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Molecular and cellular insights into T cell exhaustion

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Abstract

In chronic infections and cancer, T cells are exposed to persistent antigen and/or inflammatory signals. This scenario is often associated with the deterioration of T cell function: a state called ‘exhaustion’. Exhausted T cells lose robust effector functions, express multiple inhibitory receptors and are defined by an altered transcriptional programme. T cell exhaustion is often associated with inefficient control of persisting infections and tumours, but revitalization of exhausted T cells can reinvigorate immunity. Here, we review recent advances that provide a clearer molecular understanding of T cell exhaustion and reveal new therapeutic targets for persisting infections and cancer.

During acute infections or vaccinations, naive T cells are activated and differentiate into effector T cells over the course of 1–2 weeks[1,2]. This differentiation is accompanied by robust proliferation, transcriptional, epigenetic and metabolic reprogramming, and the acquisition of cardinal features of effector T cells such as effector function, altered tissue homing and dramatic numerical expansion[1,2]. Following the peak of effector expansion, the resolution of inflammation and the clearance of antigen, most activated T cells die, but a subset persists and transitions into the memory T cell pool. These memory T cells downregulate much of the activation programme of effector T cells, yet they maintain the ability to rapidly reactivate effector functions upon restimulation[2]. In addition, memory T cells develop a key memory property of antigen-independent self-renewal, which is a type of stem cell-like, slow division that is driven by interleukin-7 (IL-7) and IL-15. There is considerable diversity and complexity of memory T cell subsets and differentiation following acute infections or vaccinations (for example, effector memory T cells versus central memory T cells)[2]. However, a key aspect of the development of functional, persisting memory T cells is that after the effector phase, memory development occurs in the absence of ongoing antigen stimulation and high levels of persisting inflammation.

By contrast, during chronic infections and cancer — which involve persistent antigen exposure and/or inflammation — this programme of memory T cell differentiation is markedly altered[3]. An altered differentiation state, termed T cell exhaustion, usually manifests with several characteristic features, such as progressive and hierarchical loss of

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effector functions, sustained upregulation and co-expression of multiple inhibitory receptors, altered expression and use of key transcription factors, metabolic derangements, and a failure to transition to quiescence and acquire antigen-independent memory T cell homeostatic responsiveness[3–5] (FIG. 1). Although T cell exhaustion was first described in chronic viral infection in mice[6,7], it has also been observed in humans during infections such as HIV and hepatitis C virus (HCV), as well as in cancer[3,5]. Importantly, while T cell exhaustion prevents optimal control of infections and tumours, modulating pathways overexpressed in exhaustion — for example, by targeting programmed cell death protein 1 (PD1) and cytotoxic T lymphocyte antigen 4 (CTLA4) — can reverse this dysfunctional state and reinvigorate immune responses[3,5,8,9]. Whereas T cell exhaustion and the reversal of this state of dysfunction have considerable relevance for tumours, an in-depth discussion of T cell exhaustion in cancer is beyond the scope of this Review and has been covered elsewhere recently[10,11].

Of note, exhausted T cells are not inert (BOX 1). These cells retain suboptimal but crucial functions that limit ongoing pathogen replication or tumour progression. Despite this host–pathogen ‘stalemate’ mediated by exhausted T cells, these cells are not effective in eradicating pathogens or tumours, and there has been considerable interest in avoiding or reversing exhaustion. The demonstration that T cell exhaustion is reversible (at least at the population level) rather than a terminal or irreversible fate provides a substantial clinical opportunity to use immunotherapy to improve immunity[9]. Although the immunological effects of these human treatments remain to be fully defined, emerging results support the notion that reversal of T cell exhaustion in humans is a causative mechanism for the marked antitumour effect that is seen in many patients receiving agents that block the PD1 pathway.

In this Review, we outline the characteristic features of altered functionality that are hallmarks of T cell exhaustion and focus on major advances in three key areas: first, inhibitory receptors and negative regulatory pathways; second, lack of canonical memory T cell properties and maintenance; and third, altered transcriptional control including recently defined progenitor and terminal progeny subsets of exhausted T cells controlled by T-bet and eomesodermin (EOMES)[12]. Where relevant, we also discuss similarities and differences between $CD4^+$ and $CD8^+$ exhausted T cells.

Developmental pathways of exhaustion

Persistent antigen exposure

A central question in this field has been: what causes T cell exhaustion? The development of $CD8^+$ T cell exhaustion probably integrates information from altered inflammatory and tissue microenvironments, other lymphocyte populations such as $CD4^+$ T cells, B cells and regulatory T (T_{Reg}) cells, and also inhibitory signals from cytokines and cell surface inhibitory and co-stimulatory receptors (FIG. 2). However, one key feature seems to be the chronic and probably continuous exposure to antigen rather than acutely terminated or intermittent exposure. Additional factors, including lack of $CD4^+$ T cell help[13] and perhaps instructive signals directly from inhibitory receptors[14], probably also contribute to T cell exhaustion. Early studies in the chronic lymphocytic choriomeningitis virus (LCMV) model demonstrated that the severity of exhaustion (and ultimately the deletion of antigen-specific

T cells) correlated with the level of antigen stimulation[15]. The importance of the level of antigen persistence in exhaustion was also confirmed in other mouse models and in human HIV-1 infection[16–18]. Indeed, if $CD8^+$ T cells are primed during infection with a chronic strain of LCMV but removed from persisting antigen stimulation early (approximately 1 week after infection), these cells can differentiate into fully functional $CD8^+$ memory T cells[19,20]. However, if these T cells are instead exposed to a persisting antigen for 2–4 weeks, T cell exhaustion becomes established, and these cells do not recover normal memory differentiation simply by removal from antigen exposure[19,20]. These observations are consistent with the notion that early antiviral treatment of HIV infection, and possibly HCV infection, in humans can preserve functional T cell responses[17,21]. In addition, specific T cell receptor (TCR)-dependent pathways, including those mediated by nuclear factor of activated T cells (NFAT) and sprouty homologue 2 (SPRY2), have been implicated in T cell exhaustion, consistent with a role for ongoing TCR stimulation[22–24]. Furthermore, chronic antigen stimulation also leads to sustained expression of PD1 through NFAT cytoplasmic 1 (NFATc1)[25]. It is likely that PD1 further modulates the level of TCR signalling and provides an important feedback loop or rheostat to temper chronic antigen stimulation[26,27] (FIG. 3). Thus, the level and duration of chronic antigen stimulation and infection seem to be key factors that lead to T cell exhaustion and correlate with the severity of dysfunction during chronic infection.

Inhibitory receptors

Inhibitory receptors are crucial negative regulatory pathways that control autoreactivity and immunopathology[28]. Although inhibitory receptors are transiently expressed in functional effector T cells during activation, higher and sustained expression of inhibitory receptors is a hallmark of exhausted T cells. The inhibitory signalling pathway mediated by PD1 in response to binding of PD1 ligand 1 (PDL1) and/or PDL2 offers an illustrative example[27,29,30]. Whereas our understanding of the molecular mechanisms by which the inhibitory receptor PD1 controls T cell exhaustion remains incomplete, there are several mechanisms by which inhibitory receptors such as PD1 might regulate T cell function[5,29] (FIG. 3): first, by ectodomain competition, which refers to inhibitory receptors sequestering target receptors or ligands and/or preventing the optimal formation of microclusters and lipid rafts (for example, CTLA4 (REF. 31)); second, through modulation of intracellular mediators, which can cause local and transient intracellular attenuation of positive signals from activating receptors such as the TCR and co-stimulatory receptors[32–34]; and third, through the induction of inhibitory genes[14].

