

## Mechanism of T cell exhaustion in a chronic environment

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**T cell exhaustion develops under conditions of antigen-persistence caused by infection with various chronic pathogens, such as human immunodeficiency virus (HIV) and mycobacterium tuberculosis (TB), or by the development of cancer. T cell exhaustion is characterized by stepwise and progressive loss of T cell function, which is probably the main reason for the failed immunological control of chronic pathogens and cancers. Recent observations have detailed some of the intrinsic and extrinsic factors that influence the severity of T cell exhaustion. Duration and magnitude of antigenic activation of T cells might be associated with up-regulation of inhibitory receptors, which is a major intrinsic factor of T cell exhaustion. Extrinsic factors might include the production of suppressive cytokines, T cell priming by either non-professional antigen-presenting cells (APCs) or tolerogenic dendritic cells (DCs), and alteration of regulatory T (Treg) cells. Further investigation of the cellular and molecular processes behind the development of T cell exhaustion can reveal therapeutic targets and strategies for the treatment of chronic infections and cancers. Here, we report the properties and the mechanisms of T cell exhaustion in a chronic environment. [BMB reports 2011; 44(4): 217-231]**

### INTRODUCTION

Persistent or chronic viruses and bacteria have evolved a variety of strategies for escaping host immune responses. First, some chronic pathogens depend on specific mechanisms of tropism and pathogenesis, which deteriorate immune systems by direct infection of immune cells. For example, human immunodeficiency virus (HIV) infects either T cells or macrophages, Epstein-Barr virus (EBV) targets B cells, and lymphocytic choriomeningitis virus clone 13 (LCMV clone 13) can infect dendritic cells, which can lead to total loss of immune system functionality. Secondly, chronic pathogens often target non-immune

cells such as hepatocytes and fibroblastic reticular cells, but these cells are not appropriate environments for inducing immune responses. For example, hepatitis C virus (HCV) and hepatitis B virus (HBV) target hepatocytes, whereas LCMV clone 13 infects fibroblastic reticular cells. Third, some proteins expressed by chronic pathogens can directly inhibit specific signaling pathways that induce the innate or adaptive immune response. Therefore, it is impossible to explain the decrease in immune function in response to chronic pathogen infection as one single cause. However, virologists and immunologists recently found that due to the persistence of foreign antigens, there is a common immunological phenotype of defective T cells during chronic pathogen infection, even though many different chronic pathogens have specific mechanisms to evade the immune system. Here, we tried to introduce 'T cell exhaustion', a common phenotype expressed by pathogen-specific T cells during chronic pathogen infection, and also report recent developments in explaining the mechanism of T cell exhaustion.

### CHRONIC ENVIRONMENT DURING CHRONIC PATHOGEN INFECTION AND CANCER DEVELOPMENT

T cells play a major role in protective immunity against many infectious pathogens and can eradicate malignant cells. Much of our current knowledge on the development of the T cell response is based on the study of acute viral infection. The dynamic response of antigen-specific T cells to acute viral infection has been resolved at the cellular, molecular, and gene-expression levels in both mice and humans (1-6). Following acute viral infection, antigen-specific T cells undergo a program of activation, expanding dramatically in number during the first 1-2 weeks post-infection. T cells become activated and acquire effector functions, including the production of effector cytokines such as IFN- $\gamma$  and TNF- $\alpha$ , as well as cytotoxicity mediated by granzyme and perforin (6, 7). After 90-95% of the effector T cell population dies by apoptosis, the surviving T cells differentiate into a pool of long-lived memory T cells, which retain the ability to rapidly activate effector functions and expand robustly upon re-exposure to antigen (6, 7). Importantly, the memory T cell population is maintained over the long-term in the absence of antigen primarily through two common gamma-chain cytokines, IL-7 and IL-15 (6, 8). However, key aspects of T cell memory formation and function can

