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CD8 T Cell Exhaustion During Chronic Viral Infection and Cancer

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Abstract

Exhausted CD8 T (Tex) cells are a distinct cell lineage that arise during chronic infections and cancers in animal models and humans. Tex cells are characterized by progressive loss of effector functions, high and sustained inhibitory receptor expression, metabolic dysregulation, poor memory recall and homeostatic self-renewal, and distinct transcriptional and epigenetic programs. The ability to reinvigorate Tex cells through inhibitory receptor blockade, such as αPD-1, highlights the therapeutic potential of targeting this population. Emerging insights into the mechanisms of exhaustion are informing immunotherapies for cancer and chronic infections. However, like other immune cells, Tex cells are heterogeneous and include progenitor and terminal subsets with unique characteristics and responses to checkpoint blockade. Here, we review our current understanding of Tex cell biology, including the developmental paths, transcriptional and epigenetic features,

and cell intrinsic and extrinsic factors contributing to exhaustion and how this knowledge may inform therapeutic targeting of Tex cells in chronic infections, autoimmunity, and cancer.

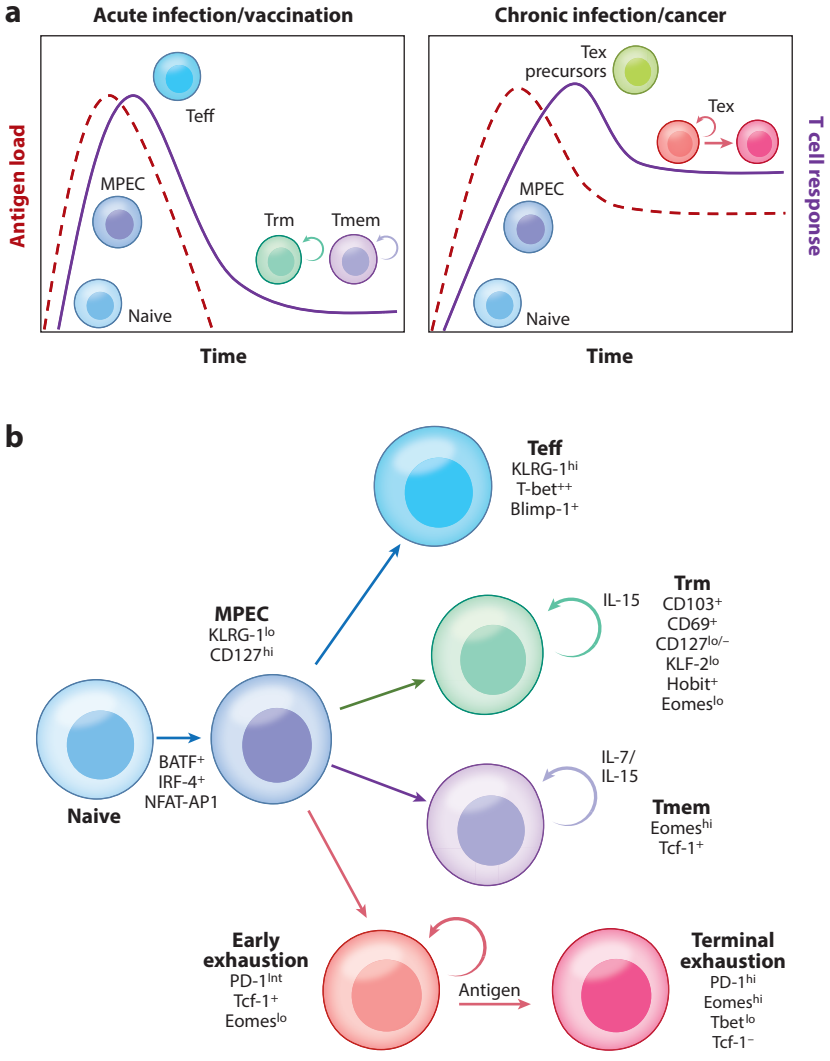
1. INTRODUCTION

The concept of immune exhaustion can be traced to experiments in the 1960s to 1970s using high doses of antigen exposure and describing depletion or exhaustion of a memory immune response (1, 2). These early studies on immune refractory states often focused on B cell responses and were performed before technological advances allowed the detailed investigation of features of cellular dysfunction. In the 1990s the advent of T cell receptor (TCR) transgenic technology and MHC tetramers allowed a new examination of the consequences of high antigen burden on lymphocytes. Early use of lymphocytic choriomeningitis virus (LCMV)-specific TCR transgenic CD8 T cells revealed exhaustion of the virus-specific CD8 T cell response through the physical deletion of these cells during chronic infection (3). The concept of a persisting yet functionally compromised CD8 T cell, however, was first revealed by Zajac et al. (4) and Gallimore et al. (5), who identified CD8 T cells responding to chronic LCMV infection that remained present and detectable by tetramer staining throughout infection but were unable to efficiently elaborate effector functions. In the subsequent two decades, it has become apparent that exhausted T (Tex) cells are a unique immune cell type and that these cells have a central role in cancer, autoimmunity, and chronic infections. Moreover, these cells are now the targets of immunotherapies, such as PD-1 (programmed cell death 1) blockade, placing them at the center of a paradigm shift in the ability to target the immune system for therapeutic benefit.

During acute infection or following vaccination, naive CD8 T cells undergo robust proliferation and clonal expansion to differentiate into effector CD8 (Teff) cells that directly kill target cells and control infections (**Figure 1*a***). Teff differentiation is accompanied by transcriptional, epigenetic, and metabolic reprogramming as well as the acquisition of hallmark effector features such as the ability to produce cytokines and cytotoxic molecules. Following antigen clearance and resolution of inflammation, the majority of activated T cells die. A small subset, however, persists and differentiates into memory T (Tmem) cells. Tmem cells downregulate their effector program and acquire a stem cell–like ability to survive, independent of antigen, as long-lived cells that undergo slow homeostatic self-renewal driven by IL-7 and IL-15 (6).

In contrast to the development of functional Teff and Tmem cells following acute infections and vaccinations, during chronic infections and cancer where antigen stimulation persists, T cell memory fails to efficiently develop and T cells become exhausted (**Figure 1*a***). Tex cells are functionally distinct from Teff and Tmem cells and are characterized by the loss of effector functions, elevated and sustained expression of inhibitory receptors (IRs), altered epigenetic and transcriptional profiles, a distinct metabolic lifestyle, and the inability to transition to the quiescent, antigen-independent state characteristic of Tmem cells (7–9). Many key discoveries about T cell exhaustion have come from work in the LCMV infection model in mice. One advantage of the LCMV infection system is the availability of laboratory strains of LCMV that result in either acutely resolved infection (e.g., LCMV Armstrong, 53b or low doses of WE) or persistent chronic infection (e.g., docile, high doses of WE or clone 13) (10, 11). Although early studies first described T cell exhaustion during chronic LCMV infection (4, 5), it is clear that T cell exhaustion occurs in many other chronic viral infections, including HIV (12–15), hepatitis C virus (HCV) (16, 17), hepatitis B virus (HBV) (18), and other persisting infections (19), as well as autoimmune disorders (20, 21).

It is also now clear that T cell exhaustion has a major role in immune dysfunction in cancer (22, 23). Indeed, tumor-specific CD8 T cells display hallmarks of T cell exhaustion and dysfunction in mouse models (24, 25) and human cancers including, but not limited to, melanoma (26–30), chronic myeloid leukemia (24), ovarian cancer (31), non–small cell carcinoma (32, 33), Hodgkin lymphoma (34), and chronic lymphocytic leukemia (35, 36). Exhausted tumor-infiltrating lymphocytes (TILs) are characterized by high expression of IRs, poor effector functions, and a common transcriptional and epigenetic program (27–31, 33, 35, 37–44). T cell exhaustion in the context of cancer is discussed further throughout this review.



(*Caption appears on following page*)

Figure 1 (*Figure appears on preceding page*)

Model for T cell activation following antigen recognition. (*a*) T cell differentiation in response to acute infection or vaccination (*left*) or chronic infection or cancer (*right*). Upon activation driven by antigen recognition, naive CD8 T cells differentiate into MPECs and eventually fully differentiate into terminal Teff cells (acute/vaccination) to control peak antigen load or Tex precursor cells (chronic/cancer). During acute infection or vaccination, the majority of Teff cells die and remaining cells differentiate into Tmem cells that patrol different sites in the body or Trm cells that are retained in tissues and epithelial regions poised for reactivation following secondary antigen exposure. During chronic infection or cancer, Tex precursors are unable to fully clear antigen and gradually develop into early Tex cells that retain some proliferative potential. In the setting of persisting antigen, Tex progenitor cells differentiate into terminal Tex cells and in some cases can be eventually deleted entirely. (*b*) T cell fates and phenotypes. The initial activation of naive T cells by antigen is driven by transcription factors including BATF, IRF-4, and NFAT-AP1, and then differentiation of these activated cells into KLRG-1^{lo}CD127^{hi} MPECs occurs. MPECs can then follow multiple paths of differentiation depending on antigen levels and disease setting. Teff cells that form from MPECs are characterized by high KLRG-1 and effector functions, such as cytokine production, driven in part by the transcription factors T-bet and Blimp-1. Differentiation to Tmem cells occurs following antigen clearance in acute infections and vaccination, and self-renewing Tmem cells use the transcription factors Tcf-1 and Eomes. Trm cells do not circulate in the body but are rather retained in tissues and are characterized by high CD103 and CD69 expression and low CD127 expression. Additionally, resident memory cells are associated with low KLF-2 and expression of Hobit. Early exhausted cells form during chronic infections and cancer, where antigen persists. Early Tex cells are characterized by intermediate expression of PD-1 and low Eomes, with a role for Tcf-1 in a progenitor population. Early Tex cells give rise to terminally exhausted T cells that are characterized by high expression of PD-1 and Eomes and loss of Tcf-1 and loss of the ability to proliferate further upon additional antigen stimulation. Abbreviations: MPEC, memory precursor T cell; Teff, effector T cell; Tex, exhausted T cell; Tmem, memory T cell; Trm, resident memory T cell. Figure created with BioRender.

It was unclear for some time whether exhaustion could be reversed; however, in 2006, Barber et al. (45) demonstrated that blocking PD-1:PD-L1 interactions in vivo revitalized Tex cells, resulting in improved proliferation and function as well as enhanced control of chronic LCMV infection. Results from the LCMV system were rapidly extended to HIV, HCV, HBV, and other chronic viral infections (46). These studies were paralleled by similar studies in tumor models (47–54). Targeting and reinvigorating Tex cells using checkpoint blockade is likely a major mechanism for clinical response to cancer immunotherapy. The mechanisms of action of these therapies as well as their long-term efficacy, however, remain areas of intense investigation that will be aided by a better understanding of the underlying mechanisms of T cell exhaustion.