Whereas there is some knowledge about PD1, our understanding of the intracellular mechanisms of action of inhibitory receptors — including those of PD1 — is incomplete. The intracellular domain of PD1 contains an immunoreceptor tyrosine-based inhibitory motif (ITIM) and an immunoreceptor tyrosine-based switch motif (ITSM)[35]. *In vitro* studies suggest a role for the ITSM in recruiting the tyrosine-protein phosphatase SHP1 (also known as PTPN6) and/or SHP2 (also known as PTPN11)[33,36]. The role of the ITIM in PD1 function remains poorly understood. Other evidence implicates a role for PD1 signalling in modulating the phosphoinositide 3-kinase (PI3K), AKT and RAS pathways[32,37], and also links PD1 to cell cycle control[38]. Notably, much of our information about how PD1 controls

T cell signalling is derived from *in vitro* studies of acutely activated T cells. *In vivo* studies of the role of PD1 during acute T cell activation and expansion suggest a possible role for PD1 signalling in either increasing mobility paralysis[39] or decreasing migratory arrest[26], depending on the context. Finally, signalling downstream of PD1 may in fact induce the expression of genes that could negatively regulate the expression of effector genes, such as *BATF*, which encodes the activator protein 1 (AP-1) family member basic leucine zipper transcription factor ATF-like[14]. Despite this elegant work, it is unclear how these observations relate to exhausted T cells exposed to chronic infection *in vivo*.

PD1 expression is rapidly upregulated upon T cell activation[30], and it may persist at moderate levels in healthy humans[40,41], indicating that PD1 expression alone is not a unique feature of exhausted T cells. However, during chronic infections PD1 expression can be substantially higher than observed on functional effector or memory $CD8^+$ T cells[42,43]. During chronic infection, sustained upregulation of PD1 is usually dependent on continued epitope recognition[44], although examples exist of residual PD1 expression even after removal of persisting antigen signalling[20,45].

In addition to PD1, exhausted T cells express a range of other cell surface inhibitory molecules (FIG. 3). Exhausted T cells can co-express PD1 together with lymphocyte activation gene 3 protein (LAG3), 2B4 (also known as CD244), CD160, T cell immunoglobulin domain and mucin domain-containing protein 3 (TIM3; also known as HAVCR2), CTLA4 and many other inhibitory receptors[18]. Typically, the higher the number of inhibitory receptors co-expressed by exhausted T cells, the more severe the exhaustion. Indeed, although individual expression of PD1 or other inhibitory receptors is not indicative of exhaustion, co-expression of multiple inhibitory receptors is a cardinal feature. These co-expression patterns are mechanistically relevant, as simultaneous blockade of multiple inhibitory receptors results in synergistic reversal of T cell exhaustion. This concept was demonstrated for PD1 and LAG3 (REF. 18) in chronic LCMV infection, and for PD1 and CTLA4 in HIV infection[46], other infections[47] and cancer[48,49]. Many other combinations of inhibitory receptors such as PD1 and TIM3 (REFS 50–53) can also co-regulate exhausted T cells. PD1 and CTLA4 blockade in patients with melanoma demonstrated impressive tumour control[54], and clinical trials of other combinations of agents blocking inhibitory receptors are underway (for example, ClinicalTrials.gov identifiers NCT01968109, NCT02210117 and NCT02408861, which are among >120 other trials involving the PD1 pathway). Overall, these data on the role of inhibitory receptors in co-regulation of T cell exhaustion suggest that these pathways are non-redundant. These molecules come from diverse structural families, bind ligands with distinct expression patterns and have distinct intracellular signalling domains (FIG. 3). Thus, there is the potential to tailor or tune the type and magnitude of exhausted T cell reinvigoration.

In addition to inhibitory receptors, it has become clear that co-stimulatory receptors are involved in T cell exhaustion[29]. For example, desensitization of co-stimulatory pathway signalling through the loss of adaptor molecules can serve as a mechanism of T cell dysfunction during chronic infection. The signalling adaptor tumour necrosis factor receptor (TNFR)-associated factor 1 (TRAF1) is downregulated in dysfunctional T cells in HIV progressors, as well as in chronic LCMV infection[55]. Adoptive transfer of $CD8^+$ T cells

expressing TRAF1 enhanced control of chronic LCMV infection compared with transfer of TRAF1-deficient $CD8^+$ T cells, which indicates a crucial role for TRAF1-dependent co-stimulatory pathways in this setting[55]. It has also been possible to exploit the potential beneficial role of co-stimulation to reverse exhaustion by combining agonistic antibodies to positive co-stimulatory pathways with blockade of inhibitory pathways. 4-1BB (also known as CD137 and TNFRSF9) is a TNFR family member and positive co-stimulatory molecule that is expressed on activated T cells. Combining PD1 blockade and treatment with an agonistic antibody to 4-1BB dramatically improved exhausted T cell function and viral control[56]. Although a simple model of positive versus negative co-stimulation during T cell exhaustion probably has mechanistic validity, the diversity of pathways and much of the experimental data suggest that specific qualitative signals may be imparted by distinct co-stimulatory and co-inhibitory pathways.

Soluble mediators

Soluble molecules are a second class of signals that regulate T cell exhaustion (FIGS 2,3); these include immunosuppressive cytokines such as IL-10 and transforming growth factor-β (TGFβ) and inflammatory cytokines such as type I interferons (IFNs) and IL-6. For example, the IL-10–IL-10 receptor (IL-10R) pathway has received considerable attention for its role in T cell exhaustion. Blockade of IL-10 restores T cell function and improves viral control during chronic viral infections, demonstrating that IL-10 promotes or fosters T cell exhaustion[57,58]. Studies of LCMV infection in mice and HIV in humans demonstrated that many cell types can be the source of IL-10 during chronic infection including dendritic cells (DCs), monocytes and/or $CD4^+$ T cells[59–61]. The most relevant source of this cytokine remains a matter of debate, although recent studies suggest that T_H1 cells expressing B lymphocyte-induced maturation protein 1 (BLIMP1; also known as PRDM1) are important IL-10 producers in chronic LCMV infection[62]. Simultaneous blockade of IL-10 and the PD1 pathway in mice synergistically reverses $CD8^+$ T cell exhaustion and enhances viral control, which indicates a role for IL-10 in controlling $CD8^+$ T cell exhaustion[63]. Some evidence suggests a potential connection between the PD1 pathway and IL-10 production through induction of IL-10 by monocytes following ligation of PD1 by PDL1 (REF. 60). Despite the clear evidence that blocking IL-10 reverses exhaustion, the molecular events downstream of IL-10 signalling (presumably via signal transducer and activator of transcription 3 (STAT3)), which shape T cell exhaustion, remain to be more precisely defined.

