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T cell exhaustion

E John Wherry

T cell exhaustion is a state of T cell dysfunction that arises during many chronic infections and cancer. It is defined by poor effector function, sustained expression of inhibitory receptors and a transcriptional state distinct from that of functional effector or memory T cells. Exhaustion prevents optimal control of infection and tumors. Recently, a clearer picture of the functional and phenotypic profile of exhausted T cells has emerged and T cell exhaustion has been defined in many experimental and clinical settings. Although the pathways involved remain to be fully defined, advances in the molecular delineation of T cell exhaustion are clarifying the underlying causes of this state of differentiation and also suggest promising therapeutic opportunities.

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T cell exhaustion was described more than a decade ago as dysfunction and subsequent physical deletion of antigen-specific T cells during chronic viral infection in mice[1,2]. Since then, T cell exhaustion has been demonstrated in a wide variety of animal models and in humans with chronic viral, bacterial and parasitic infections as well as during human cancer[3]. Although the details of T cell dysfunction differ for specific pathogens, a general phenotypic and functional portrait of T cell exhaustion is becoming clearer. Recently, two emerging themes have shed considerable light on the understanding of T cell exhaustion. The first is the understanding that both extrinsic negative regulatory pathways (such as immunoregulatory cytokines) and cell-intrinsic negative regulatory pathways (such as PD-1) have key roles in exhaustion. Second, an accurate molecular definition of exhaustion is unfolding. These studies suggest that exhausted T cells represent a distinct state of T cell differentiation and that more comprehensive molecular analyses of T cell exhaustion in different settings may identify common underlying principles and clinical opportunities.

T cell responses to acute infections

After infections that are cleared acutely, highly functional memory T cells develop and are endowed with several defining properties that distinguish them from naive, effector or other subsets of memory-like T cells[3–5]. These properties of memory T cells include rapid reactivation of effector functions after antigen re-encounter, reacquisition of homing to secondary lymphoid tissues, high proliferative potential and the ability to persist long term without antigen via homeostatic proliferation driven by interleukin 7 (IL-7) and IL-15. These properties are acquired gradually over time and allow memory T cells to confer protective immunity. More in-depth discussions of memory T cell differentiation after well-controlled infection have been published[3–5]. However, understanding of T cell exhaustion is intimately tied to concurrent analyses of functional effector and memory T cells. Most of the discussion of T cell exhaustion below reflects observations obtained from infections with high viral replication (at least in the untreated state) such as chronic infection with human immunodeficiency virus (HIV), lymphocytic choriomeningitis virus (LCMV), hepatitis C virus (HCV) or hepatitis B virus (HBV).

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Loss of effector function during T cell exhaustion

Exhausted CD8$^+$ T cells were first identified during chronic LCMV infection as virus-specific, tetramer-positive CD8$^+$ T cells that do not produce cytokines[1]. During exhaustion, loss of function occurs in a hierarchical manner, with exhausted CD8$^+$ T cells losing some properties before losing others[3,6–8] (**Fig. 1**). Typically, functions such as IL-2 production, high proliferative capacity and *ex vivo* killing are lost first. Other properties, including the ability to produce tumor necrosis factor, are often lost at more intermediate stages of dysfunction. Severe exhaustion eventually leads to virus-specific cells that partially or, in some cases, completely lack the ability to produce large amounts of interferon-γ (IFN-γ) or beta-chemokines or to degranulate. The final stage of exhaustion is physical deletion of virus-specific T cells[1,6,9]. More severe CD8$^+$ T cell exhaustion correlates with higher viral load. Even at the same viral load, epitopes presented in larger amounts lead to more extreme exhaustion and/or deletion than do epitopes presented in smaller amounts[6]. This tight link between the strength of stimulation and T cell exhaustion has important implications for mutations that prevent or diminish T cell recognition (epitope escape mutations) and for the maintenance of T cell function during some infections[10]. In addition to antigen load, longer duration of infection or loss of help from CD4$^+$ T cells leads to more severe exhaustion[3,7]. Thus, a model has emerged with hierarchical stages of T cell exhaustion characterized by specific functional parameters (**Fig. 1**).

