. This may seem counterintuitive, given the “opposite ends of the spectrum” framework of CD8+ T cell state in exhaustion versus autoimmunity, as outlined in Figure 1. However, the perturbation of some exhaustion-associated features has recently revealed that “dysfunctionality” or “hyporesponsiveness” may in fact be integral for CD8+ T cell persistence in a chronic stimulatory context—thus providing a rationale for finding these shared features in chronic viral infection, cancer, and autoimmunity. CD8+ T cells in each of these contexts have been extensively discussed in previous reviews[2–8]; here we will focus on salient aspects of CD8+ T cell dysfunction most relevant for highlighting areas of similarity and distinction. A better understanding of CD8+ T cell states in these contexts may reveal new ways to alleviate T cell exhaustion and enhance CD8+ T cell functions in cancer and chronic viral infection, as well as strategies to induce or augment exhaustion-like features to treat autoimmunity.

TCR specificity and affinity

In autoimmunity, chronic infection, and cancer, chronic TCR signaling is a critical signal in the development and maintenance of the associated CD8+ T cell state. While chronic TCR signaling is a common feature across these contexts, the target varies: foreign antigens are targeted in chronic viral infection, while T cell responses are directed towards self-antigens in autoimmune disease—and a combination of both neoantigens and self-antigens may be targeted on tumors.

In cancer and chronic viral infection, chronic TCR signaling in the context of inflammatory signals is a critical driver of T cell dysfunction: tumor antigen-specific CD8+ T cells are significantly more exhausted (by reduced cytokine production, high co-inhibitory receptor expression, and high TOX expression) than co-transferred non-reactive cells in mouse studies[9–11]. In mice infected with a chronic strain of lymphocytic choriomeningitis virus (LCMV Clone 13), significantly reducing the load of a specific antigen without altering the overall viral load is sufficient to mitigate the development of certain aspects of exhaustion (chronic high PD-1 expression, low TNF production)[12]. There is also evidence that persistent antigen recognition plays an important role in the maintenance of exhausted cells. When exhausted cells from day 30 of LCMV Clone 13 infection are transferred into mice previously infected with acute LCMV Armstrong (cleared of antigen), they persist relatively poorly compared to transfer into chronically infected mice[13]. Additionally, in mice

infected with LCMV Clone 13, blockade of homeostatic/memory maintenance signals IL-7 and IL-15 does not affect antigen-specific cell persistence; this may instead be supported by stimulation from residual antigen[14]. Furthermore, an epitope mutation in the chronic viral infection simian immunodeficiency virus (SIV) leads to the loss of responding CD8+ T cell clones[15]. However, a recent study of HCV-specific CD8+ T cells from patients treated with direct-acting antiviral (DAA) therapy identifies some HCV-specific cells that do persist in a setting of undetectable viral load (with a bias towards the progenitor-exhausted subset, discussed in more detail below)[16]. The role of IL-7 and IL-15 signaling, or very low level antigen, in the maintenance of these cells remains to be determined.

The prevalence of specific CD8+ T cell clones changes during the progression of chronic viral infection and autoimmunity, driven by stages of antigen-driven expansion and subsequent suppression or attrition. In chronic viral infection, high affinity T cells (e.g. D(b)NP396-404-specific T cells) respond to early dominant epitopes, but are extensively deleted by day 25, to a greater extent than lower affinity T cells (e.g. D(b)GP33-41, D(b)GP276-286-specific T cells)[17–19]. This pattern of overactivation leading to cell death could potentially function as a mechanism to prevent excessive damage to the host. At the same time, LCMV Clone 13 engineered to express a lower-affinity GP33 variant antigen generates an antigen-specific CD8+ T cell population with reduced exhaustion-associated features, suggesting that T cell exhaustion may be best elicited by antigens of intermediate affinity[12].

To maintain homeostasis, high affinity autoreactive CD8+ T cells that escape central tolerance normally undergo clonal deletion in the periphery, in a process known as cross-tolerization[20,21]. In autoimmunity, CD8+ T cells undergo antigen-driven expansion into oligoclonal populations that have been implicated in the pathogenesis of multiple autoimmune diseases; for the purposes of this review, we will focus on multiple sclerosis (MS)[22,23], Type 1 Diabetes (T1D)[24,25], and vitiligo[26–30]. In the nonobese diabetic (NOD) model of T1D, high affinity CD8+ T cells mediate extensive tissue damage at the later stages of disease. At 3 weeks of age, NOD mice are disease-free and few low-affinity $IGRP_{206–214}$–specific CD8+ T cells infiltrate the pancreas. By 10–15 weeks of age, the pancreata of NOD mice exhibit overt insulitis and are heavily infiltrated with high-affinity NRP-V7-specific CD8+ T cells[31–33].

While a substantial amount of work has explored the evolution of high- versus low-affinity neoantigen-MHC interactions in cancer, less is known about the impact of differential peptide-MHC-TCR affinity. A recent study demonstrated that high affinity T cell clones can exert superior effector function in a mouse model of concurrent cancer and autoimmunity, using shared tumor/self-antigen[34]. Additionally, work from human clinical trials suggests that TCR clonality may have an important role in the response to checkpoint blockade therapies. CTLA-4 blockade has been shown to augment the repertoire diversity of tumor-infiltrating lymphocytes (TILs)[35,36]. For PD-1 blockade, early work suggested that response was associated with higher intratumor clonality[37]. More recent studies have demonstrated that PD-1 blockade drives the recruitment and expansion of novel TCR clones in the tumor microenvironment (TME), termed “clonal replacement”[38]. These findings suggest that tumor-specific T cells in the TME may possess limited reinvigoration ability, and that

response to PD-1 blockade may be due to T cell clones recruited to the tumor. Indeed, TCR sequencing paired with single-cell RNA-sequencing has revealed that expanded clonotypes in the tumor tend to be more exhausted/dysfunctional[38,39].

Functional profile

In an acute viral infection, CD8+ T cells are critical mediators of a protective immune response. Activated CD8+ T cells exert effector function through direct cytotoxicity (release of granzyme B and perforin) and cytokine production (effector cytokines like IFNγ and TNF, as well as proliferation-promoting cytokines like IL-2). After clearance of the infection, the responding CD8+ T cell population contracts, with a subset persisting as memory cells that are primed to regain effector function. Chronic viral infection, cancer, and autoimmunity all elicit aspects of CD8+ T cell functionality; exhausted CD8+ T cells in the former two contexts show clear deficits, while the relative effector and memory functionality of CD8+ T cells in autoimmunity is less clear.