TGFβ has also been implicated in T cell exhaustion. Phosphorylation of SMAD2 in $CD8^+$ T cells was increased during chronic LCMV infection compared with acute infection, which suggests a role for TGFβ signalling. *In vivo* inhibition of TGFβ signalling in $CD8^+$ T cells through expression of a dominant-negative receptor improved the function of exhausted T cells[64]. However, recent studies in mice using systemic administration of a TGFβ inhibitor or a blocking antibody found little benefit of these treatments[65,66]. Although it is difficult to directly compare the genetic approach to antibody- and inhibitor-based strategies, these observations may warrant further evaluation of the role of this immunoregulatory pathway in driving $CD8^+$ T cell exhaustion.

The type I IFNs IFNα and IFNβ (hereafter referred to as IFNα/β) are crucial inflammatory cytokines that have essential antiviral functions early during infection. In most cases, acute viral control is dramatically compromised in the absence of IFNα/β signalling; this was recently confirmed in simian immunodeficiency virus (SIV) infection of macaques[67]. Moreover, IFNα/β signalling can provide a critical 'signal 3' for proper activation and differentiation of $CD8^+$ T cells following priming[68]. In addition to these crucial innate antiviral effects, however, recent studies have demonstrated a surprising role for chronic type I IFN signalling in facilitating viral persistence by promoting immune suppression and lymphoid tissue destruction during chronic infection. Despite the direct antiviral activity of IFNα/β, blocking this pathway during LCMV clone 13 infection paradoxically enhanced control of chronic viral replication and reversed and/or prevented T cell exhaustion[69,70]. Interestingly, applying this IFNα/β blockade early (within the first 1–2 days after infection) prevented later development of T cell exhaustion, suggesting a possible early role for IFNα/β signalling in 'programming' exhaustion. The IFNα/β effect seemed to operate via $CD4^+$ T cells, though the precise mechanism remains to be defined. For example, IFNα/β can induce several immunoregulatory molecules including IL-10, PDL1 and indoleamine 2,3-dioxygenase (IDO) in antigen-presenting cells (APCs) and other cell types[71]. Thus, whereas IFNα/β signals enhance viral control early during infection directly and indirectly (that is, by promoting effector $CD8^+$ T cell differentiation), chronic exposure to IFNα/β can be detrimental and/or enhance T cell dysfunction during chronic infections. Indeed, exposure to chronic inflammation alone (that is, in the absence of TCR signalling) can impair optimal $CD8^+$ memory T cell differentiation[72]. By contrast, other inflammatory pathways — including those induced by IL-6 and IL-27 — may be required for controlling chronic viral infection and avoiding $CD8^+$ T cell exhaustion[73,74]. Although there is no clear evidence that IL-6 and IL-27 have direct intrinsic effects on $CD8^+$ T cell exhaustion, these cytokines regulate $CD4^+$ T cell differentiation and/or exhaustion during chronic viral infection. Thus, among inflammatory cytokines, there are some such as IL-6 and IL-27 that may have positive effects and skew T cells away from exhaustion, whereas others such as IFNα/β have more complex roles including positive and negative effects depending on the context. Effects of these cytokines on $CD4^+$ T cell responses, such as enhanced IL-21 production, may have down-stream effects on preventing $CD8^+$ T cell exhaustion (see below).

Regulatory cells

It is well known that forkhead box P3-expressing ($FOXP3^+$) $CD4^+$ T_{Reg} cells influence immune responses during many infections[75,76] (FIG. 2). T_{Reg} cells can suppress T cell responses in acute infection and in the acute phase of chronic infection. The frequency of T_{Reg} cells can be increased in human chronic HIV and HCV infections, in which T_{Reg} cells may limit the responses of antiviral effector T cells *in vitro*[76]. However, it is still unclear whether T_{Reg} cells can directly cause $CD8^+$ T cell and $FOXP3^-CD4^+$ T cell exhaustion. As T_{Reg} cells are a source of IL-10, TGFβ and perhaps other suppressive cytokines (for example, IL-35)[76], such a scenario might exist. However, precisely how T_{Reg} cells affect the development of T cell exhaustion remains incompletely defined. Recent work in the chronic LCMV model system demonstrated an interaction between T_{Reg}cells and the PD1 pathway in regulating exhausted $CD8^+$ T cells because simultaneous depletion of T_{Reg} cells and blockade of PD1 signalling had a highly synergistic effect on viral control and reversal of

exhaustion[77]. These data suggest a role for T_{Reg} cells in $CD8^+$ T cell exhaustion. In addition to $FOXP3^+CD4^+$ T_{Reg} cells, other regulatory cell types — such as immunoregulatory APCs, myeloid-derived suppressor cells (MDSCs)[78,79], natural killer (NK) cells and even $CD8^+$ regulatory populations[80–82] — may influence viral persistence during chronic infections and directly or indirectly promote T cell exhaustion. Professional APCs can be direct targets of viruses, particularly during persisting infections, and dysfunction of DC cytokine production and cross-presentation has been reported in some chronic infections[78,83]. Indeed, early dysfunction of DCs may have a major role in facilitating viral persistence in some contexts[83]. Furthermore, destruction of tissue architecture and fibrosis of secondary lymphoid organs have been reported in both mice and human chronic infection[84–86]. Changes in lymphoid tissue architecture may contribute to poor T cell responses, but precisely how these anatomical features influence T cell exhaustion remains to be fully defined.

In addition to a possible role for T_{Reg} cells and other regulatory populations, positive signals from other cells are crucial in avoiding T cell exhaustion. It has long been known that loss of $CD4^+$ T cell help during pathogen persistence can underlie or contribute to defective $CD8^+$ T cell responses. Depletion of $CD4^+$ T cells in the chronic LCMV model results in failed containment of chronic infection and more severe $CD8^+$ T cell exhaustion[13]. In HIV infection, loss of $CD4^+$ T cells is associated with increased exhaustion of $CD8^+$ T cells and with disease progression. Although $CD4^+$ T cells can clearly become exhausted (BOX 2), the impact of changes in the $CD4^+$ T cell response on $CD8^+$ T cell exhaustion is highly relevant. One key factor produced by $CD4^+$ T cells during chronic infections is IL-21. In the absence of IL-21 signalling, $CD8^+$ T cell exhaustion is substantially worse during chronic LCMV infection[87–89], with similar observations in HIV infection[90,91]. IL-21 and $CD4^+$ T cell help are also essential for optimal antibody responses, and recent work highlights the importance of antibody, including non-neutralizing antibody, during ongoing chronic viral infections[92–95]. It is interesting in this respect that antiviral $CD4^+$ T cells generated during chronic infection demonstrate a skewing towards the T follicular helper (T_{FH}) cell phenotype[95,96]. Further investigation is required to determine whether the key role of IL-21 in limiting CD8+ T cell exhaustion is only due to a direct effect on $CD8^+$ T cells or whether it also includes a component of antibody-mediated reduction in antigen load.

Overall, although various negative and positive regulatory pathways have been implicated in exhaustion, the role of these pathways in actually causing $CD8^+$ T cell exhaustion has been less clear, in part because we are only just beginning to understand the developmental origin of exhausted $CD8^+$ T cells.