Like CD8$^+$ T cells, virus-specific CD4$^+$ T cells also lose effector function during chronic viral infection[11–14]. However, far less is know about the dysfunction of virus-specific CD4$^+$ T cells than about that of CD8$^+$ T cells during chronic infection. Chronic viral infection could have a different effect on virus-specific CD4$^+$ T cells than on CD8$^+$ T cells. For example, during chronic infection, CD4$^+$ T cells are the main source of IL-21 that sustains antiviral CD8$^+$ T cells[15–18], and CD4$^+$ T cells are probably an important source of increased IL-10 (ref. 19). The effect of chronic viral infection on the more diverse functional properties and differentiation plasticity of CD4$^+$ T cells versus its effect on CD8$^+$ T cells might not be

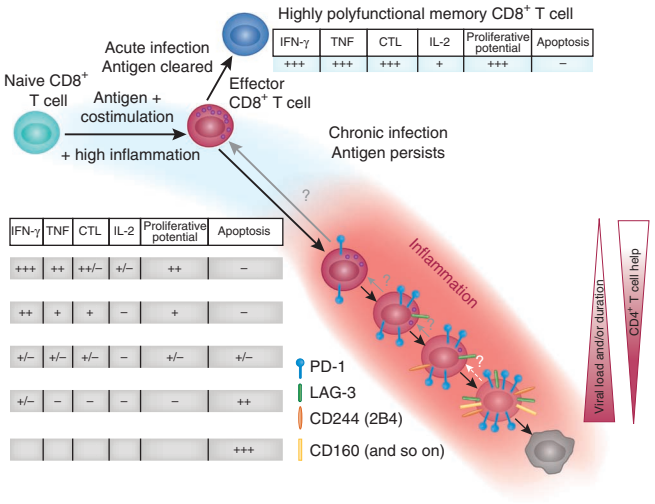

Figure 1 Hierarchical T cell exhaustion during chronic infection. During initial infection, naive T cells are primed by antigen, costimulation and inflammation and differentiate into effector T cells. Clearance of infection and antigen allows a subset of these functional effector T cells to further differentiate into highly polyfunctional memory T cells able to coproduce many cytokines (such as IFN-γ, tumor necrosis factor (TNF) and IL-2), becoming cytolytic and proliferating vigorously (top). These cells also have considerable survival capacity and are maintained long term without antigen. During chronic infection (bottom), infection persists after the effector phase. As antigen and/or viral load increases, T cells progress through stages of dysfunction, losing effector functions and other properties in a hierarchical manner. T cell exhaustion is also accompanied by a progressive increase in the amount and diversity of inhibitory receptors expressed. In addition, altered inflammation and changes in immunoregulatory cytokines such as IL-10 and/or TGF-β can have an increasingly important role. Ultimately, if the severity and/or duration of the infection is high and/or prolonged, virus-specific T cells can be completely eliminated, leading to loss of virus-specific T cell responses. The severity of T cell exhaustion is correlated with increasing inhibitory receptor expression, high viral (or antigen) load, loss of CD4+ T cell help and prolonged infection. The activity of each property is presented on a scale from high (+++) to low (—); 'CTL' indicates cytotoxic potential.

predictable from the present knowledge of CD8+ T cell exhaustion and is an important area for further investigation.

T cell exhaustion in humans

Soon after studies first described T cell exhaustion in mice, it became apparent that similar kinds of CD8+ T cell dysfunction exist in humans[3,7]. For example, HIV-, HCV- and HBV-specific CD8+ T cells that partially or, in some cases, fully lack *ex vivo* effector function have been identified[3,7]. As in mice, high viral load and low CD4+ T cell help is correlated with more severe exhaustion[3,7], although the precise features of exhaustion vary during different infections. For example, it is rare to find HIV-specific CD8+ T cells that do not make IFN-γ, although defects in polyfunctionality and cytotoxicity can occur[20]. In contrast, during chronic HCV infection, CD8+ T cells that cannot make IFN-γ have been reported[21], and prolonged infection is often associated with weak HCV-specific T cell responses in the periphery, consistent with severe exhaustion and deletion[22]. In addition to viral load and CD4+ T cell help, many features of the pathogenesis of various infections could influence the severity of exhaustion, including cellular and tissue tropism, inflammation, antigen-presenting function and lymphoid architecture integrity[23–25]. In particular, disruption of the lymphoid architecture has been highlighted in both mice and humans as a potentially critical aspect of pathogenesis and poor immunity during chronic infection. In mice, chronic LCMV infection leads to severe disruption of the lymphoid tissue that probably impairs normal trafficking and interactions between cells that are necessary for optimal T cell (and possibly B cell) function[23,26]. In humans, fibrosis and lymphoid tissue disorganization has also been reported during HIV infection and could have a role in ultimate failure of adaptive immunity in these infections[27,28]. A major goal is to define how these variables influence T cell dysfunction.

