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REVIEW

CD8$^+$ T cell exhaustion

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Abstract

CD8$^+$ T cells are important for the protective immunity against intracellular pathogens and tumor. In the case of chronic infection or cancer, CD8$^+$ T cells are exposed to persistent antigen and/or inflammatory signals. This excessive amount of signals often leads CD8$^+$ T cells to gradual deterioration of T cell function, a state called "exhaustion." Exhausted T cells are characterized by progressive loss of effector functions (cytokine production and killing function), expression of multiple inhibitory receptors (such as PD-1 and LAG3), dysregulated metabolism, poor memory recall response, and homeostatic proliferation. These altered functions are closely related with altered transcriptional program and epigenetic landscape that clearly distinguish exhausted T cells from normal effector and memory T cells. T cell exhaustion is often associated with inefficient control of persisting infections and cancers, but re-invigoration of exhausted T cells with inhibitory receptor blockade can promote improved immunity and disease outcome. Accumulating evidences support the therapeutic potential of targeting exhausted T cells. However, exhausted T cells comprise heterogenous cell population with distinct responsiveness to intervention. Understanding molecular mechanism of T cell exhaustion is essential to establish rational immunotherapeutic interventions.

Keywords CD8 T cell · Exhaustion · Chronic infection · Inhibitory receptor · Cancer immunotherapy

Introduction

Naive T cells get activated and differentiated into effector cells in 7~10 days during acute infection or vaccinations. To obtain appropriate differentiation program, naive T cells integrate signals from antigen (signal 1), co-stimulation (signal 2), and inflammation (signal 3) during priming and initial activation. This effector T cell differentiation from a naïve T cell is accompanied by robust cell proliferation, transcriptional, epigenetic, and metabolic global reprogramming, and the acquisition of cardinal features of effector T cells such as effector function, altered tissue homing, and a dramatic numerical expansion [1]. In the subsequent contraction phase, the majority of expanded effector cells die but a small fraction of effector cells persists and develops into memory T cells [1]. Memory T cells downregulate the activation program of effector cells and disarm effector molecules but keep the ability to rapidly reactivate effector functions upon re-encounter with the same antigen. During this transition, memory T cells also change homing and distribution capacity. Additionally, memory T cells develop a key characteristic of antigen-independent self-renewal, a type of stem cell-like slow proliferation driven by IL-7 and IL-15. Based on these tissue distribution preference and capability of slow proliferation in response to homeostatic cytokines, memory T cells are classified as central memory (T_{CM}), effector memory (T_{EM}), resident memory (T_{RM}), and memory stem cells (T_{SCM}) [2]. Overall, a key aspect of the development of highly functional, persisting memory T cells is that after the peak of effector expansion, this memory T cell differentiation program occurs in the absence of ongoing antigen stimulation and high levels of persisting inflammation.

On the other hand, during chronic infections or cancer where antigen and/or inflammation persist, the program of memory T cell differentiation is dramatically changed [2]. In normal setting, memory T cells undergo a transition to quiescence but still preserve potential effector capacity after effector phase. However, during chronic infection or cancer, antigen-specific T cells show progressive loss of effector functions, altered metabolism, and a unique transcriptional and epigenetic program that is characterized by an absence of a

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signature of quiescence [2, 3]. One of the major characteristics of exhaustion is co-expression of high levels of multiple inhibitory receptors, including PD-1 (CD279), cytotoxic T lymphocyte antigen-4 (CTLA-4, CD152), lymphocyte-activation gene 3 (LAG3), T cell immunoglobulin domain and mucin domain 3 (Tim-3), CD244/2B4, CD160, T cell immunoreceptor with Ig and ITIM domain (TIGIT), and others [2, 4]. Other changes are lack of antigen-independent homeostasis; altered transcriptional program including the distinct use of key transcription factors; and changes in homing and migration, signaling and cytokine and chemokine receptor expression, and metabolism [2, 5–9]. Development of T cell exhaustion is tightly associated with prolonged exposure to antigen and inflammation. Altered T cell function, differentiation, and maintenance together prevent optimal control of chronic infections and cancer. The discovery that blockade of the PD-1 pathway could partially reverse exhaustion and lead to reduced viral or tumor load was a significant breakthrough in the field [2, 10–14]. These data in animal models and clinical trials highlighted the idea that exhausted T cells were not terminally dysfunctional or irreversible, but at least a subset of exhausted T cells could be re-invigorated, with implications for the treatment of diseases including chronic infections and cancer. In this review, characteristic features of altered functionality, inhibitory receptors and negative regulatory pathways, and altered transcriptional control of exhausted CD8 T cells will be described.