Compared to CD8+ T cells generated in response to acute infection such as the Armstrong strain of LCMV, exhausted antigen-specific CD8+ T cells generated by LCMV Clone 13 are characterized by poor proliferation and persistence, in addition to reduced cytotoxicity and cytokine production (less IFNγ, minimal TNF, and almost absent IL-2)[17,18,40,41]. Exhausted CD8+ T cells also are distinguished by persistent high expression of multiple co-inhibitory receptors such as PD-1, LAG3, 2B4, TIM3, and CTLA-4[42–45]; this is distinct from the transient expression of co-inhibitory receptors elicited by an acute infection. These key features have been recapitulated in tumor-specific CD8+ T cells isolated from the TME[46–50]. T cell dysfunction begins to develop early in chronic viral infection and cancer, and progressively becomes more severe. By day 8, responding CD8+ T cells in mice infected with LCMV Clone 13 are less cytotoxic than those from LCMV Armstrong[17] and scRNA-Seq segregates T cells from mice infected with Armstrong or Clone 13 into distinct clusters[1]. Cytokine production (IFNγ, TNF) is further reduced in CD8+ T cells by day 30 of LCMV Clone 13 compared to day 7[17]. Eventually, exhausted T cells lose the capacity to regain functionality: CD8+ T cells isolated from early LCMV Clone 13 infection[13] or tumors[51] are better able to respond after transfer to an antigen-matched acute infection, compared to cells transferred at a later time point.

Despite their relatively poor functionality compared to memory and effector cells, exhausted CD8+ T cells still play a critical role in chronic infection and cancer. In a non-human primate model of HIV infection, depletion of CD8+ T cells leads to an increase in plasma viremia, even at 1–3 years of infection[52,53]. Similarly, infection of β−2-microglobulin deficient mice (which lack CD8+ T cells) results in higher LCMV Clone 13 viral titers[54]. In cancer, enhanced tumor growth is seen with CD8 depletion in mouse tumor models, and in human patients, CD8+ T cell infiltration correlates positively with prognosis (disease-free survival, overall survival) in multiple tumor types[55,56]. Furthermore, studies of the TME suggest that features of exhaustion are predictive of relatively more functionality (compared to non-exhausted, likely non-responding cells). In human tumors, tumor infiltrating lymphocytes (TILs) expressing markers of exhaustion (PD-1, LAG3, TIM3) are more likely to express IFNγ[47]. By scRNA-Seq, a “dysfunctional” cluster of TILs

from human melanoma can be defined, characterized by high expression of exhaustion-associated markers *TIGIT*, *PDCD1*, and *LAG3*; tumors with a higher proportion of these "dysfunctional" CD8+ T cells are associated with greater TIL *ex vivo* tumor reactivity and greater clonality[39]. While exhausted CD8+ T cells have reduced function compared to those elicited by an acute infection, these data suggest that it is specifically the exhausted CD8+ T cells that are exerting residual control over tumor growth.

In contrast to the limited pathological effect of responding CD8+ T cells on tissues in chronic infection and cancer, T cells effectively damage host tissues in various autoimmune diseases. Pathogenic CD8+ T cells in these contexts are primarily effector, effector memory, and resident memory cells, and are critical for disease initiation and progression. The prevalence of CD69+CD103+ resident memory T cells in the epidermis is positively correlated with active vitiligo[57,58] and these cells can recruit cytotoxic effector T cells from the circulation important for sustained disease[59]. In T1D patients, 50–60% of antigen-specific CD8+ T cells that infiltrate the exocrine pancreas are of a memory (CD45RO+) phenotype[60,61]. In MS, both effector and effector memory CD45RA+/−CCR7− CD8+ T cells are associated with active disease[62–64]. Consistently across these autoimmune diseases, pathogenic CD8+ T cells express high levels of effector molecules (granzyme B, perforin, IFNγ, TNF) *in vivo* or upon *ex vivo* restimulation. This is apparent in autoreactive CD8+ T cells that mediate vitiligo[28,58,65], NOD diabetes[25,32,66], MS[64], or central nervous system (CNS) inflammation in mouse models[22,67,68]. Encephalitogenic and diabetogenic CD8+ T cells also upregulate a combination of co-inhibitory receptors, including LAG3, TIM3, and PD-1[22,61,67–70]. In contrast, pathogenic T cells in vitiligo express relatively low levels of PD-1 and LAG3[28,58,65]. Whereas exhausted T cells in chronic viral infection are independent of IL-7/IL-15 for persistence, pathogenic effector/memory T cells in autoimmune diabetes can be inhibited by blockade of IL-7[71,72] or IL-15[73]. IL-15 blockade also can inhibit vitiligo[28] and IL-15 has been implicated in promoting CD8+ T cell effector function in MS[74,75].

Despite their maintenance of effector function, there is some evidence that autoreactive CD8+ T cells also exhibit features of exhaustion in autoimmunity. Transcriptional profiling has identified a subset of CD8+ T cells that infiltrate the pancreatic islets of T1D patients and aged NOD mice with aspects of T cell exhaustion; these exhausted-like CD8+ T cells expand as mice age and progress towards diabetes while a cytotoxic effector subset does not[69,70]. The prevalence and importance of exhausted T cells in autoimmunity remains unclear—however, exhausted autoreactive T cells could represent a suppressed but not halted autoimmune disease process. Activated islet-specific CD8+ memory T cells were prevalent in subjects with T1D who experienced rapid loss of the antigen C-peptide; in contrast, slow disease progression was associated with an exhaustion-like profile, with expression of multiple inhibitory receptors, limited cytokine production, and reduced proliferative capacity[69]. A transcriptional profile of T cell exhaustion also correlated with improved prognosis in patients with vasculitis, Crohn's disease, and Systemic Lupus Erythematosus (SLE)[76,77]. This relationship between exhaustion-associated properties in autoreactive CD8+ T cells and the rate of autoimmune disease progression make these phenotypes attractive putative biomarkers of disease trajectory and potential targets for therapeutic intervention.