Exhaustion and altered memory maintenance

A key property of memory CD8+ T cells generated after acute infections is the ability to persist long term without antigen via IL-7- and IL-15-mediated homeostatic self-renewal. During chronic infection in mice, virus-specific CD8+ T cells do not develop this property. Exhausted CD8+ T cells have low expression of CD122 (the β-chain of the IL-2 and IL-15 receptor) and CD127 (the IL-7 receptor α-chain), respond poorly to IL-7 and IL-15 and are not maintained when adoptively transferred to infection-free mice[29]. In fact, during chronic viral infection, virus-specific CD8+ T cells become 'addicted' to their cognate antigen and use epitope-specific T cell antigen receptor (TCR) signals for long-term maintenance[30]. In humans, expression of CD127 is also low on CD8+ T cells responding to chronic viral infection, and during HIV infection, highly active retroviral therapy or epitope escape mutation and loss of T cell recognition leads to loss of virus-specific CD8+ T cells, which suggests that antigen-dependent maintenance similar to that in mice exists in humans during chronic infection[29]. In mice, analysis of such exhausted CD8+ T cell populations that do not persist without antigen has provided another notable finding. Before the disappearance of these exhausted cells in antigen-free recipients, there is little recovery of normal differentiation of memory CD8+ T cells[31]. This observation suggests that once T cells are committed to exhaustion, simply removing antigen does not restart the memory T cell differentiation process. In humans, there is also evidence of persistent dysfunction after HIV infection is controlled by highly active retroviral therapy.[32] However, if epitope escape occurs early, some recovery of memory T cell differentiation may occur[10], consistent with other examples of functional recovery of HIV- or HCV-specific CD8+ T cell responses after successful therapy or escape mutation[33,34]. Those observations and the altered pattern of division-associated maintenance of virus-specific T cells during chronic infection[30] raise questions about how the size of the pool of exhausted T cells is maintained *in vivo*. Notably, in some cases new thymic emigrants can be primed in the periphery during chronic viral infection[35]. Although mice that have undergone thymectomy do not have major defects in maintaining antiviral T cell responses during chronic viral infection[36], new thymic emigrants could make quantitative or qualitative contributions to virus-specific CD8+ T cell responses in the long term. Thus, in addition to influencing effector function, T cell exhaustion can also compromise other key properties associated with highly functional memory T cells.

Negative regulatory pathways

Immunoregulation is centrally involved in T cell exhaustion. These negative pathways can be grouped into three main categories: cell surface inhibitory receptors (such as PD-1), soluble factors (such as IL-10), and immunoregulatory cell types (such as regulatory T cells (T_{reg} cells) and other cells). Identifying a role for such regulatory pathways has been important in distinguishing T cell exhaustion from other types of defects such as downregulation of CD3 (ref. 37) or the TCR[38], in which T cells become ignorant of ongoing infection.

Inhibitory receptors have a key role in many aspects of adaptive immunity, including self-tolerance and prevention of autoimmunity[39]. Although functional effector T cells can transiently express inhibitory

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receptors during activation, prolonged and/or high expression of multiple inhibitory receptors is a key feature of the exhaustion of $CD8^+$ and $CD4^+$ T cells both in animal models and in humans[3]. The axis of PD-1 and its ligand seems to be a major inhibitory receptor pathway involved in T cell exhaustion, and blocking this pathway during chronic LCMV infection reinvigorates virus-specific $CD8^+$ T cell responses and results in a lower viral load[40]. T cell exhaustion is thus an active process under the control (at least partly) of inhibitory receptors; therefore, if the appropriate pathway(s) could be targeted, the severe dysfunction of exhausted $CD8^+$ T cells could be reversed and control of infection could be improved. Shortly after the initial studies in mice, studies found that the PD-1 pathway has a central role in T cell dysfunction during HIV infection[41–43]. In addition, *in vivo* blockade PD-1 during infection with simian immunodeficiency virus leads to a substantial improvement in virus-specific $CD8^+$ T cell responses and also enhancement of B cell responses and antibody[44]. Perhaps most importantly, blocking the PD-1 pathway during pathogenic infection with simian immunodeficiency virus improves survival[44]. The PD-1 pathway also has an important role in limiting the effectiveness of antigen-specific T cells during several other persisting viral and nonviral infections as well as in cancer[3,39]. In fact, early clinical trial results suggest that blocking the PD-1 pathway could be a major immunotherapeutic strategy for achieving immunological control of tumors in humans[45].