Overview of T cell exhaustion

CD8 T cells play a pivotal role in clearing intracellular pathogens and tumors [1]. However, high and sustained antigen and inflammatory stimulation during chronic infection and tumors can lead to altered CD8 T cell differentiation or exhaustion. T cell exhaustion, first described in chronic virus infection in mice [15], and was defined as the persistence of antigen-specific T cells that lacked or possessed poor effector functions. These studies were followed rapidly by the realization that CD8 T cell exhaustion also occurred in humans during HIV, HCV, HBV, HTLV-1, and other infections as well as cancer [2, 16–19]. Although the severity of T cell dysfunction can differ for specific pathogens, the general principle originally defined during lymphocytic choriomeningitis virus (LCMV) infection in mice appears to apply in a wide variety of infectious and cancer settings. Importantly, this state of T cell differentiation prevents optimal control of infections and tumors and a better understanding of the molecular mechanism of exhaustion should lead to new clinical opportunities [17].

T cell exhaustion usually manifests in progressive and hierarchical loss of effector functions during persistent infection [2, 17]. Typically, functions such as IL-2 production and cytokine polyfunctionality, as well as high proliferative capacity are lost early, followed by defects in production of TNF, IFN-γ, and chemokines as well as degranulation capacity. Ultimately, virus-specific T cells can be physically deleted. Exhausted T cells express inhibitory receptors including PD-1, LAG-3, Tim-3, 2B4/CD244, CD160, TIGIT, and others that have a major role in regulating T cell function. The demonstration that blocking the PD-1 inhibitory receptor in vivo revigorated exhausted T cell responses and enhanced viral control was a critical advance in this field [10]. These studies demonstrated a novel concept that T cell exhaustion was reversible, rather than a, terminal or irreversible differentiation state. Moreover, these observations have become the foundation for remarkable clinical trials blocking the PD-1 pathway in human cancer and chronic infections that have resulted in impressive clinical response rates, sometimes in patients who have failed other immunotherapies [11, 13]. The immunological effects of these human treatments remain to be fully defined, but the emerging results support the notion that reversal of T cell exhaustion in humans is the causative mechanism for the profound antitumor effect seen in many patients receiving PD-1 pathway blocking reagents. In addition to loss of effector function and negative regulation by inhibitory receptors, considerable evidence such as comparative transcription analysis of functional memory versus exhausted CD4 and CD8 T cells indicates that exhausted CD8 T cells have a unique molecular signature distinct from naïve, effector, and memory T cells [3, 20, 21]. Thus, while loss of function is one of the key defining features of T cell exhaustion, recent work has also highlighted several other defining aspects of T cell exhaustion including sustained expression of inhibitory receptors, altered memory, and a unique pattern of transcriptional control [2].

Persisting antigen signaling drives T cell exhaustion

While there are clearly contributions from a variety of pathways one key feature appears to be the chronic (and likely continuous rather than intermittent) exposure to antigen. Additional factors including lack of $CD4^+$ T cell help [22] and perhaps signals from inhibitory receptors [23] also likely contribute. Early studies in the chronic LCMV model demonstrated that the severity of exhaustion (and deletion) of antigen-specific T cells correlated to antigen abundance [24]. The importance of the level of antigen persistence exhaustion was also confirmed in other murine models and HIV-1 infection [2, 25]. Thus, the level and duration of chronic antigen stimulation appears to be a key event leading to exhaustion and correlating with the severity of dysfunction during chronic infection.

Indeed, downstream of TCR signaling, NFAT, and Sprouty-2 (SPRY2) has been implicated in T cell exhaustion.