Heterogeneity within dysfunctional T cell populations

With the expansion of single-cell profiling technologies in immunology, from flow cytometry to single-cell RNA-sequencing and beyond, the fields of exhaustion and autoimmunity are now equipped to better understand the diversity of CD8+ T cell states across these contexts. In chronic viral infection and cancer, it has recently been appreciated that there are multiple subsets of exhausted cells with distinct functionalities (reviewed in more detail here[7,78,79]). Several exhausted subset frameworks have been described[39,80–82]; here we focus on one framework with substantial coherence across cancer and chronic viral infection in both mice and humans. A similar dissection of CD8+ T cell heterogeneity in autoimmunity is underway, with some clear parallels to exhaustion-defined subsets.

A “progenitor” exhausted subset was first described in LCMV Clone 13 based on intermediate PD-1 expression and high CD44 expression; this was associated with improved persistence, and more expansion in response to anti-PD-L1 treatment[83] (Table 1). This subset has also been identified in the TME[11,84]. The key feature of progenitor-exhausted cells is their self-renewal capacity[85]; they can also differentiate into a “terminal” exhausted subset in an antigen-dependent process[11,84]. The transcription factor TCF1 (encoded by the gene *TCF7*), is required for progenitor-exhausted CD8+ T cells. TCF1 has also been shown to play an important role in central memory[86,87] and stem cell memory CD8+ T cells[88], suggesting it may function to promote self-renewal and persistence. This subset plays an integral role in control of infection or tumor: TCF1-deficient mice exhibit higher viral titers and accelerated tumor growth, while transferring progenitor-exhausted cells (compared to terminal-exhausted cells) enhances tumor control[11,84,89,90]. Importantly, this stem-like population provides the proliferative burst after PD-1 pathway blockade during chronic viral infection[11,83–85]. While a “progenitor exhausted-like” subset of human memory CD8+ T cells has recently been identified[91], it is important to note that progenitor-exhausted CD8+ T cells are distinct from memory cells: they express high levels of the exhaustion-associated transcription factor TOX[92], and are more similar to terminal-exhausted cells than memory cells by chromatin accessibility profiling[11].

The second major subset, terminal-exhausted cells, has more direct cytotoxic function[11], but persists poorly and does not proliferate in response to PD-1 blockade nor tumor vaccination[84]. Within this TIM3+ subset, additional subpopulations have recently been described: CX3CR1+ “transitory” cells with greater proliferation, cytokine production and cytotoxic potential, which can develop into CD101+ “terminal” cells with the least functionality and poorest survival[93,94]. This evolving subset landscape highlights the complex role that exhausted CD8+ T cells play: balancing effector function with stem-like function for long-term pathogen/tumor control.

Subsets of stem-like autoreactive CD8+ T cells that share some features with progenitor-exhausted T cells have been identified in autoimmunity, but there is no current consensus on markers to define these populations. In NOD mice, scRNA-Seq analysis revealed terminal effector-like (*Pdcd1*^{hi}*Lag3*^{hi}) and stem-like (*Tcf7*^{hi}*Tox*^{hi}) CD8+ T cell populations, both of which showed differential expression of an exhaustion-associated gene signature by GSEA[70]. There is also evidence of non-exhausted stem-like T cells within autoimmune

effector and memory effector CD8+ T cell populations. Using dextramers for various beta-islet antigens, antigen-specific CD8+ T cells can be identified in the blood of both healthy individuals and patients with T1D. In patients with T1D, these autoreactive CD8+ T cells are primarily a stem-like effector memory phenotype characterized by the expression of CD95 (CD45RA+CD45RO−CCR7+CD95+), while the majority of beta-islet-specific T cells remain naive (CD45RA+CD45RO−CCR7+CD95−) in the blood of healthy individuals[24,95,96]. Considering autoimmune-related conditions more broadly, non-naive but stem-like CD8+ T cells (CD45RO−CD45RA+CCR7+CD27+CD95+) are elevated in the blood of patients with aplastic anemia, autoimmune uveitis, and sickle cell disease[97]. These findings suggest that stem-like subsets of autoreactive CD8+ T cells present in the blood may be important for the sustained production of more terminal populations that mediate cytotoxic destruction in autoimmune organs. Further studies are required to elucidate the significance of stem-like subsets of CD8+ T cells in autoimmune disease and the potential for durable therapeutic strategies targeting this population.

PD-1/PD-L1 pathway

The PD-1/PD-L1 pathway is a critical immunoregulatory pathway initially described in the context of autoimmunity[98–100] that is also important in chronic viral infection and cancer. The PD-1 co-inhibitory receptor can be expressed on various hematopoietic cells including activated CD8+ T cells. Binding of PD-1 to its ligands, PD-L1 or PD-L2, reduces downstream TCR and CD28 signaling and effector T cell functionality[101]. Physiologically, this pathway negatively regulates T cell activation, mediates T cell tolerance and controls resolution of inflammation. PD-L1 expression by host tissues is a protective mechanism against damage directed towards healthy tissues. For example, PD-L1 is upregulated by beta-islets in the context of T1D[102], as well as by astrocytes and microglia/macrophages in the brains of MS patients[103,104]. This pathway's critical importance is apparent in PD-1/PD-L1 deficient mice, which develop severe spontaneous autoimmunity on autoimmune-prone backgrounds (NOD, MRL)[105,106]. In addition to protecting against autoimmunity due to its effects on autoreactive CD8+ T cells[107], the PD-1 pathway is critical for preventing excess damage in the context of chronic viral infection: LCMV Clone 13 infection is lethal in PD-1-pathway deficient mice at doses that are non-lethal for wildtype mice[43]. This is due to unchecked perforin-mediated destruction of endothelial cells leading to circulatory collapse[108].

Whereas PD-1 is transiently expressed on CD8+ T cells during the early response to acute viral infection and downregulated upon viral clearance, chronic infection and cancer cause high, sustained PD-1 expression on exhausted CD8+ T cells[43]. Early in chronic infection, expression is highest on TCF1+ progenitor-exhausted CD8+ T cells[109], while later, expression becomes highest on TIM3+ cells[11,85], particularly on the CD101+ terminal-exhausted subset[93]. These temporal changes in relative PD-1-expression indicate that PD-1 may have distinct roles at various stages of the development of T cell exhaustion.