In addition to PD-1, many other cell surface inhibitory receptors coregulate T cell exhaustion[46]. Virus-specific $CD8^+$ T cells during chronic infection in animal models and in humans can also coexpress LAG-3, CD244 (2B4), CD160, TIM-3, CTLA-4 and many other inhibitory receptors[47]. The pattern of inhibitory-receptor coexpression and the number of receptors simultaneously expressed by the same $CD8^+$ T cell can substantially affect the severity of dysfunction[46]. Furthermore, the recovery of function is increased considerably by simultaneous blockade of the PD-1 pathway and LAG-3 (ref. 46), the PD-1 pathway and CTLA-4 (refs. 13,48), or the PD-1 pathway and TIM-3 (refs. 49,50). In addition, dysfunctional tumor-specific T cells have been reported to coexpress PD-1 and LAG-3 (refs. 51,52) or PD-1 and TIM-3 (refs. 53,54).

Understanding the downstream mechanisms of these diverse inhibitory receptors is a major goal. One possibility is that individual inhibitory receptors regulate distinct cellular functions. For example, the PD-1 pathway seems to strongly affect survival and/or proliferation of exhausted $CD8^+$ T cells[41,55–57]. In contrast, LAG-3 affects cell cycle progression but has less influence on cell survival or apoptosis[46,58]. CD244 (2B4) and CD160 might also affect nonoverlapping functions of exhausted $CD8^+$ T cells[46]. Although the molecular mechanism for inhibitory receptor–mediated regulation in most cases has not been defined, studies suggest that inhibitory receptors might attenuate T cell responses in more than one way. For example, CTLA-4 can compete with CD28 for costimulatory ligands[59]. In contrast, inhibitory motif–containing molecules such as PD-1 can recruit phosphatases (such as SHP-1, SHP-2 or SHIP) to TCR-proximal signaling complexes and attenuate signaling[60,61]. Both PD-1 and CTLA-4 can inhibit signaling by the serine-threonine kinase Akt but seem to do so by different molecular mechanisms[62]. Such observations suggest that inhibitory receptors mainly attenuate or blunt signaling and gene expression. However, PD-1 ligation can induce genes that encode molecules actively involved in inhibiting T cell function[63], which suggests a potential additional mechanism by which these receptors operate. Inhibitory receptors also bind a diverse array of ligands, which suggests the possibility that environmental factors, including ligand availability, could 'tune' the functionality of exhausted $CD8^+$ T cells during persisting infections. Other negative regulatory receptors and ligands, such as FasL, tumor necrosis factor receptor and TRAIL, are upregulated during or have also been linked to T cell exhaustion[64,65]. Additional studies are needed to understand the intracellular pathways coupled to inhibitory receptors and their role in various anatomic and cellular interactions during chronic infection.

In addition to cell surface inhibitory receptors, immunoregulatory cytokines also influence T cell exhaustion. For example, polymorphisms in *IL10* or *IL10RA* have been associated with differences in disease progression during chronic viral infection in humans, and IL-10 expression is higher in several chronic infections[18]. Moreover, IL-10 is linked to T cell dysfunction during persistent viral infection, as blockade of IL-10 enhances viral control and improves T cell responses[19,66]. Studies of LCMV infection in mice have identified dendritic cells and/or $CD4^+$ T cells as the main source of IL-10 during persistent infection[19,66]. In HIV-infected humans, IL-10 might also be produced by monocytes as a result of PD-1 ligation[67]. Given that monocytes and macrophages have not been identified as major sources of IL-10 in mice, further studies are needed.

Transforming growth factor-β (TGF-β) has also been linked to T cell exhaustion. Phosphorylation of the signal transducer Smad2, which is indicative of TGF-β signaling, is greater in virus-specific $CD8^+$ T cells from chronic LCMV infection than in virus-specific $CD8^+$ T cells obtained during well-controlled infection[68]. Preventing the ability of T cells to receive TGF-β signals (via a dominant negative receptor) improves the functionality of $CD8^+$ T cells during chronic LCMV infection and prevents the severe exhaustion and deletion of some $CD8^+$ T cell specificities[68]. TGF-β has also been linked to chronic viral infection in humans[69,70]. Exactly how TGF-β signaling fosters T cell dysfunction or exhaustion during chronic viral infection, however, remains unclear.