This review summarizes our current understanding of the developmental biology, molecular program, and functional characteristics of Tex cells, as well as the transcriptional and epigenetic changes associated with the development of this distinct state of T cell differentiation. Although exhaustion has been best studied for CD8 T cells, exhaustion can also occur in other immune cell populations including CD4 T cells (55), B cells (56), natural killer (NK) cells (57), and other immune cells (58). Although these cell types are also of considerable interest, detailed discussion is beyond the scope of this review. This review describes cellular and molecular characteristics of Tex cells and highlights our current understanding of how to reverse T cell exhaustion through immune therapies such as checkpoint blockade.

2. CELLULAR AND FUNCTIONAL FEATURES OF T CELL EXHAUSTION

Compared to Tmem or Teff cells, Tex cells represent a distinct population with a unique differentiation pathway, collection of phenotypes, functionality, and subset dynamics. Tex cells have operationally been defined by the following features: (*a*) progressive or hierarchical loss of effector

function and proliferative potential, (*b*) sustained and high expression of multiple IRs, (*c*) poor or altered response to homeostatic cytokines responsible for maintenance of Tmem cells, (*d*) skewed metabolism, (*e*) altered expression and use of transcription factors compared to functional Teff and Tmem cells, and (*f*) an epigenetic program distinct from that of Teff and Tmem cells. Individually, most of these features are insufficient to definitively identify Tex cells, though in many settings, high IR coexpression, low effector function, and transcription factor expression patterns can be used together. In addition, poor proliferative potential is often confused with lack of cell division. In vivo, a subset of Tex cells is actively dividing, but the progeny of these cells has low potential to undergo additional proliferation upon stimulation (59–62). Finally, recent epigenetic data have the potential to provide specific definitive markers of Tex cells that might be attractive therapeutic targets.

2.1. Progressive Loss of Effector Function

Severely exhausted CD8 T cells were originally described as cells that persist during chronic infection but are unable to efficiently produce IFN-γ (4, 5). Subsequent studies showed that Tex cells lose effector functions in a progressive and hierarchical pattern during chronic infection (63, 64). Loss of IL-2 production occurs early followed by defects in tumor necrosis factor (TNF) production (63, 64). Cytotoxicity can also be compromised at this stage of exhaustion. Defects in robust production of IFN-γ occur at more severe stages of exhaustion and correlate with terminally exhausted T cells (64–68). Tex cells may retain the ability to produce chemokines including Mip1α, Mip1β, and Rantes (69, 70) as well as IL-10 in some settings (55, 71). Additionally, even if actual cell killing is reduced, many Tex cells still retain the ability to degranulate (measured by CD107a) (64), and the more terminal subset of Tex cells (see below) retains slightly better capacity to kill targets (61, 72). The final stage of exhaustion can be physical deletion, a feature observed in some settings where antigen stimulation is high (4). In the LCMV system, deletion occurs for the D^bNP396- and K^bGP34-specific CD8 T cell responses, whereas responses to other epitopes such as D^bGP33 and D^bGP276 are physically preserved but dysfunctional (64, 68). Further, the severity of exhaustion often correlates with pathogen burden, CD4 T cell help, and/or the duration of infection; therefore, a range of exhausted patterns can occur, resulting in varying degrees of CD8 T cell dysfunction.

In general, the precise pattern of functionality of Tex cells may vary from disease to disease, perhaps related to pathogen burden, pattern and location of pathogen replication, and/or antigen expression, as well as the inflammatory environment. Indeed, different persisting chronic human viruses result in distinct phenotypes of virus-specific CD8 T cells (73). Moreover, recent high-dimensional cytometry studies have revealed approximately nine subtypes of Tex cells that likely reflect environmental modulation of the core program of exhaustion (33). These fingerprints of Tex cells might allow one to infer not only features of the Tex differentiation state but also potential immunotherapeutic opportunities to target subtypes of Tex cells with specific disease relevance.

2.2. Altered Responsiveness to Homeostatic Cytokines

A key property of Tmem cells is their ability to be maintained in an antigen-independent manner via the cytokines IL-7 and IL-15 (6, 74, 75). Following acutely resolved infection or vaccination, developing Tmem cells gradually upregulate IL-7Rα and IL-2/15Rβ and acquire the ability to undergo slow, steady homeostatic self-renewal that is essential for the quintessential Tmem property of long-term antigen-independent maintenance (76). During chronic infection, virus-specific Tex cells are unable to undergo IL-7- and IL-15-mediated homeostatic proliferation due to defects in the IL-7Rα and IL-2/15Rβ signaling pathways (4, 60, 77–81). Although IL-2/15Rβ signaling

promotes survival of developing Tex cells (82), loss of IL-7Rα expression and/or signaling (77, 83) and defects in the IL-15-sensing pathway occur in Tex cells, especially at later time points (84). Instead, Tex cells are maintained by persisting antigen signals that drive ongoing proliferation of a subset of Tex cells (60, 61). This ongoing Tex proliferation defines a proliferative hierarchy necessary for Tex maintenance. A progenitor pool of Tex cells gives rise to a proliferative event resulting in the formation of a numerically larger and extensively divided pool of terminal Tex cells (59, 61). However, although they have recently divided (and are often $Ki67^+$), these recently generated terminal Tex cells lose ability to respond to additional proliferative signals (61) and are nonresponsive to future stimulation (59). Long-term Tex maintenance in the context of chronic infection can be traced to a need for specific antigenic peptide stimulation in vivo driving this proliferative hierarchy, indicating that Tex cells have adopted a maintenance lifestyle that is dependent on continuous TCR stimulation (60, 77). In fact, when Tex cells are adoptively transferred into antigen-free mice, unlike Tmem cells, they fail to be efficiently maintained by homeostatic self-renewal, though a small subset of cells may persist (60, 77, 85). Altered memory homeostasis is also likely a feature of Tex cells in humans because in HIV and HCV infection many Tex cells are lost following antiviral therapy or viral escape, suggesting an antigen-dependent lifestyle, though a small subset of these cells may persist after viral cure or escape (86–89).

2.3. Sustained Expression of Inhibitory Receptors

A key hallmark of Tex cells is the increased and sustained expression of multiple IRs. The discovery that T cell exhaustion can be partially reversed by blocking the interaction of PD-1 with its ligand PD-L1 (45) has set in motion a wave of new discoveries and a deeper understanding of not only T cell exhaustion but also the mechanisms underlying inhibitory signaling. In the last decade, numerous IRs have been identified that can negatively regulate function, activation, or other properties of T cells and other leukocytes (**Table 1**). Among the most relevant for Tex cells is PD-1, which is discussed in further detail in Section 4. Normally, IRs are transiently expressed by Teff cells, where they function to attenuate T cell activation and help restrain immune function at the end of acute infections to limit immunopathology and/or autoimmunity. As antigen is cleared, IR expression decreases and subsequent resting Tmem cells often have low IR expression. During chronic infections and cancer, high IR expression is sustained after initial activation and developing Tex cells often express a more diverse array of IRs simultaneously than other activated T cells (33). Indeed, coexpression of multiple IRs is a common feature of Tex cells during many chronic infections and cancers in animal models and humans (15, 69, 79, 90–97). Moreover, the extent of IR coexpression and perhaps specific patterns directly correspond to the severity of exhaustion (90). Whereas blocking PD-1 signals can robustly, but incompletely, reinvigorate Tex cells, coblockade of multiple IRs can simultaneously additively or sometimes synergistically revitalize Tex cells, and such cotargeting is now a major clinical therapeutic strategy for cancer (98). Blocking IR signaling has become a point of focus in many disease settings, and its development and current use are discussed in greater detail in Section 8.

2.4. Altered Metabolic Program

A major characteristic of lymphocytes is their metabolic plasticity during activation and in response to changes in their surrounding environment (99). During acute infection, CD8 T cells transition from mitochondrial oxidative phosphorylation driving a quiescent state to glycolysis to meet the bioenergetic demands required for activated Teff cells (100). This change in metabolic lifestyle is mediated, at least in part, through the TCR-linked, phosphoinositide 3-kinase (PI3K), and mTOR signaling pathways (100). Following peak effector differentiation, developing memory

Table 1 Summary of selected costimulatory and coinhibitory receptors[a]

Mechanisms of action	Receptor	Ligands	Signaling motif	Species	Cellular expression
ITIM/ITSM	2B4	CD48	4 ITIMs	M, H	T, NK, monocytes
	CD94-NKG2A	HLA-E	ITIM	M, H	T, NK
	GP49B	Integrins	2 ITIMs	M	T, NK, macrophages, monocytes, neutrophils
	KIR family	HLA ligands	ITIM	H	NK
	KLRG-1	E cadherin	ITIM	M, H	T, NK
	Lair-1	Collagen	2 ITIMs		T, B, NK, monocytes, DC
	LILR	MHC-I, UL18	ITIM	M, H	NK
	Ly49 family	MHC-I	ITIM	M	T, NK, monocytes, macrophages
	PD-1	PD-L1, PD-L2	ITIM, ITSM	M, H	T, B, NKT, monocytes
	PECAM/CD31	PECAM1, integrin, CD38	ITIM	M, H	T, platelets, monocytes, neutrophils, vascular endothelial cells
Unconventional signaling	CD200R	CD200	NPxY	M, H	T, DC, monocytes, neutrophils, basophils, mast cells
	Lag-3	MHC-II	KIEELE	M, H	T, B, NK
	Tim-3	Galactin 9, phosphatidylserine	Multiple non-ITIM/ITSM Tyr residues	M, H	T, NK, NKT, macrophages, DC
Receptor competition	CD160	HVEM, MHC-I		M, H	T, NK, NKT, IEL
	CTLA-4	B7–1, B7–2	YVKM	M, H	T
Other/mixed	BTLA	HVEM	ITIM, ITSM, receptor competition	M, H	T, B, DC
	PD-L1	B7–1, PD-1			T, NKT, B, monocytes, DC, endothelial cells, hepatocytes, etc.
	TIGIT	CD226, CD115, CD112	ITIM, receptor competition	M, H	T, NK

Abbreviations: DC, dendritic cell; H, human; IEL, intraepithelial lymphocytes; M, mouse; NK, natural killer cell; NKT, natural killer T cell.
[a]For reviews on individual pathways see Kurachi et al. (303), Pegram et al. (304), and Odorizzi & Wherry (214).

precursors shift metabolism back to oxidative phosphorylation but also acquire the ability to use fatty acid oxidation (101, 102).