High levels of PD-1 expression contribute to the characteristic dysfunction seen in exhausted CD8+ T cells. PD-1 negatively regulates proliferation and effector function (cytokine production, cytotoxicity) at the functional and transcriptional level[43,110]. PD-1 blockade

can transiently ameliorate these functional deficits[43,110], but it does not enhance memory potential of the exhausted CD8+ T cells[110]. Interestingly, while PD-1 deficient CD8+ T cells show enhanced proliferation early during the course of chronic viral infection, this effect is accompanied by increased apoptosis and more profound deficits in cytokine production[111], illustrating a critical role for PD-1 in the maintenance of T cell functionality and persistence. During acute infection, PD-1 also plays a role in proper memory formation[112,113], highlighting the importance of early PD-1 signals in the long-term maintenance of T cell populations.

Therapeutic modulation of PD-1 was first demonstrated in the context of LCMV Clone 13: blockade of the PD-1 pathway resulted in lower viral titers across tissues[43]. Antibody-based PD-1 pathway blockade is now an FDA-approved therapy for many tumor types[114] and has a demonstrated positive effect on numerous clinical indicators, particularly long-term survival[115]. Murine tumor models have demonstrated that PD-1 blockade specifically promotes proliferation and differentiation from progenitor-exhausted to terminal-exhausted T cells[11]. Correspondingly, in human tumors, a higher proportion of progenitor-exhausted cells was associated with a greater likelihood of response to checkpoint blockade[116]. While PD-1 blockade therapies have been successful in multiple tumor types, immune-mediated side-effects, termed immune-related adverse events (irAEs), can limit their efficacy. There are many unresolved questions about how irAEs arise and their connection to classical autoimmunity (reviewed in Box 3).

Given the role of the PD-1/PD-L1 pathway in mediating T cell tolerance, the induction of tolerance through administration of checkpoint agonists is currently being investigated for the treatment of autoimmune disease. PD-1 agonism with PD-L1 has shown efficacy in a variety of preclinical animal models, including colitis mediated by dextran sulfate sodium (DSS) or adoptive T cell transfer[117], psoriasis[118], EAE[119], lupus[120], and collagen-induced arthritis[121].

Regulation of T cell dysfunction by TOX

While many transcription factors play important roles in CD8+ T cell biology, TOX has recently become of particular interest to the field of exhaustion. TOX is highly expressed by exhausted CD8+ T cells in chronic infection and cancer, particularly at later time points, but largely absent from naive CD8+ T cells and acute infection-induced CD8+ T cell populations (effector and memory)[9,92,122,123]. Overexpression of TOX is sufficient to recapitulate a significant fraction of exhaustion-associated gene expression changes, both in naive CD8+ T cells and in fibroblasts[9,122]. Most strikingly, TOX deficient T cells are unable to persist in chronic infection and tumor models[9,122], suggesting that exhaustion is an adaptation for T cells to survive in an environment of chronic antigen stimulation.

In the context of autoimmunity, TOX may play a role in controlling the pathogenicity and maintenance of self-reactive CD8+ T cells. In murine models of CNS autoimmunity, TOX expression is increased in encephalitogenic CD8+ T cells, and TOX-deficient T cells exhibit reduced encephalitogenic potential, cytokine production, and persistence[67,68]. TOX is also expressed by a subset of T cells in the pancreas of aged NOD mice, and the TOX locus

shows increased chromatin accessibility within a subset of beta-islet-cell specific CD8+ T cells from humans with T1D[70,96]. Further work is needed to understand the signals driving TOX expression in autoimmunity and the disease-modifying impact that TOX+ subsets confer. It remains unclear whether TOX (1) promotes T cell persistence and maintenance of cytotoxic function, or (2) promotes the development of exhaustion to reduce cytotoxicity and maintain tolerance.

Epigenetic regulation of CD8+ T cell dysfunction

Along with the expression of transcription factors, a cell regulates *when* and *how much* of a gene transcript is expressed through epigenetic regulatory mechanisms. There are many types of epigenetic regulation, but thus far CD8+ T cells in exhaustion and autoimmunity have primarily been characterized in terms of chromatin accessibility, DNA methylation, and histone modifications that affect the rate of transcription of genes.

Recent advances in epigenomic profiling have revealed that exhausted CD8+ T cells in chronic infection and cancer have a distinct epigenetic landscape compared to other CD8+ T cell states. In both LCMV Clone 13 and tumor contexts, progenitor- and terminal-exhausted subsets show significant chromatin accessibility differences from naive, effector, and memory populations[11,51,110,124,125]. These findings are conserved in human disease contexts: exhaustion-associated chromatin accessible regions (ChARs) from human CD8+ T cells in HIV and HCV significantly overlap with orthologous regions from T cells isolated from LCMV Clone 13[124]. This distinct epigenetic profile develops early: exhausted versus effector CD8+ T cell chromatin accessibility patterns diverge by day 7 of LCMV Clone 13 infection[110,124], as do H3K27 acetylation patterns[1], a histone modification marking active promoters/enhancers. These epigenetic changes have transcriptional consequences: multiple regions near the *Tox* locus show significant increases in chromatin accessibility as well as H3K27 acetylation, consistent with increased *Tox* expression specifically in exhausted cells.

DNA methylation profiling also reveals distinctions between exhausted CD8+ cells and naive, effector, or memory T cells. While naive CD8+ T cells are highly methylated, corresponding to a state of relative transcriptional quiescence, many regions become demethylated in the effector state and remain demethylated in memory CD8+ T cells. In contrast, a significant portion of effector demethylated regions become remethylated in exhausted cells, which could represent a mechanism to reduce gene expression and consequent effector function in chronic stimulation contexts[126]. Interestingly, the inverse of this pattern occurs at regulatory regions near the PD-1 locus (CR-B, CR-C); these regions become remethylated in memory T cells, but remain demethylated in exhausted T cells from LCMV Clone 13, consistent with high sustained PD-1 expression. This pattern is also seen at orthologous loci in human CD8+ T cells when comparing cells in chronic viral infections (Epstein-Barr virus and cytomegalovirus-specific) to memory cells (Yellow fever virus-specific)[127].