In contrast to the immunosuppressive cytokines noted above, common γ-chain cytokines are typically positive regulators of T cell responses and can enhance immunity during chronic infection[71]. Providing exogenous IL-2 enhances the responses of exhausted T cells[72]. In addition, IL-7 treatment during chronic viral infection in mice has indicated a key role for this cytokine in T cell exhaustion and in other aspects of the immune response to chronic infection[73,74]. IL-21 also seems to have an especially important role during chronic viral infection. IL-21 produced by virus-specific $CD4^+$ T cells during chronic LCMV infection is needed to sustain antiviral $CD8^+$ T cell responses[15–17]. Studies of humans also support the interpretation that IL-21 fosters more functional $CD8^+$ T cell responses during chronic viral infection[75–77]. Such results are notable given the prominent role of IL-21 produced by follicular helper $CD4^+$ T cells and suggest that IL-21 can affect antiviral immunity outside the germinal center reaction. Such studies also shed new mechanistic light on the long-established importance of $CD4^+$ T cell help for $CD8^+$ T cell responses during chronic viral infections, and suggest that IL-21 could be substituted for $CD4^+$ T cell help in some pathological settings. Thus, during chronic infection, changes in both negative and positive regulatory cytokines seem to substantially shape the quality of the pathogen-specific T cell response.

Immunoregulatory $Foxp3^+CD4^+$ T_{reg} cells affect immune responses during chronic infections, such as infection with Friend leukemia virus, HCV or HIV, during many persisting bacterial and parasitic infections and in cancer[3]. T_{reg} cells have also been linked to chronic LCMV infection, although, notably, in this setting the population expansion of these cells depends on a superantigen encoded by an endogenous retrovirus[78]. Although that study demonstrates better control of chronic LCMV infection after depletion of T_{reg} cells, another study has found a smaller role for T_{reg} cells in persistent LCMV

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infection[73], which indicates a need for further studies of the role of T_{reg} cells in this model. The mechanisms of suppression of immunity by T_{reg} cells *in vivo* are incompletely understood, although pathways such as IL-10, TGF-β and inhibitory receptors have been linked to this[79]. In addition, some populations of $CD4^+$ T_{reg} cells, such as those that produce IL-35, can induce otherwise normal effector T cells to become T_{reg} cells[80,81]. It remains unclear whether T_{reg} cells directly affect the exhaustion of antigen-specific $CD4^+$ or $CD8^+$ T cells during chronic viral infection or whether the suppression of effector functions and resulting pathogen persistence reinforce the pathway(s) to exhaustion. In addition to conventional $Foxp3^+CD4^+$ T_{reg} cells, other cell populations such as $CD8^+$ T_{reg} cells[82,83], alternatively activated macrophages[84] or altered antigen-presenting cells, such as those that produce the suppressive enzyme IDO[85], are probably present during some chronic infections. The effect of these cell types on T cell exhaustion is poorly understood at present.

Transcriptional definitions and exhaustion as a distinct lineage

A major advance in many areas of immunology has been the ability to define states of cellular differentiation not only by phenotype and function but also through the use of comprehensive molecular and transcriptional profiles. These approaches have shown that in some cases, cells that share phenotypic markers are very different, whereas in other cases, cells induced by different stimuli or even from different species share conserved molecular programs[86]. Genomic approaches have begun to provide a molecular definition of exhausted T cells that complements the phenotypic and functional assessments described above[63,87]. Such studies have defined specific molecular pathways and major features of T cell exhaustion, identifying a role for inhibitory receptors as well as changes in TCR and cytokine signaling pathways, migratory potential, chemokine expression and metabolism[87]. These genomic studies support the idea that exhausted T cells represent a unique state of T cell differentiation. For example, global transcriptional profiling has shown that exhausted $CD8^+$ T cells are as different from effector and memory T cells as those cells are from each other[87]. Although a lineage-specific transcription factor has not yet been identified for exhausted T cells, some transcriptional pathways are used differently by exhausted $CD8^+$ T cells than by effector and memory $CD8^+$ T cells[87,88] (discussed below). Together these studies suggest that exhausted $CD8^+$ T cells represent a distinct lineage fate for T cells generated during some chronic infections. A key future goal will be to determine whether exhausted T cells represent a fixed lineage or retain the flexibility to become fully functional effector or memory T cells.