Transcriptional profiling of Tex cells revealed substantial changes in genes involved in metabolism, including the citric acid cycle (69), suggesting that metabolic dysregulation accompanies T cell exhaustion. In vitro, PD-1 cross-linking attenuates PI3K, Akt, and mTOR signaling with impacts directly on metabolic pathways including a suppression of glycolysis (103–105). In vivo, developing Tex cells display metabolic derangements, including suppressed cellular respiration, reduced glucose uptake, and dysregulated mitochondrial energetics (106). These developing Tex cells appear to have little metabolic reserve and do not acquire a metabolic lifestyle with Tmem-like spare respiratory capacity (106). Several key pathways have been implicated in

these metabolic defects, including PGC1α (106, 107) and Foxo1 (108) pathways. One potential link for these effects is that some IRs, such as CTLA-4 and PD-1, may negatively regulate signaling through CD28, a positive regulator of glycolysis (105, 109–111). At least some of these metabolic changes during T cell exhaustion have been observed in human disease such as HBV (112). In addition, metabolic dysregulation also contributes to exhaustion in the tumor microenvironment. Tumor growth is typically supported by aerobic glycolysis, the same metabolic lifestyle used by efficient Teff cells (100, 113, 114). Further, immunosuppressive metabolic byproducts of the tumor itself may inhibit T cell function, and competition between tumor cells and immune cells for glucose, other fuel sources (e.g., fatty acids), and oxygen may promote exhaustion.

Because of the link between PD-1 signaling and metabolic pathways, the effects of PD-1 blockade on metabolism of Tex cells have been investigated. Indeed, PD-1 blockade reengaged anabolic metabolism and glycolysis in Tex cells in an mTOR-dependent fashion (108, 115). Moreover, blocking PD-1 signaling enhanced glucose uptake, mainly by the progenitor Tex subset (106). In addition, blockade of the PD-1 pathway results in an increase in glucose in the tumor microenvironment that also likely contributes to improved TIL function and tumor regression (116). These findings suggest that cellular metabolism might represent a crucial aspect of immunotherapeutics aimed at reversing T cell exhaustion, and indeed, metabolic drugs such as rapamycin (106, 117–119) and metformin (120) are being investigated.

2.5. Transcriptional and Epigenetic Landscape of Tex Cells

Recent advances in genomic profiling have defined the underlying transcriptional and epigenetic landscape of Tex cells, and these cells are now understood to have a distinct transcriptional program compared to Teff and Tmem cells (8, 43, 55, 69, 121). Changes in gene expression include significant alterations in genes encoding IRs and transcription factors and genes controlling TCR signaling pathways, costimulatory and cytokine signaling, and cellular metabolism. Transcriptional profiles of Tex cells are also distinct from those of functionally defective anergic cells, though some shared features of exhaustion and anergy may exist (69, 122). A major finding from network analysis of Tex transcriptional profiles was that Tex cells can often reuse transcription factors used by other T cells (e.g., Teff or Tmem cells), but Tex cells use these transcription factors in novel ways, with distinct transcriptional network connectivity for these transcriptional circuits (8). The altered use of transcription factors involved in or required for other steps of T cell differentiation is a hallmark of Tex cells and is discussed in Section 6.

Beyond transcription factor expression patterns, chromatin landscape has a major role controlling cellular differentiation patterns and fate commitment. It is now clear that the epigenetic landscape of Tex cells is distinct from that of Teff and Tmem cells (42–44, 123). Tex cells differ from other CD8 T cells by ~6,000 differentially accessible chromatin regions (42, 43), consistent with the scale of difference between other distinct hematopoietic lineages (124, 125). The epigenetics of Tex cells is discussed in detail in Section 7.

3. DEVELOPMENT OF T CELL EXHAUSTION

The pathways involved in the initial development of T cell exhaustion remain poorly understood. The general three-signal model (126) of signal 1 from antigen, signal 2 from costimulation or, in this case, negative costimulation by IRs, and signal 3 from inflammation and soluble mediators is a useful framework for discussing the development of T cell exhaustion (**Figure 2**). Persisting antigen stimulation is at the core of what drives T cell exhaustion, but other factors clearly also

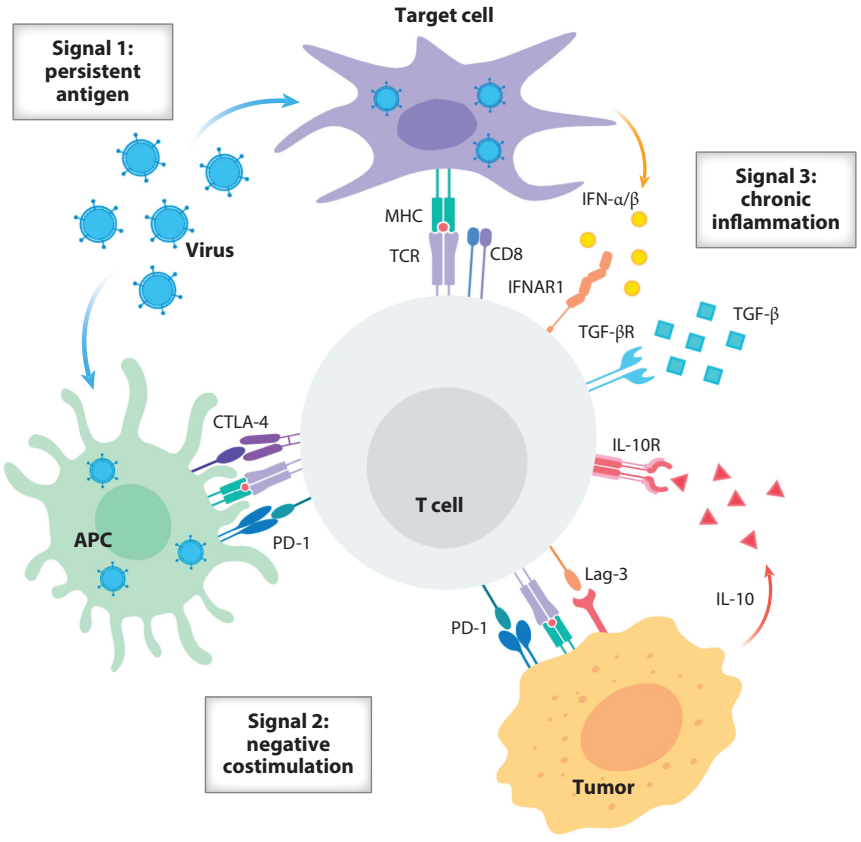


Figure 2

Three-signal model of development of T cell exhaustion. Persistent antigen (signal 1) from virus or tumor drives hyperactivation of T cells that eventually leads to sustained coexpression of multiple inhibitory receptors on T cells and their ligands on antigen-presenting cells (APCs), virally infected cells, and tumors. Inhibitory receptors provide negative costimulatory signals to T cells (signal 2) that prevent optimal T cell effector responses and result in an inability of T cells to mount a robust immune response. In response to persistent antigen, virally infected cells, immunoregulatory cells, APCs, and tumors contribute to a chronic state of inflammation by producing both proinflammatory cytokines (such as IFN-α/β) and inhibitory cytokines (such as IL-10 or TGF-β) (signal 3) that further drive exhaustion by, either directly or indirectly, eliciting negative regulatory signals on T cells. Figure created with BioRender.

play a role. One of the earliest events known to foster T cell exhaustion during chronic viral infections was absence of (or poor) CD4 T cell help (127). However, additional factors including IR signaling, soluble cytokines that promote or antagonize exhaustion, and immunoregulatory cells also contribute to the development of T cell exhaustion. Here we discuss alterations in signal 1 and signal 3; discussion of IRs and costimulation (signal 2) is in Section 4.

3.1. Antigen Load and Persistence

A key feature shared between many mouse models of chronic infections or cancer, chronic human infections, and human cancer is the persistent exposure of T cells to antigen (60, 69, 90, 128–131). Indeed, both high antigen load and long duration of antigen exposure contribute to more severe

T cell exhaustion (19). Persisting infections such as chronic LCMV and untreated viremic HBV, HCV, and HIV are commonly characterized by severe exhaustion consistent with high antigen stimulation (4, 12, 13, 78, 132–134). Further, the duration of antigen exposure appears to contribute to driving exhaustion. Using adoptive transfer approaches, several groups have shown that CD8 T cells primed during chronic infections can recover and develop into Tmem cells if adoptively transferred into uninfected mice during the first one to three weeks of infection (135, 136). However, longer exposure to chronic infection results in irreversible commitment to exhaustion (89, 135, 136). This timing also fits with an inflection point in transcriptional and epigenetic programming where the molecular program of exhaustion becomes established, at approximately two to three weeks of chronic infection or tumor development (8, 44, 55). Even during established chronic infection, CD8 T cell responses can continue to progressively erode over time, especially when viral load is high (61, 137). These observations support the idea that the severity of exhaustion is progressive and connected to both antigen burden and the duration of antigen exposure.

3.2. Soluble Mediators Contributing to T Cell Exhaustion

Cytokine and inflammatory environments play a major role in shaping T cell activation and differentiation (138). During acutely resolved infections, a highly proinflammatory environment promotes Teff development, including KLRG-1$^+$, short-lived effector cells, whereas memory precursors and Tmem cells develop in a less proinflammatory environment (139, 140). During chronic infections and cancer, high levels of some proinflammatory cytokines can induce the release of negative regulatory cytokines that each contribute to T cell exhaustion. These proinflammatory cytokines alone are not sufficient to drive T cell exhaustion (141), however, indicating a connection between inflammatory signals and TCR and/or other signals. Nevertheless, a number of cytokines have been implicated in fostering or antagonizing exhaustion.

3.2.1. Cytokines that promote exhaustion. IL-10 is a STAT-3-inducing cytokine often associated with attenuating T cell activation (142). IL-10 is often induced in chronic infections and cancer, and blocking IL-10 can prevent and/or reverse T cell exhaustion (23, 143–145). Multiple immune cell types including dendritic cells (DCs), B cells, monocytes, CD8 T cells, and nonregulatory CD4 T cells can produce IL-10, and although there are some data implicating CD4 T cells or antigen-presenting cells (APCs) as a source of IL-10 in some settings, the cellular origin of this regulatory cytokine relevant for Tex cells in most settings is not clear. The effects of IL-10 may be direct on T cells through STAT-3, indirect via modulation of APCs, or both (146). Several chronic infections, such as LCMV, HIV, HBV, and HCV, are associated with increased IL-10 production (147), and polymorphisms in the IL-10 promoter that result in lower IL-10 expression can lead to better control of chronic infections (148–150). During chronic LCMV infection, blocking IL-10 in combination with PD-1 results in the maintenance of robust Teff responses, development of functional antiviral memory cells, and faster control of viral replication (143, 144, 151). In addition, the use of neutralizing IL-10 antibodies in combination with therapeutic vaccination enhanced CD8 and CD4 T cell responses and reduced viral load (144). Similar approaches are being investigated for cancer immunotherapy (23, 152).

TGF-β has also been implicated in promoting T cell exhaustion. TGF-β, like IL-10, can attenuate or restrain immune cell activation by activating downstream SMAD transcription factors (153). During acute infection, TGF-β acts as a negative regulator of effector function (154) through the repression of T-bet (T-box expressed in T cells) (155) and results in the upregulation of the proapoptotic factor Bim (156). During chronic viral infections, TGF-β expression and/or downstream SMAD2 activation are features of Tex cells (156–158). In this setting, TGF-β

regulated the magnitude of the T cell response, though the functional ability of T cells appeared less impacted. Nonetheless, attenuation of TGF-β signaling increased antigen-specific CD8 Tex cell numbers, enabling enhanced viral control (156). Similar to publications on IL-10, a wealth of literature has implicated TGF-β in immunoregulation of tumor immunity, suggesting a similar role in cancer for deviating T cell responses toward exhaustion.