Recent studies suggest that both encephalitogenic and diabetogenic CD8+ T cells also exhibit distinct epigenetic profiles compared to naive, effector, or memory T cells. Beta-islet-specific CD8+ T cells were tetramer-sorted from the blood of T1D

patients for whole-genome methylation analysis. These profiles were compared with the methylation profiles of sorted naive (CCR7+CD45RO−CD45RA+CD95−), stem-cell memory (CCR7+CD45RO−CD95+), central memory (CCR7+CD45RO+), and effector memory (CCR7−CD45RO+) from T1D patients and healthy controls, as well as exhausted tetramer+ T cells from the blood of HIV patients. The methylation profile of tetramer+ T1D T cells most closely resembled stem-cell memory cells, with both naive-associated and effector-associated features including demethylation at *PRF1, GZMK, IFNG,* and *TBX21.* Single-cell ATAC-Seq analysis confirmed that this hybrid epigenetic profile coexists in a subset of T cells. In the NOD T1D mouse model, the methylation profile of beta-islet-specific CD8+ T cells varied between tissue compartments: the epigenetic profile of tetramer+ T cells in the pancreas was distinct from effector-memory T cells and most comparable to exhausted P14 T cells isolated from mice with LCMV Clone 13 infection, while splenic tetramer+ cells shared epigenetic characteristics of both effector-memory and central-memory T cells[96]. This location-based difference mirrors a bias seen in exhausted subsets from chronic viral infection: progenitor-exhausted cells (which relatively enrich for memory signatures) tend to reside in lymphoid organs, while terminal-exhausted cells tend to reside in non-lymphoid tissues[85]. Chromatin accessibility profiling has also revealed epigenetic features of exhaustion in encephalitogenic CD8+ T cells in a murine model of CNS autoimmunity. In mice expressing the LCMV glycoprotein in myelin-forming CNS cells (MOG-GP mice), infection with an attenuated LCMV variant initiates antigen-specific CD8+ T cell-mediated neurologic disease, whereas in WT mice, it causes a transient choriomeningitis that resolves by day 10. At day 21 post-infection, antigen-specific CD8+ T cells from the CNS of autoimmune MOG-GP mice, compared to matched cells from infected wild-type mice, exhibited increased chromatin accessibility at a majority of exhaustion-associated ChARs[68]. These data suggest that autoreactive CD8+ T cells exist in a stem-like state in the blood and lymphoid organs, but develop epigenetic features of exhaustion upon infiltration into the target organ.

Beyond deepening our understanding of CD8+ T cell state and heterogeneity, epigenetic profiling also has the potential to reveal mechanisms of existing therapies, as well as offer new therapeutic strategies. Despite its important clinical utility, PD-1 blockade does not alter the exhausted epigenetic profile of T cells, as demonstrated by chromatin accessibility profiling[10,11,110] and methylation profiling[126]. This finding reinforces our understanding of the mechanism of action of PD-1 blockade as promoting proliferation and differentiation from progenitor- to terminal-exhausted. Reversing exhaustion-associated epigenetic changes may represent a new class of therapeutics to augment T cell functionality in cancer and chronic viral infection. Consistent with this concept, genetic or pharmacologic inhibition of exhaustion-associated enhanced remethylation can increase CD8+ T cell numbers as well as effector function. This treatment synergizes with anti-PD-L1 blockade to further improve control of LCMV Clone 13 infection and tumor growth[126].

Conclusion and future perspectives

In an acute infection, a productive CD8+ T cell response is generated, characterized by appropriate effector function (leading to clearance of the pathogen) followed by relative CD8+ T cell quiescence in the memory state, with minimal damage to self-tissue. In

comparison, CD8+ T cells develop dysfunctional states in response to chronic antigen stimulation in the context of cancer, chronic infection, and autoimmunity. Autoimmunity and chronic viral infection/cancer are traditionally exemplified as the pathological consequences at opposite ends of a spectrum of CD8+ T cell functionality (Figure 1). Despite the distinct consequences of reduced T cell effector function in chronic infection and cancer compared to sustained effector function in the context of autoimmunity, CD8+ T cells exhibit some key similarities across these conditions (Figure 2). Notably, both mouse and human CD8+ T cells in these different contexts share some molecular programming at both the transcriptional and epigenetic levels. Recent work suggests that exhaustion-like states may be found in an even broader set of pathological states, including infections like SARS-CoV2[128].

While much has been learned about the dysfunctional states in chronic infection, cancer, and autoimmunity, there are still critical gaps in our knowledge. TCR affinity and self-antigen specific T cell responses are well studied in the context of autoimmunity, but have been less well studied in the context of cancer, with most work focused on neoantigen responses. Self-antigen responses may have important potential relevance to checkpoint-therapy induced irAEs. Moreover, our understanding of the function of non-specific “bystander” CD8+ T cells in contexts of chronic antigen stimulation is limited. Intriguingly, “bystander” CD8+ T cells that do not recognize the relevant self-antigen have the potential to inhibit an autoimmune response[129]. Although non-reactive bystander cells have also been identified in tumors[10,130,131], their role in anti-tumor responses is unknown.

Likewise, while exhausted subsets have been defined epigenetically, transcriptionally, and functionally in cancer and chronic viral infection, CD8+ T cell heterogeneity on these three levels remains incompletely explored in autoimmunity. Further research using clonotype, epigenetic, and single-cell analyses is needed to define the presence and significance of exhausted and stem-like T cell subsets in a broader set of autoimmune diseases. It is not yet clear whether exhausted autoreactive CD8+ T cells in T1D remain important effectors of tissue destruction, or whether a progenitor-exhausted subset might be responsible for the sustained production of terminal-exhausted effectors. Alternatively, exhausted autoreactive CD8+ T cells might represent a minority population that does not effectively mediate tissue destruction in the autoimmune pancreas. If so, it could be desirable to induce a more wide-spread, profound, and durable state of exhaustion with strategies such as PD-1 agonism to ameliorate autoimmune disease.

In contexts of exhaustion, we are still working to disentangle what constitutes features of dysfunction versus preservation of residual function. Exhausted CD8+ T cells in chronic infection and cancer are regulated by a set of complex mechanisms intended to maintain homeostasis, which includes protection against pathogens and cancer as well as protection from aberrant self-destruction. Genetic deletion of dysfunction-associated molecules PD-1 and TOX in exhausted contexts results in a transient increase in proliferation/effector function, but dramatically reduces T cell persistence. Conversely, loss of effector-associated transcription factors (IRF4) promotes the maintenance of T cell functionality and persistence at the cost of reduced cell numbers[132]. These findings suggest that T cell exhaustion may

represent a trade-off between proliferation and cellular persistence/maintenance of function in a chronic immunostimulatory environment.