Several specific transcriptional pathways have been implicated in T cell exhaustion. For example, the transcriptional repressor Blimp-1 is centrally involved in $CD8^+$ T cell exhaustion[88]. Blimp-1 controls the terminal differentiation of cells in a variety of immunological settings and outside the immune system[89,90]. During acute infection that is well controlled, Blimp-1 is associated with terminal differentiation of effector $CD8^+$ T cells, whereas smaller amounts of Blimp-1 are found in memory precursors and mature memory $CD8^+$ T cells[91,92]. During chronic infection, Blimp-1 expression is very high in exhausted $CD8^+$ T cells, much higher than its expression in functional effector $CD8^+$ T cells during the acute phase of a cleared infection[88]. This high expression of Blimp-1 is associated with the upregulation of many inhibitory receptors, including PD-1, LAG-3, CD160 and CD244 (2B4)[88]. Genetic ablation of Blimp-1 reverses inhibitory receptor expression and the pattern of memory differentiation (such as CD127 expression)[88]. These results suggest that moderate or small amounts of Blimp-1 promote the formation of memory T cells, and intermediate amounts of Blimp-1 promote the terminal differentiation of highly functional effector T cells, but very large amounts of Blimp-1 foster the expression of inhibitory receptors and exhaustion. High expression of the transcription factor T-bet has an analogous role to that of Blimp-1 in fostering the terminal differentiation of functional $CD8^+$ T cells after cleared infections[93]. Notably, the role of T-bet is distinct during viral persistence, and T-bet promotes sustained responses during chronic viral infection and represses transcription of the gene encoding PD-1 and the expression of other inhibitory receptors[94]. Blimp-1 and T-bet are therefore key transcriptional 'nodes' involved in the exhaustion of $CD8^+$ T cells, and the precise function of these transcription factors might depend on context (such as acute versus chronic infection).

Transcriptional profiling indicates higher expression of the transcription factor NFATc1 (NFAT2) in exhausted $CD8^+$ T cells[87]. That observation is notable given the connections among NFAT, the calcineurin pathway and T cell anergy[95], although T cell anergy and $CD8^+$ T cell exhaustion seem not to be entirely overlapping processes[87] (discussed below). Accordingly, translocation of NFAT to the nucleus is impaired in exhausted $CD8^+$ T cells during chronic LCMV infection in mice[96]. Lower NFATc1 function is associated with poor cytokine production, whereas cytotoxicity is preserved[96]. That same study reported that HIV-specific $CD8^+$ T cells have a similar 'split functionality' in which degranulation is preserved but cytokine production is impaired[96]. Other studies have demonstrated that the preservation of the cytotoxic activity of HIV-specific T cells from long-term nonprogessors is associated with more efficient translocation of NFATc1 to the nucleus by HIV-specific $CD8^+$ T cells from those patients than by HIV-specific $CD8^+$ T cells from patients with normal progression of disease[97]. These data suggest that dysregulated NFATc1 function is involved in the poor cytotoxicity of $CD8^+$ T cells from patients with normal progression of disease resulting from HIV infection[97]. More transcription of *Nfatc1* in exhausted $CD8^+$ T cells[87], therefore, might be associated with poor activation or translocation of this transcription factor during T cell exhaustion in some settings. NFATc1 can also regulate PD-1 expression after *in vitro* activation of T cells[98], although how altered nuclear translocation of NFATc1 and PD-1 expression are connected in exhausted T cells remains to be determined. In addition, because NFAT often works together with other proteins[99,100], cofactors or other transcription factors could modify the functionality of NFAT in various contexts. It will be interesting to determine how the global set of NFATc1 target genes is affected in exhausted $CD8^+$ T cells.

An integrated genomics approach has been used to define genes that are induced by PD-1 ligation and also involved in T cell exhaustion in mice (chronic LCMV infection) and in humans (HIV infection)[63]. Such studies have identified BATF as a common transcriptional pathway downstream of PD-1 in exhausted T cells[63]. BATF forms dimers with the transcription factor c-Jun, displacing the transcription factor c-Fos, and can inhibit canonical AP-1-mediated transcription[101], although BATF–c-Jun dimers might actively promote transcription of other genes[102]. Overexpression and short interfering RNA–mediated knockdown of BATF have confirmed a key role for BATF in T cell dysfunction during HIV infection[63]. Understanding how BATF promotes exhaustion and whether it does this by negatively regulating AP-1 or by positively regulating the transcription of other genes in exhausted T cells is an important future goal.

In addition to defining an exhaustion-associated transcription factor, the studies of BATF are important for several reasons. First, an integrated genomics approach has shown that although inhibitory receptors such as PD-1 might attenuate proximal signals from the TCR, ligation of PD-1 can also induce the transcription of key genes involved in dysfunction. That finding suggested new ways of considering the mechanisms downstream of inhibitory receptors

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expressed by exhausted T cells (and other cells) during infection. Second, this approach embraces cross-species analysis of genomic data. Although there are unique aspects of the immune response to different viruses, the ability to compare across species and types of infection allows the definition of fundamental common pathways. Finally, that study and other work has taken advantage of considerable advances in the ability to generate and then extract meaning from large genomic data sets to understand the pathogenesis of chronic infections and immune system failure in such cases. It is now possible to define exhausted T cells in very specific and comprehensive transcriptional terms. In the future, such an approach should allow researchers to determine how much of a core program of T cell exhaustion is shared during different infections and also what unique features distinguish T cells responding to LCMV, HIV, HCV, HBV and other pathogens.