Origin, homeostasis and durability

Questions have arisen about the origin of exhausted $CD8^+$ T cells. Are these cells simply persisting effector T cells? How are exhausted $CD8^+$ T cells, different from other dysfunctional T cells, such as senescent T cells or anergic T cells (TABLE 1)? Whereas there are some similarities between exhausted and $CD8^+$ T cells, the transcriptional profiles of these two subsets are distinct[4,97]. Phenotypic markers can be identified that distinguish effector T cells from exhausted T cells, including markers that are highly expressed on

effector CD8$^+$ T cells — such as CD44, LY6C and killer cell lectin-like receptor subfamily G member 1 (KLRG1) — but are expressed at low or intermediate levels on exhausted T cells, as well as markers such as inhibitory receptors that are expressed at substantially higher levels by exhausted CD8$^+$ T cells (TABLE 1). In addition, whereas CD8$^+$ effector T cells co-express the transcription factors T-bet and EOMES[98], exhausted T cell subsets express either T-bet or EOMES in a somewhat mutually exclusive pattern[12] (see below).

Recent studies have directly compared the transcription programmes of exhausted CD4$^+$ and CD8$^+$ T cells in the lymphocytic choriomeningitis virus (LCMV) model system[96]. These studies confirmed a core transcriptional signature of T cell exhaustion common to both CD4$^+$ and CD8$^+$ T cells that included some transcription factors, inhibitory receptors, IFNα/β signature genes, and dysregulated pathways such as proliferation. However, despite this similar core programme, exhausted CD4$^+$ T cells were distinct from exhausted CD8$^+$ T cells. For example, whereas PD1 expression was common to both lineages, the expression pattern of other inhibitory receptors was distinct for each subset[46,96]. Of note, the expression pattern of transcription factors differed extensively; exhausted CD4$^+$ T cells had altered expression of, for example, GATA-binding protein 3, B cell lymphoma 6 (BCL-6) and Helios, which was not observed for exhausted CD8$^+$ T cells. Together with altered expression of IL-10 and IL-21 and the redirection of CD4$^+$ T cell responses from T helper 1 cells to T_{FH}-like cells[95,96,146], these data suggest an altered and perhaps more complex differentiation programme for exhausted CD4$^+$ T cells than previously appreciated. Indeed, transcription factor co-expression patterns in exhausted CD4$^+$ T cells reveal substantial heterogeneity in this population[96]. Thus, although our understanding of exhaustion in CD4$^+$ T cells still lags behind that in CD8$^+$ T cells, the existing data suggest a distinct pattern of differentiation that may include enhanced skewing towards T_{FH} cell-like cells, altered immunoregulatory pathways and a lineage-specific network of transcription factor control[96]. Future studies are needed to explore this heterogeneity in differentiation in more detail and to define the pathways and mechanisms that might be used to reverse dysfunction and reinvigorate exhausted CD4$^+$ T cells.

Some of these observations also suggest a distinction between phenotypically defined senescence and exhaustion (TABLE 1). Whereas senescent CD8$^+$ T cells are often defined by high expression of markers such as KLRG1 and/or CD57 (REFS 41,99,100), exhausted CD8$^+$ T cells tend to have low expression of these markers[97]. Furthermore, exhausted CD8$^+$ T cells have high expression of PD1, whereas senescent cells do not[101]. Although both exhausted and senescent CD8$^+$ T cells have low proliferative capacity, this difference in phenotype suggests a potential distinction between these two cell states[100]. Analysis of the lineage origin of exhausted CD8$^+$ T cells in the chronic LCMV model has demonstrated that exhausted T cells do not derive from the ‘senescent’ KLRG1$^+$ subset of effector CD8$^+$ T cells but rather from the CD127$^+$ subset of memory precursors that are present in the effector phase[20] (FIG. 1). These studies reinforce the notion that exhaustion and senescence are distinct and that exhausted CD8$^+$ T cells arise from a skewed pattern of memory T cell differentiation rather than from a residual population of effector CD8$^+$ T cells.

Exhausted CD8$^+$ T cells have maintenance characteristics that are distinct from memory T cells that are generated after acute infection. Following acute infection, CD8$^+$ memory T

cells can persist long term in the absence of antigen via IL-7- and IL-15-driven slow, steady homeostatic turnover (see below). This self-renewal capacity develops after the effector phase as $CD8^+$ memory T cells acquire high co-expression of both CD122 (also known as IL-2Rβ) and CD127, which are the key receptor subunits for responsiveness to IL-7 and IL-15 (REFS 1,3,102). By contrast, exhausted $CD8^+$ T cells have lower expression of CD122 and CD127 during chronic infection and are unable to respond efficiently to IL-7 or IL-15. As a result, exhausted T cells are maintained poorly when adoptively transferred to infection (antigen)-free recipients, although in some settings small numbers of these cells may persist[45,103–107] (TABLE 1). Virus-specific $CD8^+$ T cells become 'addicted' to their cognate antigen, and continuous TCR stimulation is needed for the maintenance of exhausted T cells[103,104]. A similar scenario is also likely to occur in humans based on apparent loss of virus-specific $CD8^+$ T cells after antiviral treatment or epitope escape[21,108,109]. This antigen-dependent maintenance is accompanied by extensive ongoing division of a subset of exhausted $CD8^+$ T cells; for example, a subset of exhausted T cells gives rise to cells that divide at least 5–7 times per week for a prolonged period of time in chronic LCMV infection[12,104]. This maintenance pattern is in stark contrast to the slow homeostatic turnover of memory $CD8^+$ T cells, for which there are only one or two divisions in a month. Similar maintenance phenotypes have also been reported for T cells responding to persisting herpesviruses[110,111].

A key unanswered question, therefore, is: what happens to exhausted $CD8^+$ T cells in clinical settings in which chronic infections are cured? Will these exhausted $CD8^+$ T cells disappear? Will they differentiate into functional T cells? As we rapidly move towards a drug-mediated cure of HCV infection — a setting that often shows marked T cell exhaustion — understanding the fate of exhausted T cells after cure, and determining whether these cells can provide protection if re-exposed to HCV, will become critical. Similar scenarios may also exist in cancer immunotherapy. Better defined cellular and molecular mechanisms that underlie the persistence of exhausted $CD8^+$ T cells will be important in answering these questions.

Homeostatic cytokines

In contrast to the immunosuppressive cytokines described above, common γ-chain cytokines can positively affect T cell responses during chronic viral infections. IL-2 treatment during chronic LCMV infection resulted in an increase in the number of antigen-specific $CD8^+$ T cells and increased viral control, consistent with reversal of T cell exhaustion[112]. Combining IL-2 treatment with blockade of the PD1-mediated inhibitory pathway had striking synergistic effects for re-invigorating exhausted $CD8^+$ T cells and decreasing viral load[113]. IL-7 treatment early during chronic LCMV infection augmented T cell responses and enhanced viral clearance, effects that were in part dependent on inhibition of suppressor of cytokine signalling 3 (SOCS3) and on endogenous IL-6 (REFS 114,115). This early IL-7 treatment probably occurred at time points before severe loss of IL-7 responsiveness during chronic LCMV infection. As discussed above, IL-21 also has an important role during chronic infection. IL-21, produced primarily by antigen-specific $CD4^+$ T cells, is required to sustain antiviral $CD8^+$ T cell responses and prevent exhaustion during chronic LCMV infection[87–89,91]. However, IL-21 may also restrict virus-driven T_{Reg} cell populations[116].

Despite many important insights about the role of common γ-chain cytokines in chronic infection, it remains unclear where and when these signals are important *in vivo* during chronic infections, what regulates their availability and how signalling via IL-21R, for example, alters the cellular and molecular mechanisms that maintain exhausted $CD8^+$ T cells.