3.2.2. Cytokines that antagonize exhaustion. IL-2 is a key cytokine required for T cell survival and activation and robust immune responses to infections and tumors. Indeed, IL-2 was the first cytokine used for cancer treatment to augment T cell function (38), and it remains a focal point for cancer immunotherapy. Developing Tex cells that lack the IL-2 receptor are rapidly lost, suggesting that IL-2 responsiveness is important to maintaining Tex cells in settings of chronic antigen stimulation (159). Therapeutic administration of IL-2 to reinvigorate Tex cells has yielded mixed results. During chronic LCMV infection, IL-2 boosted the number of the LCMV-specific Tex cells and enhanced viral control (160). In combination with PD-1 pathway blockade, IL-2 administration has dramatic synergy in chronic LCMV infection (161), suggesting an attractive strategy for cancer immunotherapy. However, IL-2 can also cause expansion of suppressive T regulatory cells (Tregs) during persisting infections (162); and in the context of HIV infection, IL-2 treatment boosted CD4 T cell numbers, but the effects on CD8 T cells and viral replication were modest (163–165). Nevertheless, newer strategies to augment effects of IL-2 treatment on Tex cells while minimizing the effects on Tregs could have considerable value in modulating exhaustion in chronic infections and cancer.

IL-21 is another γ-chain cytokine with similarities to IL-2 that has been implicated in exhaustion. In the absence of IL-21R signaling, CD8 T cells fail to control chronic viral infections, suggesting a role for IL-21 in sustaining CD8 T cell responses and/or antagonizing exhaustion (166–168). Indeed, treatment with IL-21 can enhance CD8 T cell responses in chronic simian immunodeficiency virus (SIV) infection (169). IL-21 is produced mainly by follicular helper CD4 T (Tfh) cells (170)—a relevant observation, because there is often a skewing of CD4 T cell responses towards Tfh during chronic infections (171) and in some cancers (172, 173). IL-21 directly promotes the expression of BATF (basic leucine transcription factor ATF-like), a transcription factor involved in initiating or sustaining antiviral CD8 Teff function (174, 175) and, in some cases, fostering exhaustion (176). Therefore, during chronic infections, IL-21 may sustain CD8 T cell responses and, at least partially, antagonize exhaustion.

3.2.3. Cytokines with complex roles in exhaustion. Although some proinflammatory cytokines are likely critical for both preventing T cell exhaustion and controlling viral replication, others have more complex roles. Type I interferons (IFN-α/β) are perhaps the prototypical cytokines with a dual role in this context. IFN-α/β are critical proinflammatory cytokines that can suppress viral replication through direct induction of antiviral activities and by activating innate immune cells (177). IFN-α/β signaling is also essential for optimal priming of T cells and for the generation of functional Teff and Tmem cells (138). Though all cells can make IFN-α/β, plasmacytoid DCs are the major producers during chronic infections. IFN-α/β are usually expressed early during infections, and expression typically decreases following antigen clearance (178). During chronic infections and perhaps some cancers, IFN-α/β can remain elevated and induce the expression of IL-10, IDO, PD-L1, and other negative regulators of T cell responses (179). IFN-α/β can also foster attrition of activated T cells via Fas/FasL-mediated T cell death and perhaps other mechanisms (180, 181). There is also evidence that high IFN-α/β signaling can promote terminal exhaustion by antagonizing the Tex progenitor pool through effects on the transcription factor Tcf-1 (T cell factor-1) (182).

Blockade of the interferon pathway through targeting IFN-α/β receptor (IFNAR1) prior to chronic LCMV infection reduced IL-10, decreased the number of PD-L1$^+$ DCs, restored lymphoid tissue architecture, and ultimately enhanced control of viral infection (183, 184). These results were paradoxical given the antiviral role of type I interferons but suggested that interferon signaling has a role in fostering T cell exhaustion in some settings, likely through the induction of immunosuppressive circuits. Administration of anti-IFNAR1 following establishment of T cell exhaustion also enhanced viral control (183, 184), highlighting the complexity of the antiviral versus proexhaustion balance for these cytokines. However, blocking interferon signaling during acute SIV infection in rhesus macaques led to increased SIV replication and accelerated disease progression (185), highlighting the challenges with acute-phase targeting of such a complex cytokine pathway with dual roles in pathogen control and immune regulation. Appropriately navigating this balance could relate to the timing or disease state. For example, in HIV-infected humanized mice, INFAR1 blockade in established chronic infection rescued HIV-specific T cells, prevented hyperimmune activation, and reduced the size of HIV viral reservoirs (186, 187), suggesting that blocking interferon signaling in the context of human chronic diseases late in chronic infection might have therapeutic benefit.

3.2.4. Noncytokine mediators of T cell exhaustion. In addition to cytokines, other soluble mediators have been implicated in T cell exhaustion. Prostaglandin E2 (PGE2) is a lipid synthesized from arachidonic acid that has a dual role as both a proinflammatory molecule (188) and an immunosuppressor and can limit immune responses (188–190). PGE2 suppresses Teff survival and function in vitro (189) as well as in vivo during chronic LCMV infection where blockade of PGE2 synergizes with PD-1 pathway blockade (191). Adenosine can also act as an immunoregulatory signal in some settings where extracellular ATP/ADP is hydrolyzed to release adenosine that can signal through A2AR and other adenosine receptors to suppress T cell function and contribute to exhaustion (192, 193). This pathway may be particularly relevant for tumors where CD73 and CD39, two ectoenzymes responsible for converting extracellular ATP and ADP to adenosine, are expressed prominently by APCs, Tregs, and CD8 T cells (194). Indeed, more severely exhausted CD8 T cells express high levels of CD39 (195), and CD39 may mark the true tumor-reactive exhausted TIL (196). Local availability of metabolic fuel sources and metabolites such as glucose, amino acids, and fatty acids may also have a role in exhaustion (197, 198). In the tumor microenvironment, the ability of T cells to produce or acquire these necessary metabolites may be restricted by hypoxic conditions and/or competition with tumor cells (199, 200).

Collectively, IFN-α/β, IL-10, TGF-β, and many other cytokine and noncytokine inflammatory and immunoregulatory pathway components can influence T cell exhaustion. Other immunoregulatory cytokines such as IL-6 (201, 202), TNF (203), IL-27 (204), IL-35 (205), and chemokines such as CXCL9/10 and CXCL13 may also have a role in CD8 T cell exhaustion and are of considerable interest as promoting, antagonizing, or having more complex patterns of immunoregulation in chronic infections and cancer. Targeting these molecules, or the receptors that they bind to, in the context of chronic infections and cancer is a potentially attractive strategy to reverse the effects of the microenvironment on T cell function and exhaustion.

3.3. Immunoregulatory Cells

Several types of immunoregulatory or suppressive cells have been shown to have an impact on T cell exhaustion. First, conventional CD4 T cell help is critical for, at least partially, antagonizing T cell exhaustion (4). Loss of CD4 T cell help in chronic LCMV infection or in HIV infection leads to more severe T cell exhaustion and, in the case of chronic LCMV infection, lifelong viremia

and uncontrolled infection (127). Precisely what signals are provided by optimal CD4 T cell help remains enigmatic, likely because CD4 T cells can provide multiple levels of support for CD8 T cell responses, including IL-2 and/or IL-21 production, APC activation, and coordination of APC interaction via chemokine production, fostering the generation of antibodies and other events (206). In addition, the importance of individual signals or pathways may vary depending on both the disease setting and the duration of infection/antigen exposure.

$CD4^+$ Tregs have immunosuppressive properties that repress Teff activation and proliferation during acute and early chronic infection. Indeed, Tregs have been implicated in fostering T cell exhaustion. Tregs produce suppressive cytokines, such as IL-10 and TGF-β (207), and the overall number of Tregs at sites of infection increases during both chronic HIV infection and chronic HCV infection as well as in cancer (207). In addition to conventional CD4 Tregs, other immunoregulatory cells such as exhausted APCs, myeloid-derived suppressor cells, CD8 Tregs, and NK cells may contribute to T cell exhaustion (208–212). Thus, although persistent and high-level antigen stimulation is likely the major event driving exhaustion, numerous other environmental factors clearly play a major role in shaping, fostering, or antagonizing T cell exhaustion.

4. INHIBITORY AND COSTIMULATORY RECEPTORS ON EXHAUSTED T CELLS

T cells express a wide array of costimulatory and coinhibitory receptors that can provide either positive signals that drive T cell activation or inhibitory signals that attenuate T cell function and/or differentiation (**Figure 3**). As discussed in Section 2, Tex cells have high and sustained expression of IRs, and the cognate ligands for many of these IRs are upregulated on APCs, tumor cells, and other cells during chronic infections and cancer (49, 213) (**Table 1**). Coexpression of multiple IRs has been linked to T cell exhaustion in both animal models and patients with chronic infections including HIV, HCV, Epstein-Barr virus (EBV), and malaria; autoimmune disorders; and cancers (214). The best-characterized inhibitory and costimulatory molecules belong to the immunoglobulin superfamily and the TNF receptor (TNFR) superfamily and are discussed below.

4.1. Inhibitory Receptors and Negative Regulatory Mechanisms in Tex Cells

IRs are thought to have evolved to prevent overactivation of immune cells during an immune response and/or to help terminate immune responses after effector activation. However, during chronic infection and cancer where antigen persists, IRs are expressed at high and sustained levels and have deleterious effects on the ability of antigen-specific T cells to mount or sustain robust responses. Below, we highlight the best-studied IRs and the mechanisms through which they contribute to T cell exhaustion.