Impaired NFAT nuclear translocation results in split exhaustion of virus-specific $CD8^+$ T cell functions during chronic viral infection [26]. Sprouty-2, a negative regulator of the MAPK/ERK pathway, is upregulated by strong TCR signals and regulated T cell polyfunctionality [27]. Inhibition of Sprouty-2 in HIV-specific T cells increased polyfunctionality independently of PD-1, suggesting a central role for ongoing direct attenuation of T cell signaling in exhausted T cells. However, chronic antigen stimulation also leads to sustained expression of PD-1 through NFATc1 [28] and it is likely that PD-1 further modulates the level of TCR signaling [29]. Together, persistent antigen stimulation (signal 1) is key factor that initiates and leads T cell exhaustion and correlate with the severity of dysfunction during chronic infection.

Inhibitory signals in T cell exhaustion

Negative regulatory pathways that are responsible for T cell exhaustion can be classified in three major categories: (1) cell surface inhibitory receptors, (2) soluble factors and environmental factors, and (3) immunoregulatory cell populations.

Inhibitory receptors and co-stimulatory pathways

Inhibitory and co-stimulatory receptors play critical roles in adaptive immune cell responses [30]. Inhibitory receptors are critical negative regulatory pathways that function to control autoreactivity and immunopathology [4, 31]. Although inhibitory receptors are transiently expressed in functional effector T cells during activation, higher and sustained expression of inhibitory receptors is a central feature of the T cell exhaustion. The PD-1:PD-L1/L2 inhibitory pathway is the best studied inhibitory receptor pathway in T cell exhaustion [30, 32, 33]. The observation that blocking the PD-1 pathway reinvigorates exhausted $CD8^+$ T cell responses during chronic LCMV infection and enhances viral control indicates that T cell exhaustion is under active control by inhibitory receptors such as PD-1 [10]. This observation originally from LCMV infection was rapidly confirmed in HIV infection [18, 19, 34], and PD-1 pathway signals are now known have a major role in negatively regulating immunity during a wide variety of human chronic infections and cancer [30, 32, 33]. Indeed, with the FDA approval of PD-1 pathway inhibitors for treatment of human cancer, the importance of the PD-1 pathway and reversal of T cell exhaustion for treatment of human disease cannot be understated.

Despite the promise of clinical targeting of the PD-1 pathway, the molecular mechanisms by which this inhibitory receptor controls T cell exhaustion remain poorly understood. There are several mechanisms by which inhibitory receptors such as PD-1 might regulate T cell function [32]. (1) Ectodomain competition: inhibitory receptors sequestrate target receptors/ligands and/or prevent the optimal formation of microclusters and lipid rafts (CTLA4 [35]), (2) modulation of intracellular mediators: local and transient intracellular attenuation of positive signals from activating receptors such as T cell receptors and co-stimulatory receptors [36], PD-1 [37] and Tim3 [38], (3) Induction of inhibitory genes: some inhibitory receptors upregulate expression of genes that inhibit T cell function. (4) Alteration in T cell motility [39]: PD-1 decreases exhausted T cell motility. The PD-1 intracellular domain contains an immunotyrosine inhibitory motif (ITIM) and an immunotyrosine switch motif (ITSM) [40]. Current evidence suggests an role for the ITSM in recruiting SHP1 and/or SHP2 [37, 41] in the ability of PD-1 signals to attenuate TCR signaling in vitro. The role of the ITIM in PD-1 function remains poorly understood. Other evidence suggests a role for PD-1 signals in modulating PI3K, AKT, and Ras pathways [36, 42] and also link PD-1 to control of cell cycle progression [43]. Notably, nearly all our information about how PD-1 controls T cell signaling is derived from in vitro studies of acutely activated T cells. In vivo studies of the role of PD-1 during acute T cell activation and expansion suggest a possible role for PD-1 signals in arresting T cell migration [29], which could have important implications for viral control. Finally, there is some evidence that signals from PD-1 may, in fact, induce expression of genes such as the transcription factor BATF that could negatively regulate gene expression in some settings [23]. Nevertheless, despite this elegant work, how these observations relate to exhausted T cells exposed to chronic stimulation through the TCR remains unclear.