Maintaining exhausted T cell populations

The identification of subsets of exhausted $CD8^+$ T cells defined by expression of PD1, CD44, other phenotypic markers and the transcription factors T-bet and EOMES has enabled the lineage dynamics of these populations to be defined[12,42] (FIG. 1). Specifically, T-bet and EOMES delineate key subsets of exhausted $CD8^+$ T cells and a proliferative hierarchy that maintains exhausted T cell populations[12]. Exhausted $CD8^+$ T cells are under considerable strain to maintain ongoing antigen-driven activation and proliferation without depleting the 'clone' of antigen-specific $CD8^+$ T cells. T-bet and EOMES cooperate to sustain the overall exhausted $CD8^+$ T cell pool. Specifically, two distinct subpopulations exist: a small T-$bet^{hi}PD1^{mid}$ progenitor pool with residual proliferative potential and a numerically larger population of $EOMES^{hi}PD1^{hi}$ terminal progeny with higher co-expression of other inhibitory receptors and limited proliferative capacity[12] (FIG. 1). This role for T-bet is interesting because whereas high levels of T-bet expression are closely associated with terminal differentiation in acute infection, high T-bet expression in chronic infection partially represses the expression of PD1 and other inhibitory receptors and identifies a subset of exhausted $CD8^+$ T cells that retains some (albeit weakened) proliferative capacity during chronic infection[12,42,117]. Persistent antigen causes T-$bet^{hi}PD1^{mid}$ to lose T-bet expression, undergo proliferation and convert to $EOMES^{hi}PD1^{hi}$ cells[12]. The resulting terminal progeny have weaker cytokine production but enhanced killing ability. They also accumulate in peripheral tissues and have a total population size that is ~20-fold greater than the T-bet^{hi} progenitor pool. Importantly, PD1 pathway blockade seems to only reinvigorate the T-bet^{hi} subset, while having little to no impact on the $EOMES^{hi}$ cells[42], indicating an important aspect of population dynamics in checkpoint blockade-mediated reversal of T cell exhaustion. Importantly, genetic deletion of either transcription factor leads to loss of the respective subset (that expresses high levels of the transcription factor), failed long-term maintenance of the exhausted $CD8^+$ T cell pool and loss of the ability to establish a host–pathogen stalemate, resulting in high viral loads. Although these lineage relationships were defined in the chronic LCMV model, central aspects of this model — including the existence of exhausted $CD8^+$ T cells subsets defined by T-bet and EOMES, and a bias towards the $EOMES^{hi}$ terminally differentiated subset with more severe infection — have been confirmed in human HCV and HIV infection[12,118]. Persistently high levels of antigen might be anticipated to cause continuous proliferation and depletion of the progenitor pool. Although there is some evidence for erosion of the T-bet^{hi} progenitor pool in prolonged viraemia[12], whether this population self-renews is unclear. It is also possible that replenishment could occur from new thymic emigrants primed on persisting antigen[119,120]. Although mice that have undergone thymectomy do not have major defects in maintaining antiviral $CD8^+$ T cell responses during chronic infection[121], and adoptively transferred TCR-transgenic cells become exhausted and are maintained long-term without thymic

contribution[117,122], new thymic emigrants could make quantitative or qualitative contributions to virus-specific $CD8^+$ T cell responses in the long term. Thus, the existence of a proliferative hierarchy for the maintenance of exhausted $CD8^+$ T cells provides a framework for understanding the durability of these cells over time, but many important questions remain about the signals involved and molecular mechanisms underlying this process. In addition, how these events change during infections with different levels and/or types of persistence remains to be explored.

Transcriptional control of exhaustion

Recent studies have applied genomic approaches to investigate the transcriptional circuitry underlying T cell exhaustion. Both $CD4^+$ and $CD8^+$ exhausted T cells have a transcriptional profile that is markedly different from effector and memory $CD4^+$ or $CD8^+$ T cells, including major changes in the expression of inhibitory and co-stimulatory receptors, transcription factors, signalling molecules, cytokine and chemokine receptors, and genes involved in metabolism[4,96,97]. Thus, in addition to phenotyping, fate-mapping and functional analysis, genomic studies also support the concept that exhausted T cells represent a unique state of T cell differentiation. These studies have also revealed key differences between the exhausted phenotypes of $CD4^+$ and $CD8^+$ T cells (BOX 2).

Although considerable progress has been made in defining centrally important transcription factors, a lineage-specific transcription factor for exhausted T cells has not been identified. Nonetheless, in addition to T-bet and EOMES discussed above, the transcription factors BLIMP1, NFAT, BATF, von Hippel–Lindau disease tumour suppressor (VHL), FOXO1 and FOXP1 have been implicated in $CD8^+$ T cell exhaustion[12,14,22,24,117,123–126], and it is likely that other transcription factors involved in T cell exhaustion remain to be identified. A key concept that has emerged, however, is that although these transcription factors can have roles in other T cell populations, the expression pattern, the genes controlled and the manner in which key transcription factors operate can be distinct in exhausted T cells compared with in other T cell subsets (FIG. 4). In other words, there are exhaustion-specific functions for key transcription factors. For example, whereas T-bet is expressed by, and has a functional role in the formation of, terminally differentiated $CD8^+$ T cells in acute infection[1,127], during chronic infection T-bet controls the population of non-terminal progenitor cells within the exhausted T cell pool[12] (see above and FIG. 1). Similarly, EOMES is involved in central memory T cells following acute infection, when it is involved in controlling quiescence and homeostatic turnover[128–130]. However, during chronic infection EOMES controls the formation of a terminally differentiated (non-quiescent) subset of exhausted T cells that is highly enriched in peripheral tissues[12]. This distinct context-specific reuse of transcription factors was further interrogated using transcriptional network analysis, which demonstrated that the same transcription factors can have markedly different functions in memory $CD8^+$ T cells versus exhausted $CD8^+$ T cells[4]. The concepts of context-specific transcription factor function and combinatorial transcription factor control of cellular differentiation and/or lineage development have also been observed in other settings[131,132]. These different functions could occur for various reasons including the availability of distinct cofactors, concentration-dependent binding to different genomic sites and altered genomic accessibility due to epigenetic changes (FIG. 4).

The epigenome provides the context in which transcription factors function. Although global epigenetic landscape information does not yet exist for exhausted T cells, studies of the *Pdcd1* locus (which encodes PD1) have been informative (FIG. 4b). Analysis of the *Pdcd1* promoter region in acutely resolved LCMV infection demonstrated that these regions were largely demethylated in the effector phase and then became remethylated as infection resolved and $CD8^+$ T cell memory formed[122]. By contrast, the *Pdcd1* locus became completely demethylated in chronic LCMV infection and no remethylation was observed, even when viral titres and PD1 protein expression by exhausted $CD8^+$ T cells decreased[122]. Similar data were obtained in studies examining well-controlled HIV infection[133]. These observations and other studies[134] suggest not only an important layer of epigenetic regulation on gene expression in $CD8^+$ T cell exhaustion but also provide evidence for a durable imprint of exhaustion in the epigenome. Future studies are clearly warranted to define global epigenetic changes in more detail, to determine how such an imprint will affect the success of immunotherapies and the development of memory following cure of chronic infections.