Exhaustion, anergy and senescence

What is the relationship between T cell exhaustion and T cell anergy or T cell senescence? Anergy can arise when T cells are stimulated *in vitro* with antigen in the absence of costimulation[103]. Such cells become nonresponsive to subsequent stimulation. Notably, anergy or adaptive tolerance (that is, *in vivo* anergy) seems to be a state of nonresponsiveness molecularly distinct from CD8$^+$ T cell exhaustion[87]. First, nonresponsiveness in anergic T cells is induced at the time of first antigen stimulation and is initiated rapidly. T cell exhaustion, in contrast, is progressive, with dysfunction worsening over time[87]. Unlike anergy, during initial T cell activation in chronic infection, T cells probably receive costimulation and undergo robust initial activation. Second, the gene-expression profiles of anergy and exhaustion seem to be at least partially distinct[87]. Although NFAT has a role in both situations, other anergy-associated genes such as *Rnf128* (also known as *Grail*), *Egr2* and *Egr3* do not seem to be upregulated in exhausted CD8$^+$ T cells[87]. Other transcriptional 'modules' could be shared between anergy and exhaustion, and it is important to examine this issue directly. Because most information about anergy has been provided by studies of CD4$^+$ T cells and much more is known about the exhaustion of CD8$^+$ T cells, these states of dysfunction could themselves be biased toward CD4$^+$ T cells or CD8$^+$ T cells, respectively. Understanding this issue in more detail could have substantial implications for therapeutic interventions during chronic infection.

Senescence and terminal differentiation are important features of many biological systems, including T cell responses. Markers such as KLRG1 in the mouse and CD57 in humans can be used to identify T cells with low proliferative capacity that seem to be terminally differentiated or senescent[93,104]. Many latent or 'smoldering' infections, such as infection with cytomegalovirus or Epstein-Barr virus, generate virus-specific T cells that express markers of senescence and/or terminal differentiation[105]. The precise definitions of cellular senescence, replicative senescence and terminal differentiation can vary in different biological systems. In a T cell response to infection, some T cells become terminally differentiated and lose proliferative potential, consistent with a simple definition of senescence. Exhausted CD8$^+$ T cells also have defects in proliferative potential. However, severely exhausted CD8$^+$ T cells have low expression of immunological markers of senescence such as KLRG1 (ref. 87). Similarly, the expression of CD57 is not strongly correlated with that of PD-1 during HIV infection[43], although there is a connection between PD-1 and telomere length in HIV-infected subjects[106]. In addition, in most settings CD8$^+$ T cells that express markers of senescence such as KLRG1 or CD57 can still carry out effector functions robustly, unlike exhausted CD8$^+$ T cells[107]. In contrast, the transcriptional profiles of repetitively stimulated CD8$^+$ T cells that have some characteristics of senescence or terminal differentiation also undergo some changes reminiscent of T cell exhaustion[108,109]. Although most of these data suggest that exhaustion and senescence are distinct mechanistic processes, the molecular relationships between exhaustion and senescence remain largely to be defined[107].

Reversing exhaustion

There has been considerable interest in reversing T cell exhaustion during chronic viral infection and cancer. Reversing T cell exhaustion by blocking the PD-1 pathway[40] is proof of principle that exhausted T cells (at least as a population) are not completely terminal. Combining blockade of inhibitory receptors (such as PD-1) or suppressive cytokines (such as IL-10) with blockade of other inhibitory receptors, cytokine blockade (such as blocking PD-1 and IL-10) or therapeutic vaccination are promising approaches for enhancing immunity during chronic infections[46,48,110–112]. The efficacy of these approaches probably depends on several factors, including the characteristics of the starting population of exhausted T cells. For example, these approaches will probably be more effective in situations in which antigen-specific T cells are present than in scenarios such as congenital carriers of HBV in whom central tolerance or peripheral deletion might have occurred, although this topic warrants direct examination. In addition, distinct subsets of exhausted CD8$^+$ T cells exist with different potentials for recovering function after blockade of the PD-1 pathway. Exhausted CD8$^+$ T cells with intermediate expression of PD-1 (PD-1^{int} cells) can be reinvigorated by blockade of the PD-1 pathway, whereas those with high expression of PD-1 (PD-1^{hi} cells) cannot[57] (**Fig. 2**). Potent reversal of exhaustion by therapeutic intervention might depend on the proportion of PD-1^{int} exhausted T cells versus PD-1^{hi} exhausted T cells present. Alternatively, the influence of other