Indeed, PD-1 expression is rapidly upregulated upon T cell activation and expression of PD-1 may persist at moderate levels in humans. For example, in healthy adult humans, many functional effector memory cells express PD-1 [44, 45], indicating that PD-1 expression alone is not a unique feature of exhausted T cells. However, during chronic infections, PD-1 expression can be substantially higher than observed on functional effector or memory $CD8^+$ T cells [19, 46]. During chronic infection sustained upregulation of PD-1 and the functional inactivation of virus-specific T cells during is dependent upon continued epitope recognition [47], although examples exist of residual PD-1 expression even after removal of persisting antigen signaling [48, 49]. This latter observation may relate to epigenetic changes in the control of expression of the *Pdcd1* gene encoding PD-1 [50].

In addition to PD-1, exhausted T cells express an extensive suite of other cell surface inhibitory molecules. Exhausted T cells co-express PD-1 together with LAG-3, CD244 (2B4), CD160, TIM-3, CTLA-4, and many other inhibitory receptors [25]. Typically, the higher the number of inhibitory receptors co-expressed by exhausted T cells the more severe the exhaustion. Indeed, while individual expression of PD-1 or other inhibitor receptors is not indicative of exhaustion, co-expression of multiple inhibitory receptors is a key-defining

feature of exhaustion. These co-expression patterns are highly functionally relevant since simultaneous blockade of multiple inhibitory receptor pathways results in synergistic reversal of T cell exhaustion. This concept was first demonstrated for PD-1 and LAG-3 [25] in LCMV and then other infections [51] or cancer [52, 53], but also for many other combinations of inhibitory receptors including PD-1 and CTLA-4 [54, 55] and PD-1 and TIM-3 [56–59]. Again, these observations from mice are translating to humans and making significant clinical impact in the last decade. PD-1 and CTLA-4 blockade in human melanoma patients demonstrated impressive tumor control [60], and other combination inhibitory receptor clinical trials in multiple settings are underway. It is noteworthy that PD-1 pathway blockade is typically included as one arm of these combination therapies consistent with the central role of this inhibitory receptor in T cell exhaustion. Overall, these data on inhibitory receptor co-regulation of T cell exhaustion suggest that these pathways are non-redundant in how they control T cell function and differentiation in chronic infection and cancer. These molecules come from diverse structural families, bind ligands with distinct expression patterns and properties and possess impressively different intracellular signaling domains. Thus, substantial potential may exist to tailor or tune the type and magnitude of re-invigoration of exhausted T cell responses by appropriate co-blockade approaches. Indeed, an extensive set of additional potential blockade targets exist and are currently being explored for combination therapies to reverse T cell exhaustion [32].

Although inhibitory receptors draw considerable attention in cell-to-cell interaction mechanism of exhaustion, it has become clear that co-stimulatory receptors, which normally play a positive role in acute infection, are also involved for T cell exhaustion [4]. For example, desensitization of co-stimulatory pathway signaling through the loss of adaptor molecules can serve as a mechanism of T cell dysfunction during chronic infection. TNFR-associated factor 1 (TRAF1), a signaling adaptor of TNF superfamily, is downregulated in dysfunctional T cells in progressor of HIV and chronic phase of LCMV Cl-13 infection [61]. Adoptive transfer of TRAF1 expressing, but not TRAF1 deficiency, $CD8^+$ T cell enhanced viral control of Cl-13 infection, indicating the essential role of co-stimulatory pathway for T cell exhaustion. Co-stimulatory pathways can also regulate T cell exhaustion in an indirect fashion. CD27 signaling on $CD4^+$ T cells enhances TNF and IFN-γ secretion, which can lead to destruction of splenic architecture and immunodeficiency [62]. CD40 agonistic antibody can rescue PD-1 mediated $CD8^+$ T cell exhaustion perhaps due to myeloid DC activation [63]. In pancreatic cancer, such CD40 targeting may overcome a substantial T cell extrinsic barrier leading to enhanced T cell responses and better tumor control [64]. It has also been possible to exploit this concept of agonizing a positive co-stimulatory pathway while blocking an inhibitory pathway. For example, combined blockade of and PD-1 and treatment with an agonistic antibody to 4-1BB dramatically improved exhausted CD8 T cell function and viral control [65]. In addition, PD-1 pathway blockade has been combined with other "positive" regulators of immune responses including therapeutic vaccination [66], delivery of IL-2 [67], or regulatory T cells (T_{reg}) depletion [68]. Since combined therapy of blocking antibody to inhibitory receptors and agonistic antibody to co-stimulatory receptor showed synergistic effect, detailed mechanism of co-stimulatory pathway in T cell exhaustion would be of great interest.