4.1.1. PD-1. PD-1 is expressed transiently during T cell activation on functional Teff cells, and normally expression returns to baseline after activation. In contrast, in chronic infections and cancer PD-1 expression is maintained at high levels on Tex cells (15, 45, 69, 95, 134, 215). Barber et al. (45) demonstrated that blocking PD-1 during chronic LCMV infection resulted in the reinvigoration of Tex cells and reduced viral load. These observations were important for two fundamental reasons. First, the revitalization of Tex cells by PD-1 blockade challenged the notion that Tex cells were terminally dysfunctional. Second, these data provided support for the idea that Tex cells were an independent differentiation state and not simply anergic cells or Teff cells that were unable to signal through their TCR. Therapeutic use of PD-1-blocking antibodies was soon

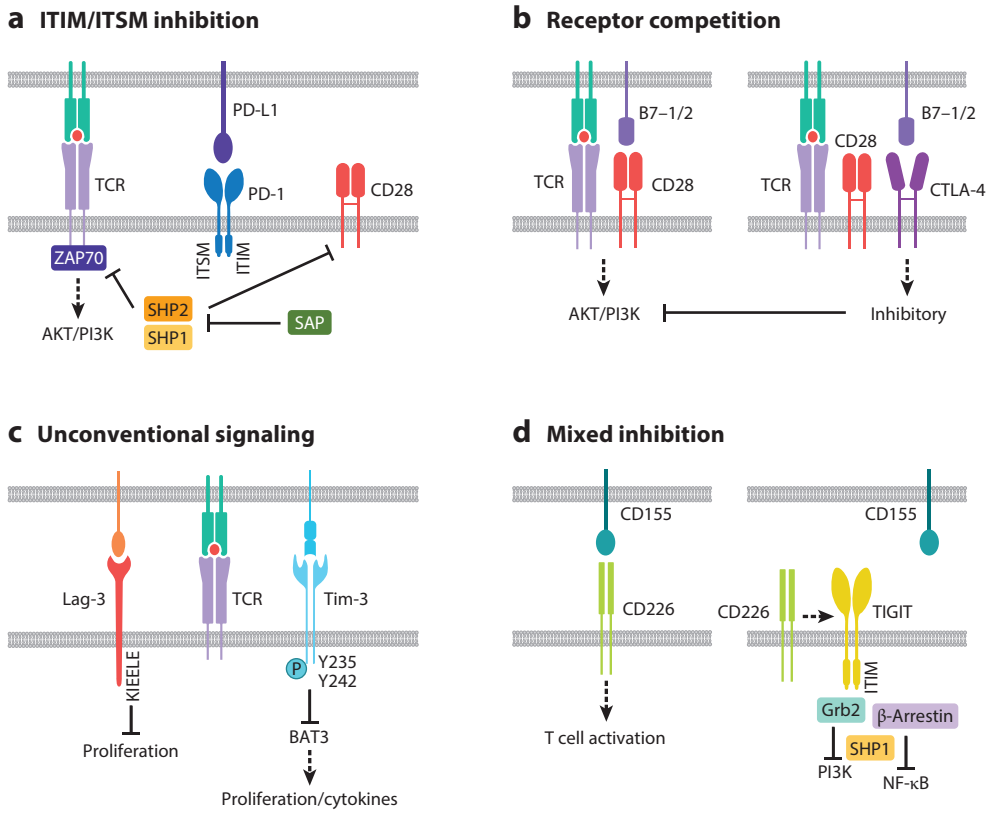


Figure 3

Inhibitory receptors can mediate their negative regulatory effects on T cell activation and effector function via multiple mechanisms, including conventional and nonconventional signaling motifs on their cytoplasmic tails, competition with costimulatory receptors and ligands, or a combination of both. Many inhibitory receptors, such as PD-1, contain ITIM or ITSM motifs (*a*) in their intracellular tail that can recruit SHP proteins and other adaptors that can interfere with positive signals from the T cell receptor (TCR) and costimulatory molecules such as CD28. Other inhibitory receptors, such as CTLA-4, compete directly for binding to costimulatory molecules or their ligands (*b*). Under normal conditions, TCR engagement, in combination with CD28 binding to its ligand, B7–1 or B7–2, activates the AKT/PI3K pathway resulting in T cell proliferation and cytokine production. CTLA-4 can also bind B7–1 and B7–2, preventing CD28-mediated T cell activation. Other inhibitory receptors, such as Lag-3 and Tim-3, provide negative signals to T cells using non-ITIM or non-ITSM motifs (*c*). In both cases, Lag-3 and Tim-3 can interfere with T cell proliferation and cytokine production through the inhibition of positive signaling cascades. Inhibitory receptors can also use a combination of negative signaling and competition to inhibit T cell activation (*d*). The inhibitory receptor TIGIT can sequester, in *cis*, the receptor CD226, preventing its binding to its ligand CD155 and, thus, inhibiting T cell activation. Additionally, TIGIT contains an ITIM that can recruit SHP-1, via Grb2 and β-arrestin in natural killer cells, to inhibit PI3K and NF-κB-mediated activation. Figure created with BioRender.

extended to humans with chronic infections and provided insights for effects being observed using PD-1 pathway blockade in cancer. These findings are discussed further in Section 8.

Two ligands exist for PD-1: PD-L1 (B7.H1) and PD-L2 (B7.DC). PD-L1 is expressed by immune and nonimmune cells, including tumor cells, whereas PD-L2 is expressed by DCs, macrophages, and germinal center B cells (49, 216). The wide expression pattern of PD-1 ligands allows local regulation of immune responses likely to help protect from tissue damage. These immunoregulatory effects have been co-opted by tumors and in chronic infections where tumor

cells, tumor-associated myeloid cells, or infected cells expressing PD-L1 contribute to exhaustion of TILs or responding CD8 T cells.

Some of the negative signaling pathways initiated upon ligation of PD-1 have been investigated. Upon receptor engagement, tyrosine residues on the cytoplasmic domain of PD-1 are phosphorylated, resulting in recruitment of SHP-2, which then attenuates proximal and/or more distal signaling intermediates, including the PI3K-Akt and Ras-MEK-ERK pathways (103, 217, 218). PD-1 recruitment of SHP-2 has also been recently implicated in the dephosphorylation of CD28 as a major mechanism through which PD-1 promotes T cell exhaustion (110, 219). However, recent data have also demonstrated that SHP-2 is not required for T cell exhaustion (220), suggesting a compensatory role for SHP-1 or potentially additional mechanisms downstream of PD-1. Tex cells can also form in the complete absence of PD-1 (221), demonstrating that PD-1 does not program the development of exhaustion. Although initially able to respond vigorously to antigen challenge, PD-$1^{-/-}$ CD8 T cells ultimately become more severely exhausted, and their ability to adequately respond to antigen erodes over time with poor long-term maintenance (221, 222). These data are consistent with the notion of antigen stimulation driving exhaustion and PD-1 antagonizing antigen/TCR signaling such that in the absence of PD-1, stronger signaling drives more severe exhaustion.

Early studies in tumor models (22) and the observations of reversal of T cell exhaustion in the LCMV model (45) helped form the foundation for clinical targeting of the PD-1 axis in chronic infections and cancer. Indeed, PD-1:PD-L1 is now a transformational therapy with impressive response rates in many tumor types, though notably higher response rates in cancers with higher mutational burden (51, 223–226). This latter observation likely reflects the higher abundance of neoantigens for T cell recognition (227). Despite these promising clinical advances, most patients still do not have long-term benefit from PD-1 blockade, and our understanding of the molecular and mechanistic events underlying response or failure to PD-1 blockade is incomplete. Thus, deeper interrogation of PD-1 biology and the underlying mechanisms of action on Tex cells would be invaluable for more effective immunotherapy.

4.1.2. Other inhibitory receptors in T cell exhaustion. Beyond ITIM-containing IRs, other mechanisms of inhibition employed by IRs include nonconventional signaling domains, competing for binding to ligands, and other undefined mechanisms (**Table 1**). Examples of other Tex-relevant IRs include TIGIT, Lag-3, Tim-3, and others that are reviewed elsewhere (98, 228).

TIGIT negatively regulates T cell function both via an ITIM and through competition with CD155 for the costimulatory ligand CD226, thus preventing T cell stimulation (98, 229). The TIGIT-CD155 interaction simultaneously induces IL-10 production and reduces IL-12 production by DCs, further promoting Tex development (230). Blockade of TIGIT shows significant synergy with PD-1 blockade and provides superior control of tumors and chronic viral infection in mouse models (229) and also has a role in human cancer (231), making it a high-priority clinical target.

Lag-3 is a non-ITIM IR that may function through a KIEELE motif in its intracellular tail to negatively regulate cell cycle progression and other cellular functions (232). Lag-3 is highly expressed by Tex cells during chronic infections (90) and by TILs in many cancers (31, 228). Blocking Lag-3 alone during chronic LCMV infection has little effect on Tex function (90, 233); however, coblockade of Lag-3 and PD-1 results in robust and synergistic reinvigoration of Tex cells (90), with similar results in cancer (31).

Tim-3 is an IR with a complex extracellular structure that includes multiple domains that can interact with different ligands that might confer context-specific activity (234). Expression of Tim-3 is highly upregulated on Tex cells and marks the most severely exhausted subsets during

chronic LCMV infection (235, 236) and chronic HCV and HIV infection (237, 238). Tim-3$^+$ Tex cells typically coexpress PD-1, and coblockade of both receptors improved virus-specific T cell immunity during chronic LCMV infection (235) as well as during chronic human infections (238–242).

4.2. Costimulatory Signals Implicated in T Cell Exhaustion

Generally, costimulatory receptors function to activate T cells by driving the expansion, proliferation, and differentiation of resting or naive T cells. During exhaustion, however, costimulatory signaling can be improperly dampened or enhanced, both of which can drive T cell exhaustion.

4.2.1. CD28. CD28 is indispensable for T cell activation and is required for initial T cell priming. Engagement of CD28 provides unique signals that instigate wide transcriptional, metabolic, and epigenetic changes impacting T cell activation, expansion, and differentiation (243). The ligands for CD28 are members of the B7 family of receptors that also bind the IR CTLA-4; thus, the signaling effects of these molecules are compromised during chronic infection and cancer where high levels of CTLA-4 are present. Moreover, Hui et al. (110) showed that PD-1 specifically targets and dephosphorylates CD28, suggesting that PD-1 suppresses T cell activation through direct repression of CD28 signaling. Further, CD28 was shown to be essential for effective PD-1 blockade therapy during chronic LCMV infection and in mouse tumor models, since conditional deletion of CD28 diminished the efficacy of PD-1 pathway blockade (111). Thus, CD28, a fundamental costimulatory pathway for T cells, has a clear role in T cell exhaustion. Additional immunoglobulin superfamily members such as ICOS and CD226 also likely have a role.

4.2.2. TNFR family. TNFR family members OX40, 4–1BB, and CD27 are important costimulatory molecules involved in clonal expansion, differentiation, and survival of Teff cells (244). During chronic LCMV infection, OX40 expression correlates with improved viral control (245); however, continual CD27 signaling results in more profound T cell exhaustion (246), perhaps by driving overstimulation. In addition, agonizing 4–1BB synergizes with PD-L1 blockade to reinvigorate Tex cells (247). These observations may be consistent with the benefit of using the 4–1BB signaling domain in chimeric antigen receptor (CAR) T cells (248). However, why some TNFR pathways appear to benefit Tex cells while others result in more severe dysfunction is poorly understood. Where expression of these molecules is already high, further driving their activity may yield a limited benefit. However, in the case where costimulatory expression is low, such as in some tumors (27), additional stimulation might improve T cell function.

Thus, both IRs and costimulatory receptors contribute to T cell exhaustion. The differential expression pattern of individual IRs on Tex cells as well as the diversity of mechanisms employed by different IRs highlight the complexity of the inhibitory-costimulatory system that may have evolved to fine-tune T cell responses. Other layers of complexity are reflected by the interactions between different IRs as well as the dynamic interaction between IRs and transcription factors (see Section 7). This complexity represents a major therapeutic opportunity that is now beginning to be exploited clinically.