In all three contexts, epigenetics is an area that requires a deeper understanding of the biology and consequent therapeutic implications. As we come to appreciate the importance of multiple subsets with distinct roles in development and maintenance of exhaustion and autoimmune disease processes, more work is also needed to understand the epigenetic landscape of dysfunction over time and at the single-cell level. Such epigenetic profiling studies may provide mechanistic insight into the capabilities of exhausted and autoreactive T cell subpopulations. Because epigenetic modulation provides finer tuning of gene expression, modulating dysfunction-associated epigenetic features may permit the amelioration of dysfunctional aspects without perturbing appropriate effector and memory functionality. More broadly, further investigation into the similarities and differences in CD8+ T cell fate decisions and dysfunction across autoimmunity, chronic infection, and cancer will provide critical insight into the remarkably diverse roles of T cells in health and disease, and reveal new therapeutic strategies.

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Box 1:

CD4+ T cell help

CD4+ T cells can “help” CD8+ T cells by upregulating B7 costimulatory molecule expression on APCs to augment T cell activation, and by secreting multiple immunoregulatory cytokines including IL-2, which supports CD8+ T cell proliferation. In the context of chronic inflammation, CD4+ T cells play a critical role in modulating T cell dysfunction.

In autoimmunity, CD4+ T cells are considered the primary mediators of disease. In T cells from a varied set of human autoimmune diseases, a transcriptional signature of CD4+ T cell help was inversely correlated with clinical outcome, suggesting an important pathogenic role. This transcriptional signature of CD4+ T cell help was also inversely correlated with a signature of CD8+ T cell exhaustion[77]. Indeed, CD4+ T cell depletion in the setting of chronic viral infection results in more severe CD8+ T cell dysfunctionality (including reduced proliferation, cytokine production, and cytotoxicity), corresponding to extended viral persistence[18,133]. The CX3CR1+ CD8+ T cell population, which relatively maintains effector function in exhaustion, is particularly reduced upon depletion of CD4+ T cells in LCMV Clone 13 infection[94] without significant depletion of the TCF1+ progenitor-exhausted population[94,134]. CD4+ T cell help can also play an important role in supporting CD8+ T cell driven anti-tumor immunity. In one assessment of tumor vaccination strategies, inclusion of CD4 epitopes presented on MHC Class II improved CD8+ T cell recruitment to the microenvironment; this effect appeared to be mediated by CD4+ modulation of dendritic cells[135]. Similarly, enforced cancer cell expression of an MHC Class II predicted epitope enhanced the number and functionality of CD8+ T cells responding to a co-expressed MHC Class I predicted epitope[136].

Together, these findings highlight the critical role of CD4+ T cells in supporting CD8+ T cell functionality across chronic infection, cancer, and autoimmunity.

Box 2:

Dysfunction of CD4+ T cells in chronic disease

T cell dysfunction is not limited to CD8+ T cells—various subsets of CD4+ T cells (both FoxP3− conventional and FoxP3+ regulatory) develop altered functional capacity in the context of chronic viral infection, cancer, and autoimmunity. Conventional CD4+FoxP3− T cells (Tcon) subsets, including Th1, Th2, and Th17, secrete cytokines that support the activation and maintenance of CD8+ T cells (see box 1: CD4+ T cell help) and B cell production of antibodies. Tcon are also critical for the formation of tertiary lymphoid structures associated with sustained T cell responses in chronic infection, cancer, and autoimmunity[137]. Similar to CD8+ T cells, Tcon in chronic infection and cancer can develop features of T cell exhaustion, including upregulation of multiple co-inhibitory receptors such as *Pdcd1*, *Havcr2*, and *Ctla4* and poor cytokine production[138–140] resulting from chronic antigen stimulation[141]. This may be beneficial in autoimmunity, as a transcriptional signature of Tcon exhaustion is positively correlated with reduced severity of lupus nephritis[142], T1D, and optic neuritis[76]. A subset of IFNγ^{low} TCF1+ colitogenic Tcon with enhanced survival capacity and a gene signature comparable to progenitor-exhausted CD8+ T cells may be responsible for the chronicity of disease[143]. Stem-like features in Tcon are associated with expression of TCF1 which may serve as a marker of progenitor-exhausted Tcon similarly to progenitor-exhausted CD8+ T cells[143,144].

CD4+ FoxP3+ Tregs have a critical role in the maintenance of peripheral tolerance by suppressing effector responses towards host tissues. Dysfunctional Tregs have been described with an IFNγ+ Th1-like phenotype and reduced suppressive capacity despite maintaining high FoxP3 expression[145–147], and have been identified in autoimmunity[147,148], chronic viral infection (HCV)[149,150], and cancer[145,146]. These aberrant Tregs show canonical features of T cell exhaustion including impaired proliferative capacity[151] and enhanced co-inhibitory receptor expression (*PDCD1*, *HAVCR2*, *LAG3*)[145,147]. In particular, the PD-1/PD-L1 pathway has an important role in the progressive development of Treg dysfunction. In patients with chronic HCV infection, a higher fraction of intrahepatic PD-1^{hi} Tregs was correlated with lower viral burden and greater hepatic damage, consistent with reduced immune suppression[150]. In patients with glioblastoma multiforme (GBM), compared to Tregs in the peripheral blood, tumor-resident Tregs enriched for a transcriptional signature of exhaustion. Circulating PD-1^{hi} Tregs also displayed reduced suppressive capacity, a transcriptional profile of exhaustion, and produced IFNγ; treatment with PD-1 blockade skewed circulating Tregs towards the exhausted IFNγ+ phenotype[145]. Mice with PD-1 deficient Tregs are protected from both EAE and T1D due to their enhanced suppressive capacity[152]. These studies indicate that PD-1 on Tregs acts to constrain their suppressive capacity and may promote a dysfunctional phenotype characterized by a transcriptional profile of exhaustion and an IFNγ+ Th1-like phenotype.

In summary, like CD8+ T cells, CD4+ T cell subsets also exhibit altered phenotypes including features of exhaustion across cancer, chronic infection, and autoimmunity. Further integration of our knowledge of CD8+ and CD4+ T cell dysfunction will allow us

to better understand the complex mechanisms defining T cell states in both homeostasis and disease.