Soluble pathways and environmental factors

A second class of signals that regulates T cell exhaustion is from soluble molecules. Broadly, three distinct classes of such soluble mediators can be discussed including immunosuppressive cytokines such as IL-10 and TGF-β, inflammatory cytokines such as type I IFN and common-γ chain cytokines (such as IL-2, IL-7, and IL-21).

IL-10 The IL-10/IL-10R pathway has received considerable attention for its role in T cell exhaustion [17]. Blockade of IL-10 restores T cell function and improves viral control during chronic viral infections, indicating that IL-10 facilitates T cell exhaustion [69, 70]. Studies of LCMV infection in mice and HIV in humans demonstrated that during chronic infection, IL-10 can be secreted from many cell types including dendritic cells, monocytes, and/or $CD4^+$ T cells [71–73], though the important or most relevant source of this cytokine remains a matter of debate. Simultaneous blockade of IL-10 and PD-1 axis significantly enhances T cell response and viral control when compared with either blockade alone, indicating that the immunosuppressive mechanism of IL-10 in T cell exhaustion is mechanistically distinct from PD-1 [74]. Interestingly, however, some evidence suggests a connection between the PD-1 pathway and IL-10 production through induction of IL-10 by monocytes following PD-L1 ligation [72]. Despite the clear evidence that IL-10 contributes to exhaustion, the molecular events downstream of IL-10 signaling (presumably via STAT3) that shape T cell exhaustion remain to be more precisely defined.

TGF-β Another suppressive cytokine implicated T cell exhaustion is transforming growth factor-β (TGF-β). Earlier studies indicated that phosphorylation of Smad2 (indicator of TGF-β signaling) in $CD8^+$ T cells was increased during chronic infection compared with acute infection, and inhibiting TGF-β signaling in $CD8^+$ T cells using dominant negative receptor improved the function of exhausted cells [75]. However, studies using systemic administration of TGF-β inhibitor/blocking antibody in mice found little benefit of these treatments [76, 77]. While it is difficult to directly compare the genetic

approach to antibody- and inhibitor-based strategies, these observations warrant perhaps further evaluation of this immunoregulatory pathway in T cell exhaustion.

IFN-α/β Type I interferons (IFN-α/β) are critical inflammatory cytokines that have essential antiviral effects in the early stages of infection. In most cases, in the absence of IFN-α/β signaling, acute viral control is dramatically compromised. Moreover, IFN-α/β signals can provide a critical "signal 3" for proper activation and differentiation of $CD8^+$ T cells following priming [78]. In addition to these critical innate antiviral effects, however, recent studies demonstrated a surprising and crucial role for chronic type I interferon signaling in promoting immune suppression and lymphoid tissue destruction. Surprisingly, blockade of this pathway reversed and/or prevented T cell exhaustion [79, 80]. This effect appeared to operate via $CD4^+$ T cells, though the precise mechanism remains to be defined. For example, IFN-α/β can induce several immunoregulatory pathways including IL-10, PD-L1, and indoleamine dioxygenase (IDO) (ref). It is also interesting that the signal 3 cytokines IL-12 and type I IFN differentially program $CD8^+$ T cells for PD-1 re-expression levels and tumor control in a cancer re-challenge model [81], suggesting an important role for inflammatory signals in perhaps modulating the availability of other immunoregulatory pathways that influence T cell exhaustion. Indeed, exposure to chronic inflammation in the absence of TCR signaling can dramatically skew the pattern of memory $CD8^+$ T cell differentiation, although overt exhaustion does not occur without persisting TCR signaling [82].