5. DIFFERENTIATION AND SUBSET DYNAMICS OF EXHAUSTED T CELLS

Development of exhaustion is progressive and dynamic, and although it is clear that Tex cells represent a distinct immune lineage, heterogeneity within this lineage also exists. Although the precise

subset of heterogeneity is still being defined, the underlying biology likely reflects a proliferative hierarchy necessary to maintain Tex cells long-term. During initial development, CD8 Tex cells arise from the same pool of KLRG-1^{lo} CD8 Teff cells that give rise to Tmem cells following acute infection—the CD127^{+} memory precursor pool (136) (**Figure 1*b***). In chronic infections (136) and likely in cancers (44), these precursors develop into Tex cells in the context of persistent antigen stimulation and prolonged inflammation. One key observation was that senescent KLRG-1^{+} Teff cells could not give rise to Tex cells (136), suggesting that T cell senescence and exhaustion are distinct paths of differentiation. Indeed, these observations are consistent with at least partially non-overlapping expression patterns for markers of senescence (e.g., CD57 and KLRG-1) and exhaustion (high PD-1, other IRs, etc.) in humans (33, 249) and recent studies distinguishing senescence from exhaustion of T cells during aging (250). Thus, Tex cells form from a precursor that also has memory potential rather than from the more senescent T cells in the effector pool. Tex precursors then progressively lose memory potential such that by approximately two weeks of chronic viral infection, the potential for memory development is highly compromised, and by day 30 postinfection, memory potential is essentially ablated (136), with similar data in tumor models (44). Notably these time points match well to major transcriptional and epigenetic inflection points (see Sections 6 and 7) (8, 42–44, 55).

In addition to having a progressive developmental path, Tex cells are also heterogeneous. PD-1 and CD44 were the first markers used to distinguish Tex subsets with different biological functions. Blackburn et al. (90) showed that PD-1^{int}CD44^{hi} Tex cells were less functionally exhausted than their PD-1^{hi}CD44^{int} counterparts, in part due to lower coexpression of IRs. Moreover, this PD-1^{int} subset contained the cells capable of responding to PD-1 pathway blockade whereas the PD-1^{hi}CD44^{int} subset was essentially terminally exhausted and unable to mount a therapeutic response to checkpoint blockade (90). Subsequent studies demonstrated that the PD-1^{int}CD44^{hi} subset consisted of cells that were largely Eomes^{lo} and functioned as a progenitor population continuously giving rise to a numerically larger PD-1^{hi}CD44^{int}Eomes^{hi} terminal Tex pool (61). Although the PD-1^{hi}CD44^{int} subset had markers of recent proliferation (e.g., Ki67), lineage-tracing experiments demonstrated that these cells were terminal and that the proliferation signature arose from the recent conversion from the PD-1^{int}CD44^{hi}Eomes^{lo} progenitor pool (61). Together, these studies identified heterogeneity in the Tex compartment, defined how these subsets are related in a proliferative hierarchy, identified the stem-like Tex population that sustains the numerically larger terminal Tex subset, and defined the progenitor population that mediates the response to checkpoint blockade.

Additional studies further refined the understanding of Tex subsets by identifying roles for CXCR5 and the transcription factor Tcf-1. A CXCR5^{+}Tcf-1^{+}Tim-3^{-} Tex subset was defined that functioned to mediate the response to PD-1 pathway blockade and also to give rise to a more terminal CXCR5^{-}Tcf-1^{-}Tim-3^{+} Tex subset (236). It is notable that this CXCR5^{+}Tcf-1^{+} subset and the Tim-3^{+} terminal progeny did not differ in expression of the transcription factors T-bet and Eomesodermin (Eomes) (236). These data likely indicate heterogeneity in the original PD-1^{int} progenitor pool described above and possibly also indicate heterogeneity in the terminal progeny. Nevertheless, these studies affirmed the principle concept of a Tex progenitor pool and a larger terminally exhausted population. Mechanistically, Tcf-1 was shown to be essential for the CXCR5^{+} Tex population (182, 236, 251), consistent with Tcf-7 mRNA (encoding Tcf-1) expression in the PD-1^{int}CD44^{hi} subset described above (8). The two key results of these studies identifying the proliferative hierarchy of Tex cells are that they define the population of Tex cells that gives rise to the therapeutic benefit of PD-1 pathway blockade and define the underlying transcriptional control of this process through Tcf-1 and perhaps the T-box transcription factors T-bet and/or

Eomes. They also raise numerous questions about the regulation of the proliferative dynamics in the steady state, the possible role of CXCR5 in guiding Tex cells to relevant anatomical microenvironments, and the ability to design immunotherapies to reinvigorate the more numerous, but more terminal, population of Tex cells.

6. TRANSCRIPTIONAL CONTROL OF EXHAUSTED T CELLS

Transcriptional profiling has investigated the underlying molecular circuits of T cell exhaustion. A major concept that emerged from transcriptional and integrated network analysis of Tex cells was the altered expression pattern and/or transcriptional connectivity of key transcription factors. In other words, transcription factors such as T-box factors, Tcf-1, and others commonly used by functional Teff and Tmem cells were also expressed by Tex cells, but in Tex cells these same transcription factors connected to distinct genes and transcriptional circuits (8, 61) (**Figure 1*b***). Many transcription factors can display context-specific activities, and this distinct biology can be due to cofactors, concentration-dependent binding to different gene loci, differences in subcellular localization of transcription factors, or altered epigenetics that change gene accessibility. Although multiple transcription factors have been implicated in exhaustion, here we focus on selected transcription factors with well-studied roles in Tex cells.

6.1. T-bet and Eomes

T-bet and Eomes are T-box family transcription factors and are critical for effector and memory CD8 T cell development (252–255). During acute infection, T-bet and Eomes are upregulated following T cell activation, when they promote expression of effector molecules, such as IFN-γ (139, 252, 255–257). During acute infections, T-bet, in response to inflammatory cues, drives Teff cells and development of KLRG-1$^+$ terminal Teff cells. Conversely, Eomes fosters expression of IL-15Rβ and memory development, quiescence, and homeostasis (6, 61, 252, 253, 255, 258). In acute infections, loss of T-bet compromises the KLRG-1$^+$ population but KLRG-1$^-$ Teff cells form and Tmem cells are generated (139, 252, 259). Similarly, Eomes loss has a relatively mild effect, though memory maintenance is compromised (252, 255). During chronic infection, the function of both T-bet and Eomes is distinct from their role in Teff and Tmem cells (8). Unlike acute infection where responses can be mounted in the absence of either T-bet or Eomes, during chronic infection each T-box transcription factor is indispensable, and upon genetic elimination of either transcription factor, the Tex pool fails to form (61). Moreover, elevated T-bet expression was associated with the progenitor PD-1^{int}Eomes^{lo} Tex subset (61), likely due to its ability to repress transcription of IR genes, such as *Pdcd1* encoding PD-1 (70). However, higher T-bet was not associated with the CXCR5$^+$Tcf-1$^+$ population described by Im et al. (236), perhaps reflecting a role for T-bet in an intermediate event between the Tcf-1$^+$ progenitor state and the terminal progeny that would be contained in the broader definition of PD-1^{int} cells. The higher expression of Eomes in the more terminal Tex subset (26, 61, 221) is distinct from the expression of this transcription factor in the more quiescent self-renewing Tmem cells during acutely resolved infections. Indeed, transcriptional network analysis indicated that Eomes was involved in nearly completely distinct transcriptional networks in developing functional Teff and Tmem cells compared to Tex cells (8). These studies suggest a rewiring of the function of T-bet and Eomes within Tex cells that likely extends to other transcription factors, such as Tcf-1, Blimp-1, and Tox, and may serve to further the understanding of Tex cells in humans.

6.2. Tcf-1

Tcf-1 is a transcription factor involved in initial T cell development in the thymus and is also reused in Tmem cells where Tcf-1 orchestrates an Eomes-IL-2Rβ circuit (255, 258). Similar to its role in Tmem cells, Tcf-1 maintains progenitor capacity in Tex cells (89, 182, 260). However, how Tcf-1 is connected to Eomes expression in Tex cells remains unclear. Tcf-1 may, together with Bcl6, antagonize the proexhaustion effects of type I interferon (182). It remains unclear if the Tcf-1-dependent transcription program in Tex progenitors overlaps with the Tcf-1-dependent Tmem circuit and how, if at all, Tcf-1 biology has been adapted or altered to the Tex transcription circuit.

6.3. NFAT

NFAT is a family of transcription factors with well-established roles in T cell activation (261, 262), T cell anergy (263), and T cell exhaustion (7, 9, 122). Following activation of naive T cells during acute infection, calcium influx from TCR ligation results in the nuclear localization of NFATs, where these transcription factors form heterodimers with AP-1 family members to promote the transactivation of functional Teff effector genes (264, 265). During early T cell infection, a balanced ratio of NFAT:AP-1 fosters binding to a specific set of genes involved in T cell activation; however, during late effector responses, the ratio of NFAT:AP-1 becomes unbalanced and shifts toward higher NFAT (partnerless NFAT), which promotes the attenuation of effector and activation signals (122). During chronic LCMV infection, the balance of NFAT and AP-1 also favors high NFAT and low AP-1 (7, 69), perhaps due to BATF antagonizing formation of canonical Fos/Jun AP-1 complexes (175, 176), triggering partnerless NFAT nuclear accumulation independent of AP-1 (122). This partnerless NFAT binds to the PD-1 promoter and induces the expression of genes including *Pdcd1* (encoding PD-1) and also induces other IRs such as Lag-3 and Tim-3 and a subset of additional Tex genes (122, 176, 266). NFAT, in concert with Irf-4 and BATF, can also promote anabolic metabolism and effector function, while cooperatively repressing Tmem genes during chronic viral infection, further fostering exhaustion (260). It is notable that miR155 can foster long-term durability of the terminal Tex subset at least in part through regulation of the AP-1 family member Fosl2 (267).

6.4. Additional Transcription Factors Involved in T Cell Exhaustion

A number of other transcription factors or transcriptional pathways have been implicated in T cell exhaustion. Forkhead transcription factors, such as Foxo1 (108, 268), as well as Blimp-1 (61, 66, 269), BATF (122, 175, 176, 270), and IRF-4 (266, 271–273) can antagonize or promote T cell exhaustion by regulating effector or exhaustion-specific genes, respectively, depending on their cellular context. These transcription factors act as cellular rheostats, as their expression level is directly linked to their ability to drive different transcriptional programs. Indeed, expression of Blimp-1 (61, 66), IRF-4 (260, 274), and Foxo1 (108, 275) is elevated in exhausted T cells where they function as positive regulators of T cell exhaustion.