Open questions and future directions

Although advances over the past decade have shed considerable light on the mechanisms of T cell exhaustion and have revealed novel therapeutic opportunities, there are still many important unanswered questions. The recognition of potent and diverse negative regulatory pathways, such as the PD1 pathway, that can be targeted experimentally and clinically has been a major step forward with the potential to revolutionize immunotherapy of chronic infections and cancer. Despite this promise, we still understand surprisingly little about the molecular mechanisms by which these pathways operate, and where, when and how these negative pathways signal and initiate or execute a programme of exhaustion. Moreover, we still lack a true molecular understanding of the intriguing observations of synergy when targeting multiple pathways simultaneously for reversal of T cell exhaustion. In addition to inhibitory receptors, one of the emerging defining features of T cell exhaustion is the altered use of key transcription factors (FIG. 4). In general, we are only just beginning to understand the transcriptional coordination of T cell exhaustion, how these transcriptional networks change over time and what happens to these events upon reversal of exhaustion.

In addition to understanding the molecular mechanisms of inhibitory receptor and transcription factor function in T cell exhaustion, several other key areas are likely to reveal substantial new insights into fundamental aspects of T cell exhaustion. First, we lack an understanding of the epigenetic landscape of T cell exhaustion (FIG. 4). A second related issue is to define the nature of terminal differentiation in the exhausted T cell pool. Cellular reprogramming studies have demonstrated that, if taken back to an embryonic stem cell, exhausted $CD8^+$ T cells can give rise to new naive T cells[135]. Although this is important in demonstrating the concept of reprogramming, such an approach may be challenging to implement clinically. Thus, it will be important to define the nature of terminal differentiation versus reprogramming that can occur for exhausted T cells at the epigenetic level. Given the clinical opportunities with PD1 blockade and also the ability to cure chronic infections such as HCV, it will be crucial to investigate these issues. Finally, numerous recent studies have addressed the critical importance of metabolism in regulating the T cell

differentiation[136]. Although emerging studies have begun to investigate these issues for chronic infections[124,125], a more comprehensive understanding of metabolic alterations in exhausted T cells will probably be highly informative.

In summary, T cell exhaustion represents a distinct state of T cell differentiation with considerable clinical relevance. Recent work defining the mechanisms of T cell exhaustion has provided many insights as well as clinical opportunities. Despite this progress, our understanding of T cell exhaustion and how to most effectively reverse this state remains incomplete. Future mechanistic and clinical studies are needed to develop the next generation of immune-based interventions for chronic infections and cancer.

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Glossary

Effector memory T cells	A subset of memory T cells that resides in peripheral tissues and retains high levels of effector function such as the production of interferon-γ and granzymes.
Central memory T cells	A subset of memory T cells that expresses CD62L and CC-chemokine receptor 7 and resides mainly in the lymph nodes and spleen. These cells have high proliferative potential upon antigen re-encounter.
Lymphocytic choriomeningitis virus (LCMV)	A virus that induces a strong T cell response and therefore provides good mouse model of infection. Although they only differ by two nucleotides, the Armstrong strain of LCMV causes acute infection and the clone 13 strain causes chronic infection. As major antigenic epitopes are conserved between these two strains, the fate of antigen-specific T cells can be easily compared (that is, memory after infection with the Armstrong strain versus exhaustion after infection with the clone 13 strain).
Antigen-presenting cells (APCs)	T cells can be activated when antigen is displayed by major histocompatibility complexes (MHC restriction). This antigen presentation is largely executed by professional APCs, particularly dendritic cells. APCs also influence T cell differentiation by producing cytokines such as type I interferons and interleukin-12.

Senescent T cells	Cells that enter a terminal differentiation state owing to excessive cell replication. This state is associated with irreversible cell cycle arrest and telomere shortening.
Anergic T cells	An unresponsive state that is induced by suboptimal stimulation (that is, signal 1 without signal 2) at the time of priming by antigen.
Epigenetic landscape	Historically, this is proposed to refer to embryonic development and how genes might interact with their surroundings to lead to the expression of a certain phenotype. The current view of the epigenetic landscape is that overall epigenetic changes such as (but not limited to) DNA methylation and histone modifications, are accumulated genome wide and have strong impact on cellular differentiation.

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Box 1

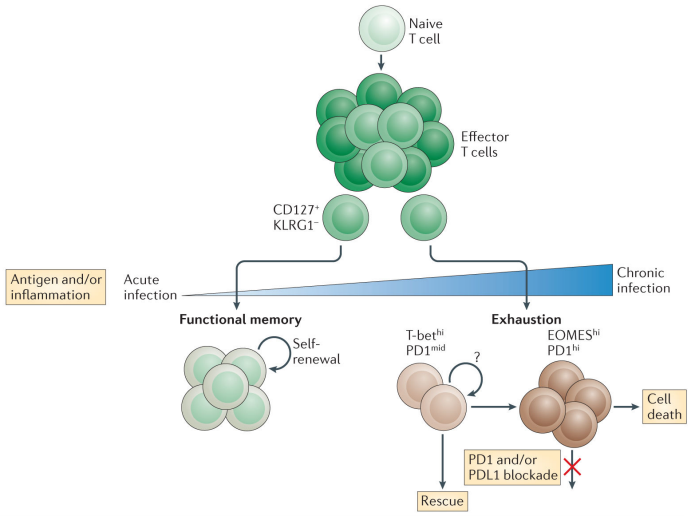
Evolutionary perspective on T cell exhaustion

What is the biological significance of exhausted T cells to the host? First, it is important to point out that exhausted T cells are not inert. In nearly all cases, exhausted T cells have some level of residual function, and this residual function (or other as yet unappreciated properties of exhausted T cells) may be important *in vivo*. For example, exhausted T cells can drive epitope mutation during chronic infections[109], and depletion of $CD8^+$ T cells during the chronic phase of simian immunodeficiency virus (SIV) infection results in rapid and marked exacerbation of viraemia and progression to AIDS[137,138]. This result indicates that although exhausted $CD8^+$ T cells are poorly functional, they are not functionally inert and have a crucial role in maintaining a virus–host stalemate by restraining viral replication. Moreover, whereas epitope escape in SIV, HIV and hepatitis C virus (HCV) infection often occurs early, escape in the chronic phase of infection — and also probably in the setting of tumour immune editing — may be driven by exhausted T cells. Finally, when the ability to sustain exhausted $CD8^+$ T cell populations is compromised, all containment of chronic infection can be lost[12]. In settings of chronic infection where immunopathology can occur, T cell exhaustion may serve as a mechanism to protect against tissue damage[139]. In this context, one might envision that for viruses that cannot be fully eradicated, at least containing these pathogens to low levels might be the evolutionary driver of T cell exhaustion. For herpesviruses, for example, continually driving a virus back into viral latency may require local effector function, but a robust proliferative burst such as might occur for central memory T cells (or even effector memory T cells) might prove pathogenic. Evidence for such a scenario may exist for herpes simplex virus (HSV)[140,141], although whether this applies for other herpesviruses remains a subject of some debate. Many persisting pathogens have evolved strategies to establish a host–pathogen balance, and there are many examples of viruses that cause chronic infections by evading immunity through the expression of immune evasion genes and through other strategies. However, in many if not most cases, uncontrolled pathogen replication does not occur, and a balance between pathogen replication and immune control is usually achieved. Overall, despite decreased functionality, the *raison d'être* for exhausted T cells may be to establish a host–pathogen stalemate for some persisting infections. Other persisting pathogens seem to have exploited this immunological 'niche' to continue to replicate, spread and cause disease.