Box 3:

The role of CD8+ T cells in immune-related adverse events (irAEs)

Autoimmune-prone mouse models clearly demonstrate that disruption of the PD-1/PD-L1 and/or CTLA-4 pathways can promote the development of various autoimmune diseases such as spontaneous lymphoproliferative disorder[153], autoimmune diabetes[105,154], peripheral neuropathy[155], and myocarditis[105,156,157]. Thus it is not surprising that checkpoint blockade therapy in cancer patients can lead to autoimmune conditions known as "immune-related adverse events" (irAEs). There is immense diversity in the clinical presentation of irAEs, ranging from mild dermatological and gastrointestinal events to serious and life-threatening pulmonary, endocrine, cardiovascular, and neurological events with time of presentation from immediately after treatment to years later[158]. Even when mild or moderate, irAEs may require immune suppression and/or culminate in treatment discontinuation. Patients treated with PD-1/PD-L1 blockade tend to develop different irAEs than those treated with CTLA-4 blockade, while combination therapy increases both the incidence and severity of irAEs compared to either therapy alone[159–161].

CD8+ T cells are present in the lymphocytic infiltrates of affected organs in patients with checkpoint blockade-induced pneumonitis[162], dermatological conditions[163,164], myocarditis[165], and colitis[166,167]. A recent study used high-dimensional flow cytometry and scRNA-seq to characterize checkpoint inhibitor-induced colitis in patients. TCR sequencing revealed that a significant fraction of colitis-associated CD8+ T cells originated from tissue-resident memory cells, explaining the frequently early onset of colitis following the start of checkpoint therapy. Colitis was associated with major changes in T cell and myeloid populations, in particular a highly proliferative and cytotoxic CD8+ T cell population that expresses *PDCD1*, *LAG3*, *HAVCR2* (TIM3), and *CTLA4*[167].

Multiple mechanisms may contribute to the generation of irAEs, including a pre-existing susceptibility to autoimmunity, aberrant presentation of self-antigen in the tumor microenvironment, and generation of a new, cross-reactive repertoire to self post-therapy. Such autoreactive T cells may also be exerting anti-tumor effects directly towards self-antigens expressed by the tumor. Studies in mouse models of vitiligo and melanoma show that autoreactive CD8+ T cells towards shared self/tumor antigens such as gp100, MART1, or tyrosinase can also be found in the TME[168] and clonal expansion of these populations can confer protection against the development of cancer[65,169–172]. irAEs have been correlated with improved durable therapeutic responses to checkpoint blockade towards tumors with low mutational burden and few neoantigens[172–174], but further work is needed to understand if this association has predictive value. Vitiligo and paraneoplastic neurological syndromes are thought to occur largely due to CD8+ T cell responses towards self-antigens common to both the tumor and affected organ[65,168,170,175,176]. This mechanism is supported by findings that high affinity TCR transgenic CD8+ T cells are capable of mediating both anti-tumor effects and autoimmunity in mouse models exhibiting shared tumor and self-antigens[34,169,177].

While autoreactive CD8+ T cells likely have a significant role in eliciting irAEs, the mechanisms leading to irAE development remain elusive. A deeper understanding of the pathogenesis of irAEs may allow us to promote effective anti-tumor immunity without concomitant autoimmunity.

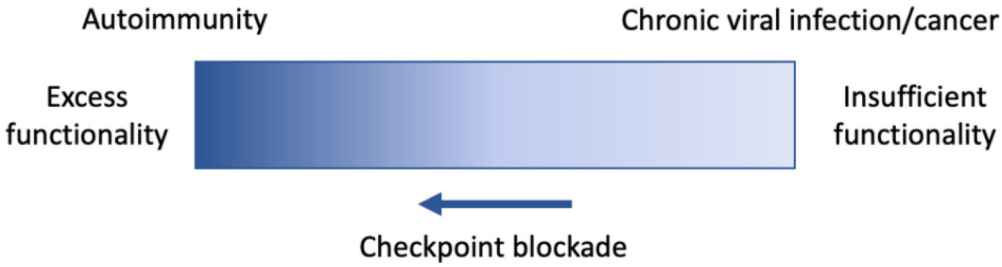

Figure 1: "Opposite ends of the spectrum" framework of CD8+ T cells in autoimmunity versus chronic viral infection.

CD8+ T cells in autoimmunity (left) exhibit excessive and inappropriate effector functionality. In some autoimmune diseases, CD8+ T cells cause extensive damage of self-tissue by overcoming numerous tolerance mechanisms that normally prevent and/or halt this reaction in homeostasis. In contrast, CD8+ T cells in chronic viral infection and cancer (right) become "exhausted" and exhibit reduced effector functionality, as compared to effector function elicited by acute infection. This dysfunction contributes to viral persistence and continued tumor growth. Blockade of immune checkpoints such as co-inhibitory receptors PD-1 and CTLA-4 can augment effector functionality of CD8+ T cells in both contexts, leading to better control of chronic viral infection/cancer and exacerbated autoimmunity.