In addition, other metabolism-related transcription factors such as hypoxia-induced factors (HIFs) and the von Hippel-Lindau (VHL) complex may have a role in driving T cell exhaustion in some settings (276). Moreover, network analysis of transcription factors with major roles in Tex cells compared to Tmem cells (8) identified not only Eomes but also Tox, Ikzf2, and Irf7 as well as several others as potential transcription factors with key distinct roles in Tex cells. There is evidence that the transcription factor Id3, a negative regulator of E2A proteins, is required for the survival of at least a subset of Tex cells expressing the IR 2B4 during chronic LCMV infection

(277). Thus, the transcriptional control of Tex cells is complex, with transcription factors used by Teff and Tmem cells employing different mechanisms or functions in Tex cells. Future transcriptomic studies of different Tex subpopulations should provide a better understanding of the central or Tex-defining transcription factors as well as the interplay between these transcription factors in the control of gene regulation in Tex cells.

7. EPIGENETIC LANDSCAPE OF T CELL EXHAUSTION

Recent technological advances in epigenetic profiling, including ATACseq (278), have allowed for epigenetic landscape analysis of Tex cells in chronic infections and cancer in both mice and humans (42–44, 123, 279). These studies demonstrated several key findings about T cell exhaustion. As discussed in Section 2, epigenetic studies revealed that Tex cells are a unique lineage of cells that are as epigenetically different from Teff and Tmem cells as B cells are from monocytes. Indeed, the epigenetic landscape of Tex cells contains ~6,000 differentially accessible chromatin regions when compared to Teff and Tmem cells (42, 43). These new insights provide a potential explanation for many of the distinct characteristics of Tex cells if the epigenetic landscape of gene expression control is fundamentally different in Tex versus Teff and Tmem cells (**Figure 4**). These studies also revealed several additional key points, including (*a*) similar epigenetic landscapes for Tex cells from both chronic viral infections and cancers, (*b*) a temporal link between the establishment of the Tex epigenetic signature and their commitment to exhaustion, (*c*) the stability of Tex epigenetics including after Tex reinvigoration upon PD-1 blockade, and (*d*) the potential for the unique Tex epigenetic landscape to explain altered transcription factor network connectivity and Tex-specific gene regulation (42–44, 55, 122, 279–282).

7.1. Epigenetic Control of the *Pdcd1* Locus

An example of a gene for which epigenetic analysis has shed light on Tex-specific expression patterns is *Pdcd1*, encoding PD-1 (**Figure 4**). During initial T cell activation in the early effector phase of LCMV infection, histone acetylation promotes the opening of the *Pdcd1* promoter and two proximal enhancer elements within approximately −2 kb of the transcription start site (TSS) (280, 283). These regions are then transiently demethylated, allowing for the transcription and expression of PD-1 (284). Following resolution of acute LCMV infection, the DNA is remethylated and repressive trimethylation histone marks are acquired, resulting in transcriptional repression of *Pdcd1* upon differentiation to Tmem cells (284). In contrast to Tmem cells, during chronic LCMV infection, as well as chronic HIV, cytomegalovirus, and EBV infections, the regions around the PD-1 promoter and its proximal enhancers remain fully demethylated (284). Notably, one of these TSS proximal enhancer elements is directly bound by T-bet, allowing T-bet to repress PD-1 expression (70). In addition, ATACseq of Tex cells has revealed an additional enhancer at −23.8 kb upstream of the *Pdcd1* TSS that is present in Tex cells but not Teff or Tmem cells (42, 43). Functional interrogation of this −23.8-kb enhancer revealed it was specifically required for the high and constitutive expression of PD-1 found in Tex cells but dispensable for an intermediate level of PD-1 expressed by Teff cells (43). Thus, the *Pdcd1* example illustrates the functional relevance of a Tex-specific epigenetic landscape.

7.2. Epigenetics and Immunotherapy

A major question that arises is whether the epigenetic landscape of Tex cells is fixed or flexible. Pauken et al. (42) recently showed that, despite robust functional reinvigoration by PD-1

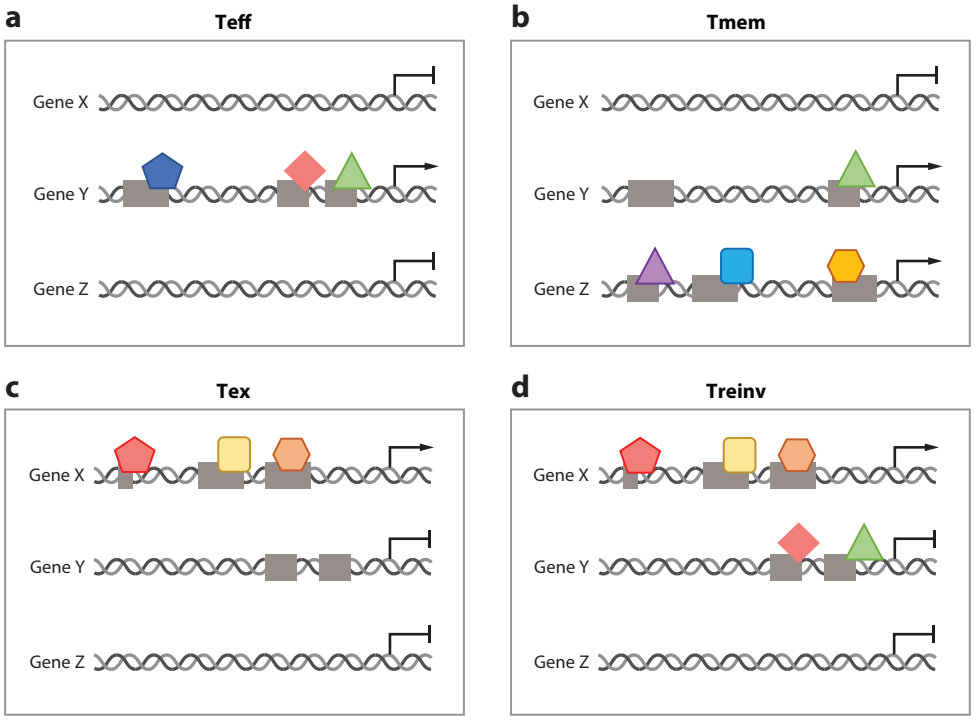


Figure 4

Model of epigenetic and transcriptional control of gene expression in different T cell populations. The development of high-throughput transcriptional and epigenetic technologies has revealed different levels of gene regulation in distinct T cell populations. In general, effector-specific genes, but not memory or exhaustion genes, are open and bound by transcription factors in Teff cells. Upon development into Tmem cells, some effector genes remain partially open and may be bound by transcription factors, whereas memory-specific genes are accessible and being transcribed by memory transcription factors. In Tex cells, exhaustion-specific genes are open and transcriptionally regulated by exhaustion transcription factors. While some effector genes remain open in Tex cells, they are not efficiently bound by transcription factors, and memory genes are closed and inaccessible to transcriptional machinery. In reinvigorated Tex cells, exhaustion genes are still expressed; however, many effector-specific transcription factors become activated and are able to bind to and drive expression of effector genes. Colored shapes indicate different transcription factors. Abbreviations: Teff, effector T cell; Tex, exhausted T cell; Tmem, memory T cell; Treinv, reinvigorated T cell. Figure created with BioRender.

pathway blockade, the epigenetic state of $CD8^+$ Tex cells changed relatively little upon PD-1 pathway blockade (**Figure 4**). As a result, after the initial burst of Tex reinvigoration induced by PD-1 blockade, these cells reverted back to the previous exhausted functional and transcriptional states (42). These data are also consistent with a single, transient peak of T cell (re)invigoration in human melanoma patients treated with PD-1 blockade (26), suggesting that in the absence of epigenetic changes, the effects of reinvigoration by checkpoint blockade may not be durable at the cellular level. The durable clinical effects observed in a subset of cancer patients following PD-1 blockade might represent settings where rapid (and essentially complete) tumor control obviates the need for durable cellular changes. Alternatively, these human clinical observations could reflect settings in humans with different cellular biology such as more profound epigenetic change or new T cell priming upon PD-1 blockade. Distinguishing these possibilities could have important clinical implications.

Early attempts to manipulate epigenetic pathways have shown some promise. For example, Zhang et al. (285) demonstrated that ex vivo treatment of Tex cells with valproic acid, a histone deacetylase inhibitor, improved Tex function. Moreover, Ghoneim et al. (281) found a key role for Dnmt3a-dependent events in limiting PD-1 blockade–mediated reinvigoration. Thus, the unique epigenetic landscape of Tex cells is central to the biology of these cells, and a more complete molecular understanding of the epigenetic landscape and underlying biochemical pathways should reveal new therapeutic opportunities.

8. APPLICATION OF T CELL EXHAUSTION BIOLOGY TO IMMUNOTHERAPY

8.1. Targeting Inhibitory Pathways on Tex Cells by Checkpoint Blockade

Preclinical models have been invaluable to our understanding of T cell exhaustion as well as therapeutic reinvigoration of these cells during chronic infections and cancer. Early work in tumor models showed the potential of blocking IR pathways, such as CTLA-4 and PD-1, to mediate tumor regression (286, 287); however, the T cells involved and the underlying mechanisms of tumor regression were unclear. One major mechanism for these effects, at least for PD-1 and now other pathways including Lag-3, TIGIT, Tim-3, and others, is directly targeting and reinvigorating Tex cells. As discussed above, the chronic LCMV infection model has been instrumental in understanding the direct effect of PD-1 pathway blockade on reinvigorating Tex cells (45). Further studies in other animal models, HIV-infected humanized mice and SIV-infected primates, and ex vivo studies in humans infected with HIV, HCV, and HBV (15, 92, 93, 97, 288, 289) have also demonstrated reacquisition of at least some effector functions and proliferation, resulting in the numerical expansion of Tex cells and, in some cases, beneficial effects on CD4 Tex and B cell responses.

In addition to being applied to infectious disease, PD-1 pathway manipulation has been applied, perhaps even more extensively, in cancer models. TILs express high levels of PD-1 in many settings (27, 29, 290), and PD-L1 is expressed on many types of cancer cells as well as myeloid cells in the tumor microenvironment in response to inflammatory signals (291, 292). Indeed, PD-1 pathway blockade or manipulation has been shown to have robust antitumor effects in many cancer models (47–54). Tumor control is often observed in these settings, and where immune responses have been examined, there is often increased T cell infiltration, T cell numbers, and/or rejuvenation of T cell function. These data are consistent with the reinvigoration of exhausted TIL populations, but in some cases, and likely for some IR blockades, effects on new T cell priming could occur. For example, treatment in the first 7–10 days after tumor inoculation could alter Teff differentiation before T cell exhaustion has been fully established.