Box 2

CD4+ T cell exhaustion

Virus-specific $CD4^+$ T cells also lose effector function during chronic viral infections[19,96,142,143]. In fact, dysregulation of $CD4^+$ T cell responses may be a crucial event in failed immune control in human chronic infections[144,145]. Exhausted $CD4^+$ T cells, similar to exhausted $CD8^+$ T cells, display reduced production of effector cytokines (such as tumour necrosis factor and interferon-γ (IFNγ)) and express high levels of programmed cell death protein 1 (PD1)[96]. In addition, exhausted $CD4^+$ T cells have characteristics that are distinct from exhausted $CD8^+$ T cells. For instance, poor effector function is often apparent earlier for virus-specific $CD4^+$ T cells than for $CD8^+$ T cells during chronic infection[19,96,143], and exhausted $CD4^+$ T cells often produce interleukin-10 (IL-10) and/or IL-21 ([REFS 57,95,96]). Moreover, there is evidence of a skewing towards T follicular helper (T_{FH}) cells during chronic viral infections[95,96] that may be dependent on chronic IFNα/β signalling[146]. These observations are intriguing and may provide insights that are relevant to cryoglobulinaemia and to altered antibody production during some human chronic infections[147–149], as well as for the importance of IL-21 signalling in sustaining exhausted $CD8^+$ T cells[87–89]. Thus, patterns of differentiation for exhausted $CD4^+$ T cells suggest perhaps more complex regulation than is required for the differentiation of exhausted $CD8^+$ T cells.



Feature	Functional memory T cell	Exhausted T cell
Proliferative potential	+++	+/–
Cytokine production	+++	+/–
Memory markers (e.g. CD44, CD62L, CD127 or CXCR3)	+++	+/–
Inhibitory receptors (e.g. PD1, LAG3, CD160 or 2B4)	–	+++
IL-7- and/or IL-15-driven self-renewal	++	–
Antigen dependency	–	+

Figure 1. Progressive development of T cell exhaustion

Upon infection, naive T cells are activated by antigen, co-stimulation and inflammation, and they exponentially proliferate to form effector populations[1,2]. Whereas the majority of effector $CD8^+$ T cells that express killer cell lectin-like receptor subfamily G member 1 (KLRG1) die during the contraction phase, a population of effector $CD8^+$ T cells that retains CD127 expression can give rise to memory or exhausted $CD8^+$ T cells. In the setting of acute infection, where antigen and/or inflammation is cleared, effector $CD8^+$ T cells further differentiate into functional memory $CD8^+$ T cells that can produce multiple cytokines (such as interferon-γ (IFNγ), tumour necrosis factor (TNF) and interleukin-2 (IL-2)) and mount robust recall responses upon secondary infection[1,2]. These memory T cells are also maintained efficiently long term without antigen via IL-7- and IL-15-driven homeostatic self-renewal[102]. By contrast, during chronic infection, antigen and inflammation persist after the effector phase. As infection progresses and T cell stimulation continues, T cells lose effector functions in a hierarchical manner and become exhausted[3]. Typically, functions such as IL-2 production and cytokine polyfunctionality, as well as high proliferative capacity, are lost early; this is followed by defects in the production of IFNγ, TNF and chemokines, as well as in degranulation. T cell exhaustion is also accompanied by a progressive increase in the amount and diversity of inhibitory receptors that are expressed, including programmed cell death protein 1 (PD1), lymphocyte activation gene 3 protein (LAG3), 2B4, CD160 and T cell immunoreceptor with immunoglobulin and ITIM domains (TIGIT). Ultimately, if the severity or duration of the infection is high or prolonged, virus-specific T cells can be lost (‘deletion’). Variables — such as the level and number of inhibitory receptors expressed, strength of antigen stimulation, availability of $CD4^+$ T cell help and the duration of infection — can all influence the severity of exhaustion. Exhausted T cell populations are heterogeneous, and subsets of $T\text{-}bet^{hi}$ $PD1^{mid}$ and $EOMES^{hi}$ $PD1^{hi}$ $CD8^+$ exhausted T cells

exist[12]. Only the T-bet^{hi} PD1^{mid} subset is responsive to reinvigoration by blockade of the PD1 pathway[42]. CXCR3, CXC-chemokine receptor 3; PDL1, PD1 ligand 1.

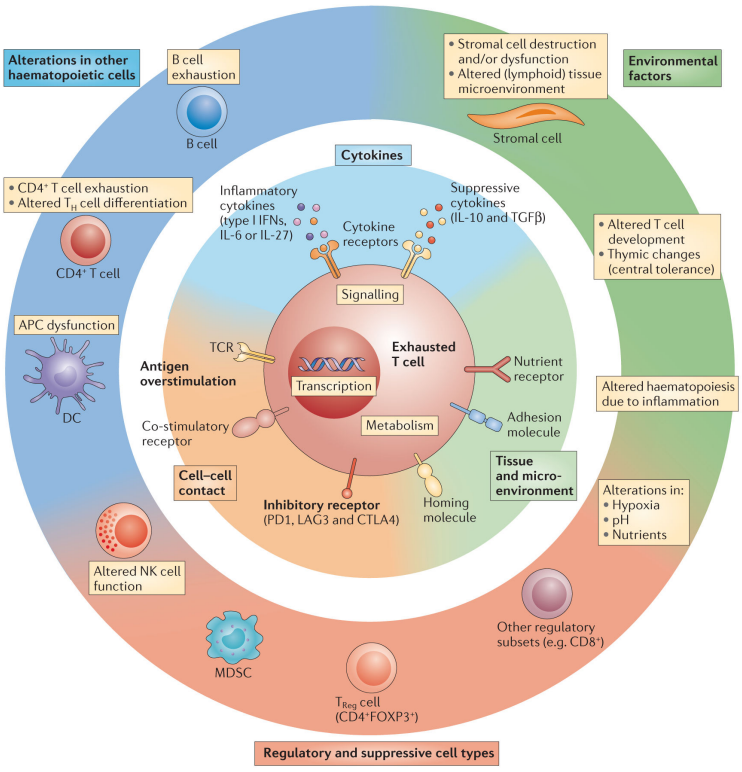


Figure 2. Overview of mechanisms of T cell exhaustion

Pathways implicated in regulating T cell exhaustion can be classified into three general categories (centre and inner circle): cell-to-cell signals including prolonged T cell receptor (TCR) engagement (signal 1) and co-stimulatory and/or co-inhibitory signals (signal 2); soluble factors such as excessive levels of inflammatory cytokines (for example, type I interferons (IFNs)) and suppressive cytokines including interleukin-10 (IL-10) and transforming growth factor-β (TGFβ); and tissue and microenvironmental influences driven by changes in the expression levels of chemokine receptors, adhesion molecules and nutrient receptors. This last class of influences may include altered tissue distribution and/or migratory patterns and lead to changes in pathways sensing oxygen tension (the von Hippel–Lindau tumour suppressor (VHL) and/or hypoxia-inducible factor (HIF) pathways), pH and nutrient levels. Tissue destruction and altered lymphoid organization may have a major role. Other immune cell types and stromal cells could be the source of many of these changes (outer circle). Cell types such as antigen-presenting cells (APCs), $CD4^+$ T cells, natural killer (NK) cells, B cells and regulatory cells (for example, myeloid-derived suppressor cells (MDSCs) and regulatory T (T_{Reg}) cells) have been implicated in $CD8^+$ T cell exhaustion. Overall, during chronic infections, cell-intrinsic and cell-extrinsic signals are probably integrated and thereby negatively influence T cell differentiation and promote exhaustion. The precise balance of these signals may determine the severity and/or qualitative aspects of T cell exhaustion in different disease settings. CTLA4, cytotoxic T lymphocyte antigen 4; DC, dendritic cell; FOXP3, forkhead box P3; LAG3, lymphocyte activation gene 3 protein; PD1, programmed cell death protein 1; T_H cell, T helper cell.