In addition to important roles for inflammatory pathways mentioned above, anatomical factors such as cellular and tissue tropism, lymphoid architecture integrity could also influence the severity of exhaustion. Given that exhausted T cells express altered pattern of trafficking and adhesion molecules [20] and expression levels of inhibitory receptors are different among organs [83], spatial and temporal regulation of T cell exhaustion should be examined in future study. Destruction of tissue architecture and fibrosis of secondary lymphoid organs have been reported in both mice and human chronic infection [84–86]. As an orchestrated immune cell trafficking and fine cell-to-cell interaction is critical for optimal immune response, a major goal is to determine how these anatomical features influence T cell exhaustion.

Regulatory subsets

Immune system has multiple subsets, and interactions among these subsets are essential to maximize immune responses against infection and cancer. For optimal responses, $CD8^+$ T cells require optimal antigen presentation from professional APC, help from $CD4^+$ T cells, and intact tissue trafficking ability to increase immune cell interaction. These events must be orchestrated in a setting that prevents excessive immune-mediated tissue damage and also appropriately shuts off T cell responses when necessary. Thus, suppressive and/or regulatory cell populations such as T_{reg} and altered APC may contribute to $CD8^+$ T cell exhaustion. DCs can be direct targets of viruses, and dysfunction of DCs for cytokine production and cross-presentation has been reported in some chronic infections [87]. In addition, persistent inflammation associated with chronic infections and cancer as well as viral targeting of hematopoietic progenitors can alter DC maturation and differentiation at multiple levels, generating suppressive subsets such as immunoregulatory APC and myeloid-derived suppressor cells (MDSCs), which can inhibitor T cell function and/or promote exhaustion [88–90]. Both immunoregulatory APC and MDSCs have been described in cancer and are thought to negatively regulate T cell responses. Analogous populations have recently been shown to promote T cell exhaustion in murine LCMV Cl-13 infection [91, 92].

Loss of $CD4^+$ T cell activity in many settings can underlie or contribute to defective $CD8^+$ T cell responses. In HIV infection, $CD4^+$ T cells are direct targets of infection and loss of $CD4^+$ T cells is associated with increased exhaustion of $CD8^+$ T cells. While $CD4^+$ T cells can clearly become exhausted, the impact of changes in the $CD4^+$ response for $CD8^+$ T cell exhaustion may be highly relevant. In the absence of IL-21 signaling, for example, $CD8^+$ T cell exhaustion is substantially worse during chronic LCMV infection [93–95] with supporting observations in HIV infection [96, 97]. Since $CD4^+$ T cells are the likely source of IL-21 signals, these observations suggest a key role for CD4 help to $CD8^+$ T cells to avoid severe exhaustion via IL-21. Furthermore, there is evidence that activated NK cells have an immunoregulatory role during chronic viral infection perhaps by directly eliminating $CD4^+$ T cells [98–100]. $Foxp3^+$ $CD4^+$ T_{reg} are well known to influence immune responses during many infection and cancer [101, 102]. Although it is relatively clear that T_{reg} have suppressive roles for T cell response in acute infection and acute phase of chronic infection (which can enhance T cell exhaustion as a result of high pathogen burden) [68], and frequency of T_{reg} is increased in some human chronic HIV and HCV infection, it is still unclear whether T_{reg} directly facilitate T cell exhaustion. As a source of IL-10, TGF-β or perhaps other suppressive cytokines (e.g., IL-35), one can envision such a scenario. However, precisely how T_{reg} affect developing T cell exhaustion remains to be more completely defined. Nevertheless, T_{reg} are an important therapeutic target since their deletion or modulation can often unleash effective antitumor or antipathogen responses [68].