These studies formed the foundation for translation to the clinic, where checkpoint inhibitors and immunotherapy are transforming cancer treatment. For example, monoclonal antibodies that target CTLA-4 (ipilimumab), PD-1 (nivolumab and pembrolizumab), and PD-L1 (atezolizumab, durvalumab, and avelumab) have been approved for treatment of melanoma, non–small cell lung carcinoma, head-and-neck cancer, urothelial carcinoma, and numerous other types of cancers. In addition, these and other monoclonal antibodies targeting IR pathways continue to see an increasing number of approvals by the US Food and Drug Administration (FDA) for treating diverse cancers, and many other checkpoint blockade molecules are in clinical trials as monotherapy or in combination (293) (**Table 2**). Checkpoint blockade has shown promising results in tumors resistant to radiation, chemotherapy, and other forms of traditional treatments, where monotherapy with αPD-1 achieved response rates ranging between 50% and 90% for Hodgkin lymphoma and

Table 2 Selected immunotherapeutic combinations targeting reversal of T cell exhaustion[a]

Therapeutic approach	Specific combination	Effect in animal model	Selected ongoing clinical trial(s)[b]
Combination of checkpoint blockers	αPD-1 + αCTLA-4	Expanded infiltrating T cells and reduces Tregs and myeloid cells within B16 melanoma tumors (225)	NCT03222076, NCT02834013, NCT02408861
	αPD-1 + αLag-3	Synergistically enhanced Tex responses during chronic LCMV infection (90)	NCT01968109, NCT03250832, NCT03005782
	αPD-1:PD-L1 + αTIGIT	Additively increased functionality of $CD8^+$ TILs (229)	NCT03119428, NCT03563716
Combination of checkpoint blockade with costimulatory agonists	4–1BB agonist + PD-1 blockade	Improves function of LCMV-specific CD8 Tex cells (247)	NCT02179918, NCT02652455
	ICOS or 4–1BB agonists + αCTLA-4	Synergistic effect in reinvigorating Tex cells in tumor mouse models (305)	NCT02904226
	OX-40 agonist + PD-1:PD-L1 blockade	Synergistically protected against tumor growth in ovarian cancer model (306)	NCT02554812, NCT02705482
Combination of checkpoint blockade with manipulation of soluble mediators	IL-2/IL-21 administration + PD-1 blockade	Synergistically enhanced Tex responses in chronic LCMV infection and mouse tumor models (161, 307)	NCT03224871, NCT02964078, NCT01629758
	IL-10 receptor blockade + PD-L1 blockade	Enhanced Tex responses and viral control during chronic LCMV infection (144)	None available
	TGF-β:TGF-β receptor blockade + PD-L1 blockade	Blocking TGF-β on Tregs prevented excessive upregulation of PD-1 on Tex cells (308)	NCT02423343, NCT02947165
	Adenosine A2a receptor antagonist + PD-1:PD-L1 blockade	Combination therapy reduced metastatic burden and prolonged survival in mouse models of melanoma and breast cancer (309)	NCT02403193, NCT03454451, NCT02655822
	IFN-α/IL-27/IL-35 + IR blockade	Efficacy remains unclear (98)	None available
Combination of checkpoint blockade with CAR T cell therapy	CD19-specific CAR T cell + PD-1:PD-L1 blockade	Combination therapy yielded positive clinical outcome with increased antitumor responses, expanded CAR 19 T cells, and decreased coexpression of PD-1 and Eomes in patient with refractory diffuse large B cell lymphoma (301)	NCT02926833, NCT02650999, NCT02706405
	Autologous T cells with CRISPR-edited endogenous TCR and PD-1	Phase 1 trials of autologous CAR T cells CRISPR-edited to eliminate endogenous TCR and PD-1 in multiple melanoma and $mesothelin^+$ multiple solid tumors	NCT03399448, NCT03545815

Abbreviations: CAR, chimeric antigen receptor; IR, inhibitory receptor; LCMV, lymphocytic choriomeningitis virus; TCR, T cell receptor; TIL, tumor-infiltrating lymphocyte; Treg, regulatory T cells.

[a]For reviews on different therapeutic approaches see Attanasio & Wherry (98), Gay et al. (310), and Ribas & Wolchok (293).

[b]**https://clinicaltrials.gov**.

Merkel cell carcinoma and an ~40% response rate for melanoma. More importantly, the rate of relapse is lower in patients treated with PD-1:PD-L1 blockade compared with most traditional therapies, and treatment is well tolerated by the majority of patients (51, 223, 293–295).

It is likely that at least some of the clinical effects of PD-1 and other checkpoint blockade arise from the reinvigoration of Tex cells. The evidence discussed above that TILs often express markers of, or the transcriptional program of, Tex cells suggests a role for these cells in response to immunotherapy (29, 196). Indeed, several groups have demonstrated that in response to PD-1 or CTLA-4 blockade in humans, Tex cells are a—if not the—major responding immune cell type (26, 296, 297). In some cancers such as melanoma, the pharmacodynamic response of Tex cells to PD-1 blockade, in combination with other disease metrics, correlates with clinical responses (26). Thus, although it is likely that many immune cell types will experience changes during checkpoint blockade and could contribute to clinical efficacy, these recent studies highlight the potential clinical relevance of Tex cells in cancer. Future studies focused on interrogating the biology of Tex cells responding to immunotherapies may shed light on clinical response versus disease progression as well as underlying mechanisms of TIL reinvigoration. Our recent efforts have developed a high-dimensional mass cytometry approach to define heterogeneity in the Tex population that has allowed the development of an exhaustion score (33). Such approaches may be of use in monitoring the effects of different immunotherapies on a specific TIL subset and within a specific disease.

One of the more impressive features of immunotherapy for cancer is the vertical shift in the survival curve. Durable responses lasting many years (e.g., effective cures) are now being seen in ~20% of melanoma patients, and there is also evidence of durable benefit in other cancers. Despite these impressive successes, a still larger fraction of patients do not experience long-term benefit. Can the biology of Tex cells provide a guide to improve upon the success of checkpoint blockade? Why do some patients have long-term benefit and others initially respond to therapies but then regress? In addition, a third set of patients do not experience even initial benefit from checkpoint blockade, and such "cold tumors" may simply not have a T cell response that can be improved by checkpoint blockade. In patients with long-term benefit, T cell effects, perhaps through Tex reinvigoration, are likely sufficient to rapidly eliminate tumors, leading to complete or near complete eradication. Alternatively, these may represent settings of true reprogramming of Tex cells into durable antitumor responses or of new T cell priming leading to more effective non-Tex responses. Whether long-term immune memory to the tumors develops in humans in these cases is often unclear, though elegant studies in pancreatic cancer have demonstrated the presence of tumor-reactive Tmem cells in long-term survivors (295). In patients who initially respond but then progress, it is possible that Tex reinvigoration is temporary and does not lead to a durable or permanent immune change. Indeed, the initiation of PD-1 therapy results in a single peak of Tex reinvigoration and rapid return to pretreatment status despite continued therapy (26). Thus, one limitation with current checkpoint blockade appears to be that reinvigoration of Tex cells, at least in some patients, does not result in the acquisition of long-term memory properties. Moreover, mouse models demonstrate that the epigenetic state of Tex cells is fixed and only slightly altered following PD-1 pathway blockade (42, 43), suggesting that permanent changes to Tex cells to achieve long-term immune control of tumors (or chronic infections) may require the addition of other types of immune or epigenetic modulators in addition to or in combination with checkpoint blockade.

8.2. Combination Immunotherapy

Coexpression of multiple IRs on Tex cells is common in the context of chronic infection and cancer, suggesting cooperative negative regulation of Tex cells by multiple IRs (Section 3). Robust

and synergistic reversal of T cell exhaustion was demonstrated by coblockade of Lag-3 and the PD-1 pathway in chronic LCMV blockade (90), with similar results following in tumor systems and other infectious models (204, 287, 298–300). Similar coblockade effects have been observed targeting many other combinations of IRs simultaneously. In addition, targeting both IRs and costimulatory receptors, the addition of cytokines or cytokine blockade with IR blockade, and the combination of checkpoint blockade and more traditional cancer therapeutics such as radiation, chemotherapies, and targeted therapies have displayed many potentially beneficial cellular effects. Further, there is emerging evidence that combining PD-1 blockade with cell-based immunotherapies, such as CAR T cells, could have enhanced benefit in reversing the effects of T cell exhaustion (301). Trials are also in development to genetically remove PD-1 from CAR T cells (302) to altogether avoid the negative regulation by this checkpoint in the exhaustion-promoting tumor microenvironment. Thus, our developing molecular, transcriptional, and genomic understanding of the mechanisms of development and control of T cell exhaustion could be used to engineer and "exhaustion proof" CAR T cells. Many of these combination therapies are now in clinical trials (**Table 2**).

8.3. Exhaustion Beyond Cancer and Chronic Infection

In 2015 McKinney et al. (21) demonstrated that the transcriptional signature of Tex cells was present in autoimmunity. Indeed, the concept that autoreactive cells that are chronically stimulated by autoantigens might display features of exhaustion fits with our current understanding of the biology of Tex cells and underscores the idea that Tex cells are not functionally inert. Residual functions of Tex cells may be important for slowing disease (in the case of cancer or chronic infections) or promoting pathology (in the case of autoimmunity). This landmark study highlights the potential to investigate the therapeutic opportunities to promote exhaustion and foster dysfunction in the context of autoimmune diseases as we better understand the underlying mechanisms.

9. CONCLUDING REMARKS AND FUTURE DIRECTIONS

Tex cells have emerged as a clinically relevant and distinct T cell type. Our understanding of the underlying mechanisms of exhaustion has revealed new T cell biology as well as therapeutic opportunities in the clinic. Nevertheless, it will be important to continue to dissect the molecular initiators of exhaustion, interrogate the pathways that restrain these cells from developing into optimal Tmem cells, and further understand the disease relevance of Tex cells in chronic infections, cancer, and autoimmunity.

SUMMARY POINTS

1. Tex cells are functionally, metabolically, transcriptionally, and epigenetically distinct from Teff and Tmem cells.
2. Tex cells develop from KRLG-1^{lo}CD127^{hi} memory precursor cells.
3. The development of T cell exhaustion is driven by persistent antigen stimulation, altered costimulation versus coinhibition by cell surface receptors, and chronic inflammation.
4. Sustained high expression of IRs is a key feature of Tex cells.
5. Tex cells can be, at least partially, reinvigorated by blocking IR signaling and/or stimulating T cells using exhaustion-antagonizing soluble molecules such as cytokines.

6. The transcriptional and epigenetic circuitry underlying T cell exhaustion is distinct from that of Teff and Tmem cells and allows for exhaustion-specific control of gene expression and transcription factor function.
7. Targeting Tex cells therapeutically via checkpoint blockade and other emerging approaches may be an effective strategy for treating some chronic viral infections and cancers.
8. A better understanding of the developmental biology, molecular mechanisms, transcriptional and epigenetic control, and reversibility of T cell exhaustion should provide a strong foundation for the development of more effective immunotherapies for chronic infections, autoimmunity, and cancer.

DISCLOSURE STATEMENT

E.J.W. has consulting agreements with and/or is on the scientific advisory board for Merck, Roche, Pieris, Elstar, and Surface Oncology. E.J.W. has a patent licensing agreement on the PD-1 pathway with Roche/Genentech. E.J.W. is a founder of Surface Oncology and Arsenal Biosciences.

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