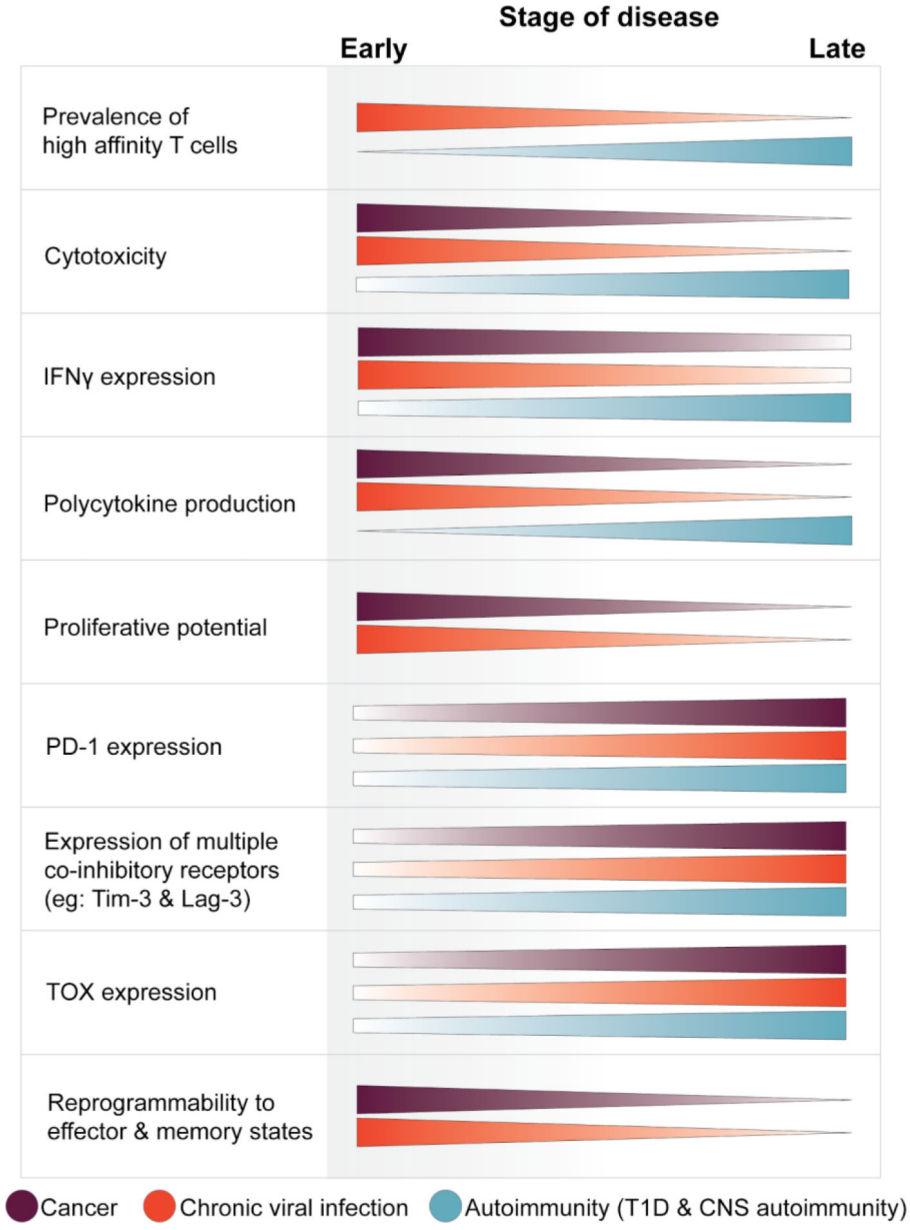

Figure 2: Temporal changes of CD8+ T cells in cancer, chronic viral infection, and autoimmunity.

Antigen-specific CD8+ T cells progressively develop distinct phenotypic changes in the context of chronic stimulation in disease. The phenotypic changes described for bulk autoreactive CD8+ T cells are generalized from various studies of diabetogenic and encephalitogenic T cells within the affected organ of mouse models. The changes in T cell properties are described as disease progresses from an early to late time point, corresponding to approximately: day 7 and day 30–60 of LCMV Clone 13 infection; TILs in newly developing tumors compared to TILs in late-stage tumors; early NOD insulitis at 3–4 weeks of age to overt diabetes at 10–12 weeks; and early day 7 of CNS autoimmunity in MOG-GP

mice compared to day 28. Changes are described for cancer (purple), chronic viral infection (orange), and autoimmunity (cyan).

Table 1:

Heterogeneity in exhausted CD8+ T cells in chronic viral infection and cancer.

Disease	**Chronic Viral Infection**		**Cancer**	
Model	**LCMV Clone 13**	**HCV, HIV**	**Various**	**Various**
Species	**Mouse**	**Human**	**Mouse**	**Human**
Target antigens	Viral antigens	Viral antigens	Model antigen (e.g. OVA), neoantigen & self-antigen	Neoantigen & self-antigen
Key markers	Progenitor: TCF1, Slamf6, CXCR5 Transitory/ intermediate: TIM3, CX3CR1 Terminal: TIM3, CD101[80,85,89,93,94]	Progenitor: Tcf1+, CD127+ Terminal: Tcf1^{-}, CD127^{-}[16,177,178]	Progenitor: TCF1, Slamf6 Terminal: TIM3[11,84]	Progenitor: TCF1, CCR7, CXCR5 Terminal: CD39, TIM[11,84,116,179,180]
Functional properties	Progenitor: self-renewal, greatest persistence, differentiate into terminal populations Transitory: greatest proliferation, differentiate into terminal exhausted[85,89,93]	Progenitor: greater persistence after loss of antigen stimulation (DAA therapy), higher percentage associated with greater ex vivo expansion capacity[16,178]	Progenitor: self-renewal, greater persistence, greater proliferative capacity Terminal: greater proliferation, greater apoptosis[11,84]	Progenitor: greater persistence in vivo (in an ACT product) and ex vivo, give rise to terminal exhausted ex vivo[179–181]
Co-inhibitory receptor expression	Progenitor: least expression of PD-1, TIM3, LAG3, CD160, TIGIT Transitory/intermediate: intermediate expression of PD-1, TIGIT, high expression of TIM3 Terminal: greatest expression of PD-1, LAG3, CD160, TIGIT, high expression of TIM3[80,85,89,93]	Terminal: greater expression of PD-1, CD39, 2B4, TIGIT[16,177]	Terminal: greater expression of PD-1, TIM3, CD244, CD39, CD101, CTLA-4[11,84]	Terminal: greater expression of PD-1, TIM3, CD39, Lag3, CTLA-4[84,116,180]
CD8+ T cell response to PD-1 pathway blockade	Progenitor: respond to checkpoint blockade through enhanced proliferation and differentiation to transitory, terminal exhausted[83,85,89]	?	Progenitor: required for response to checkpoint blockade, respond through enhanced proliferation and differentiation to terminal exhausted[11,81]	More progenitor-exhausted cells: greater response to checkpoint blockade and PFS[11,116]
Cytokine production	Progenitor: greatest IFNγ, TNF, IL-2 Transitory/ intermediate: intermediate IFNγ Terminal: least IFNγ[85,93,94]	More progenitor-exhausted cells: greater polycytokine co-production (TNF and IFNγ)[177]	Progenitor: greater TNF, greater polycytokine co-production Terminal: greater IFNγ[11,84]	Progenitor: greater TNF, IL-2, polycytokine co-production Terminal: greater IFNγ[180]
Cytotoxicity	Progenitor: least Gzmb Transitory/intermediate: greatest Gzmb Terminal: intermediate Gzmb[85,89,93,94]	Terminal: greater expression of Gzmb[177,178]	Terminal: greater Gzmb expression, ex vivo target killing capacity[11,84]	Terminal: greater expression of Gzmb[116]

ACT, adoptive cell therapy; DAA, direct-acting antiviral; Gzmb, Granzyme B; PFS, progression-free survival.