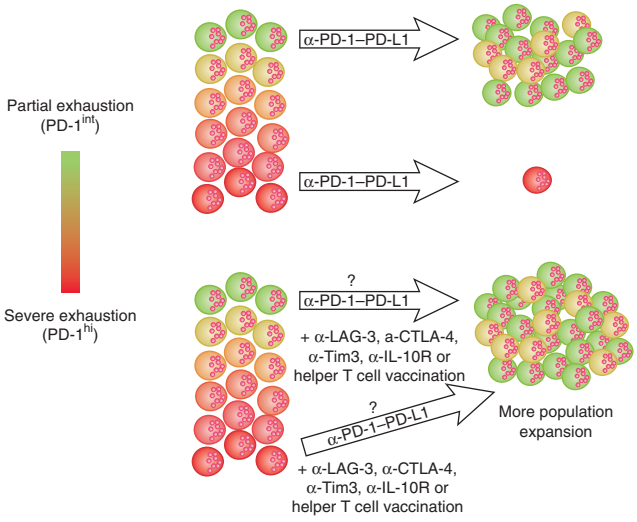

Figure 2 Subsets of exhausted T cells and combinatorial strategies to reverse exhaustion. Antibody blockade of the pathway consisting of PD-1 and its ligand (α-PD-1–PD-L1) reverses exhaustion and seems to selectively expand a subset of PD-1^{int} exhausted T cells (green and yellow cells), whereas PD-1^{hi} exhausted T cells (red cells) respond poorly (top). Many strategies have combined blockade of the PD-1 pathway with antibody blockade (α-) of other inhibitory receptors or of negative regulatory cytokines (such as IL-10) or therapeutic vaccination. Such strategies might augment the population expansion and/or survival of PD-1^{int} exhausted CD8$^+$ T cells already recovered by blockade of the PD-1 pathway or could lead to additional recovery of cells in the PD-1^{hi} subset of exhausted CD8$^+$ T cells (bottom). Tim3, inhibitory molecule; IL-10R, receptor for IL-10.

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inhibitory pathways on PD-1^{hi} exhausted T cells might allow specific combinations of interventions to target this subset of exhausted T cells. For many of these dual-blockade or combination strategies, it is unclear whether the enhanced reversal of exhaustion results from better population expansion and/or survival of PD-1^{Int} cells responding to PD-1 blockade or from the recruitment of otherwise nonresponsive (for example, PD-1^{hi}) subpopulations of exhausted T cells (**Fig. 2**). For situations such as HCV infection, in which liver cells are often poorly responsive to PD-1 blockade alone[113], determining how these combined interventions work will be important.

Remaining questions and future directions

Many important questions about T cell exhaustion remain unanswered. For example, why do exhausted CD8$^+$ T cells persist during chronic infection? One possibility is that these populations remain in case the host-pathogen balance changes. Cases of spontaneous control of HCV have been reported[114] and might reflect partial recovery of function of exhausted T cells. Another teleological idea about the reason that exhaustion evolved is one of limiting pathology. If virus-specific T cells that persist during chronic viral infections were as functionally responsive as memory CD8$^+$ T cells, even transient increases or 'blips' in viral replication would be associated with robust T cell population expansion and cytokine production; the consequence would probably be severe pathology. The existence of escape mutations indicates that T cell responses during chronic infection continue to apply some immunological pressure on persisting viruses. Perhaps (partial) exhaustion in these settings creates the balance between retaining limited ability to contain the infection and moderating immunopathology. Additional studies are necessary to test some of these ideas. Furthermore, it is not yet known whether the underlying mechanisms of the dysfunction of CD4$^+$ T cells and CD8$^+$ T cells during chronic infections are the same. Moreover, 'exhaustion' of B cells during chronic infection with HIV and in other settings has been described[115,116], and it will be interesting to compare the pathways of dysfunction of T cells, B cells and perhaps other lineages in the immune response to persisting pathogens. A central question—whether exhaustion can be fully reversed, leading to highly functional, antigen-independent, long-lived T cell memory—remains unanswered. The answer is probably related to the lineage relationships among exhausted, effector and memory T cells and also the population dynamics after reinvigoration of exhausted T cell responses. An additional key question is whether the increasing understanding of T cell exhaustion at the molecular and global transcriptional levels can be used to inform prophylactic vaccination strategies. For example, the transcriptional signature of an exhausted T cell should probably be avoided in the profiles of vaccine-induced T cells aimed at protection from HIV, HCV and other persisting pathogens. It will be useful to determine how information about T cell exhaustion can be integrated into the development therapeutic and prophylactic vaccination strategies for these diseases.

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