Transcriptional changes in T cell exhaustion

How T cell exhaustion is transcriptionally programed? Recent studies have applied genomics approached to investigating the

transcriptional circuitry that underlies development of T cell exhaustion. Exhausted $CD4^+$ and $CD8^+$ T cells have a transcriptional profile profoundly different from memory $CD4^+$ and $CD8^+$ T cells, respectively, including major changes in the expression of inhibitory and co-stimulatory receptors, transcription factors, signaling molecules, cytokine and chemokine receptors, and genes involved in metabolism [20, 21]. In addition, a major feature of CD8 T cell exhaustion is the absence of key $CD8^+$ T cell memory-associated modules of gene expression including specific coordinated gene sets associated quiescence [3]. 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 genomic studies raise questions about how key genes and molecules identified regulate the development and differentiation of exhausted T cells. Although considerable progress has been made to define centrally important transcription factors, a lineage-specific transcription factor for exhausted T cells has not yet been identified. However, the transcription factors Tbet, Eomes, Blimp1, NFAT, BATF, IRF4, von Hippel-Lindau disease tumor suppressor (VHL), FOXO1, FOXP1, and TCF1 have been shown to be involved in T cell exhaustion [2, 5, 6, 23, 26, 103–106]. Interestingly, despite the absence of an obvious unique transcription factor associated with exhaustion, a key concept that has emerged is that several of these transcription factors function in an exhaustion-specific manner in exhausted CD8 T cells [5, 6, 23, 107]. In other words, while the transcription factors can play roles in other T cell populations, the expression pattern, genes controlled, and manner in which key transcription factors operate in exhausted T cells is, in some cases, highly divergent from the function of these transcription factors elsewhere. For example, while T-bet is expressed by, and plays a functional role in the formation of terminally differentiated CD8 T cell populations in acute infection [1, 108], T-bet controls the population of non-terminal progenitor cells with the exhausted T cell pool [6]. Similarly, Eomes is involved in central memory T cells following acute infection including playing a role in being essential for central memory CD8 T cell quiescence and homeostatic turnover [109, 110], but during chronic infection, Eomes controls the formation of terminally differentiated exhausted T cells highly enriched in peripheral tissues [6]. This distinct context-specific re-use of transcription factors was initially revealed using transcriptional network analysis that revealed differential transcriptional connections for specific transcription factors with genes they control in memory versus exhaustion [3]. Together, these studies suggest that differentiation to T cell exhaustion is governed by multiple transcription factors and context-specific combination of these transcription factors might play a critical role. In addition to transcription factors, it has recently been shown that microRNA (e.g., miR-150, miR-155) play as a key regulator of development or maintenance of exhausted T cells [111].

Another important mechanism that regulates transcriptional program is epigenetic modification. As the epigenome can influence a cell differentiation through modification of transcriptional program, understanding of global epigenetic landscape of exhausted T cell appears to be one of the next fundamental steps in the field [2]. However, only limited information has been established in terms of epigenetic regulation of T cell exhaustion [8, 9, 112]. An earlier study revealed that DNA methylation status of *Pdcd1* (encoding PD-1) locus in the antigen-specific T cells is different among acute (effector and memory) and chronic (exhaustion) infection. During acute infection, Pdcd1 promotor regions were largely demethylated in the effector phase and then remethylated as T cells differentiate into memory cells. On the other hand, the Pdcd1 locus were demethylated in exhausted T cells, and this demethylation was not changed even when antigen removed and PD1 expression on exhausted T cells decreased [50]. Similarly, reinvigorated CD8+ T cells by PD-L1 blockade have a distinct epigenetic profile when compared with memory T cells that was minimally remodeled after PD-L1 blockade [9]. Thus, these epigenetic analysis also supports the idea that exhausted T cells are distinct lineage. Intervention to overcome this epigenetic fate inflexibility will be the next main target [8]. Future studies should be directed to context-dependent molecular interaction among multiple transcription factors and interaction of transcription factors and epigenetic DNA modifications, which enable context-dependent regulation of T cell exhaustion.

Conclusion

Although recent studies have provided significant advance in our understanding of T cell exhaustion, our understanding of T cell exhaustion and how to most effectively reverse this state remains incomplete. In addition, most of the research of T cell exhaustion has been utilized mouse LCMV and tumor model, and our understanding of T cell exhaustion in human chronic infection and cancer is still limited. Future mechanistic and clinical studies are needed to develop the next generation of immune based interventions for chronic infection and cancer.

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Compliance with ethical standards

Conflict of interest The author declares that he has no conflict of interest.

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