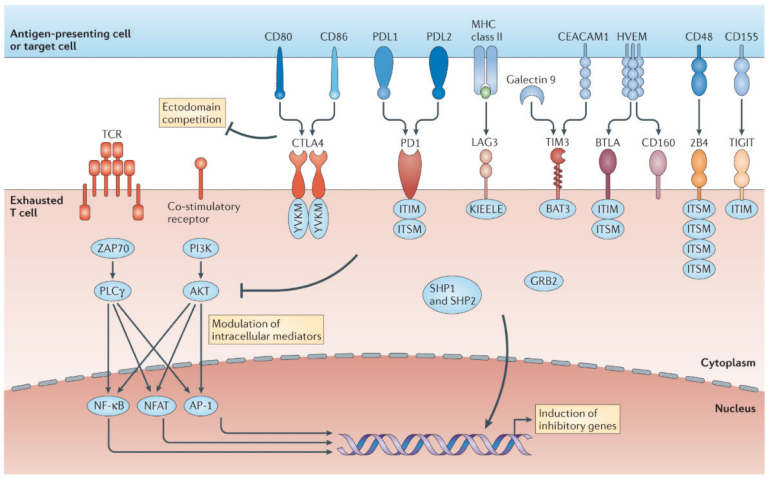


Figure 3. Molecular pathways of inhibitory receptors associated with T cell exhaustion

Ligand and receptor pairs for inhibitory pathways are depicted, showing the intracellular domains of receptors that contribute to T cell exhaustion. Many inhibitory receptors have immunoreceptor tyrosine-based inhibitory motifs (ITIMs) and/or immunoreceptor tyrosine-based switch motifs (ITSMs) in their intracellular domains; however, some receptors have specific motifs, such as YVKM for cytotoxic T lymphocyte antigen 4 (CTLA4) and KIEELE for lymphocyte activation gene 3 protein (LAG3). The molecular mechanisms of inhibitory receptor signalling are also illustrated and can be classified as: ectodomain competition (inhibitory receptors sequester target receptors or ligands); modulation of intracellular mediators (local and transient intracellular attenuation of positive signals from activating receptors such as T cell receptors and co-stimulatory receptors); and induction of inhibitory genes. Multiple inhibitory receptors are responsible for these three mechanisms. AP-1, activator protein 1; BAT3, HLA-B-associated transcript 3 (also known as BAG6); BTLA, B and T lymphocyte attenuator; CEACAM1, carcinoembryonic antigen-related cell adhesion molecule 1; GRB2, growth factor receptor-bound protein 2; HVEM, herpes virus entry mediator (also known as TNFRSF14); NFAT, nuclear factor of activated T cells; NF-κB, nuclear factor-κB; PD1, programmed cell death protein 1; PDL1, PD1 ligand 1; PI3K, phosphoinositide 3-kinase; PLCγ, phospholipase Cγ; TIGIT, T cell immunoreceptor with immunoglobulin and ITIM domains; TIM3, T cell immunoglobulin and mucin domain-containing protein 3.

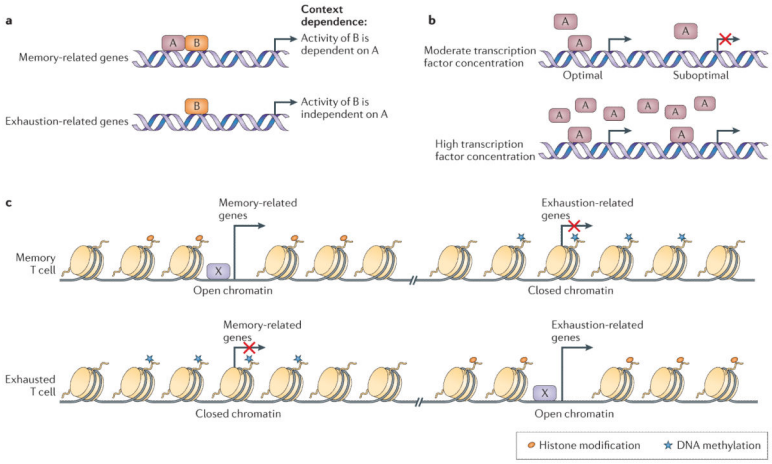


Figure 4. Transcriptional and epigenetic mechanisms of T cell exhaustion

Altered usage of key transcription factors is associated with the altered transcriptional and developmental programme of T cell exhaustion. Several potential mechanisms exist. **a** | Different use of transcription factor binding partners is one potential mechanism for distinct context-dependent transcription factor activity. In the example shown, transcription factor B is closely associated with memory-related genes in the context of acute infection where transcription factor A is abundant (top panel). However, the same transcription factor B is linked with exhaustion-related genes in chronic infection (lower panel). Post-translational modifications of transcription factors and/or their subcellular localization may also be important in this setting. **b** | The dosage or concentration of a transcription factor could also provide a mechanism for context-dependent transcriptional function. Here, a transcription factor binds only at specific high-affinity binding sites if the amount of the factor is low (top panel). By contrast, at high transcription factor concentrations binding can occur more broadly (that is, occurring also at lower affinity sites), which leads to different transcriptional activity (lower panel). **c** | DNA methylation, histone modifications and the ‘chromatin landscape’ resulting from overall epigenetic regulation could provide a mechanism for context-specific transcription factor function in exhausted T cells (transcription factor X in this example). The enhancer landscape of a cell may determine how different transcription factors function.

Table 1

A comparison of dysfunctional T cell responses

Feature	Exhaustion	Anergy	Senescence	Refs
Main cause	Excessive and continuous stimulation	Suboptimal stimulation	Repetitive stimulation	3,5,100
Nature and time frame	Persistent overstimulation of signal 1 and signal 3 leads to progressive loss of effector and memory function (typically within a few weeks)	When signal 2 and/or signal 3 are either absent or weak at priming, T cell dysfunction occurs at an earlier time point (typically within a few days)	Intermittent and multiple stimulations after the primary response lead to a limit in cell replication at later time point (typically within months to years)	3,5,15,20,100,101
Proliferative capacity	Low	Low	Low	3,100
Expression of inhibitory markers	High (and multiple markers are expressed)	?	Low	11,18,41,99,100
Expression of NK cell receptor markers	None or low	None	High	11,18,41,99,100
Expression of CD57 (human) and KLRG1 (mouse)	None or low	?	High	11,18,41,99,100
Effector function	Low to moderate	None or low	High	3,100,101
Telomerase activity	Low	?	Low	3,100
Primed properly	Yes	No	Yes	3,5

Question mark depicts unknown effects. KLRG1, killer cell lectin-like receptor subfamily G member 1; NK, natural killer.