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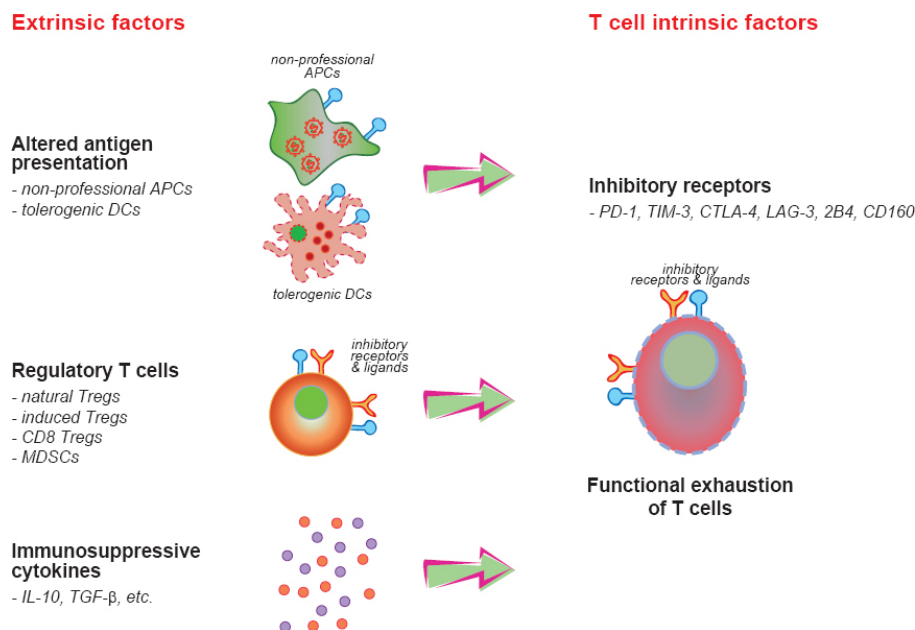
be substantially altered or impaired under settings of persistent antigen stimulation, such as chronic viral infection (9) or a tumor-bearing state (10-12). These changes are associated with step-wise impairment of effector function and proliferative capacity of responding antigen-specific T cells, and they ultimately affect the ability to confer host protection. Thus, this phenotype observed in T cells under antigen persistence is often called 'T cell exhaustion'. Several mechanisms are involved in the negative regulation of immune responses in a persistent antigen environment, wherein antigen and/or inflammation persist, as follows (Fig. 1): (i) up-regulation of intrinsic T cell factors including inhibitory receptors due to an increase in duration and magnitude of antigenic stimulation, (ii) dysfunctional/suppressive antigen-presenting cells (APCs) such as dendritic cells (DCs) and macrophages, (iii) an increase in regulatory cell populations such as regulatory T (Treg) cells and myeloid-derived suppressor cells (MDSCs), (iv) the levels of suppressive cytokines (13, 14).

Here, we first addressed inhibitory molecules up-regulated in T cells as well as those associated with impaired T cell function. Since such molecules are expressed on T cells, they would be considered as intrinsic factors. Next, we introduced extrinsic environmental factors that possibly conspire to cause T cell exhaustion during chronic virus infection or tumor growth.

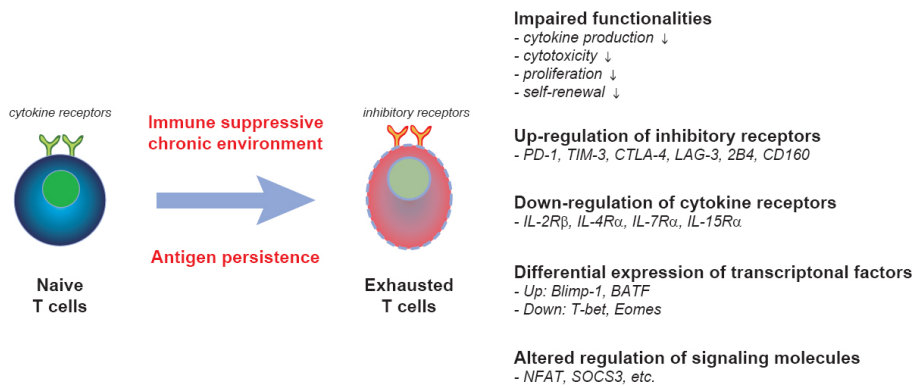
## INTRINSIC ALTERATION OF ANTIGEN-SPECIFIC T CELLS IN A CHRONIC ANTIGEN ENVIRONMENT

**Down-regulation of cytokine receptors on exhausted T cells**  
 T cell exhaustion describes a state of T cell dysfunction that

was initially observed during chronic LCMV infection in mice (15). During chronic viral infections, exhausted T cells present sequential phenotypic changes and a progressive loss of effector function (Fig. 2) (16-18). The loss of function is hierarchical, with cytotoxicity, proliferation, and IL-2 production lost first, followed by TNF- $\alpha$  and IFN- $\gamma$  production (6). Such functional impairment of responding T cells has been confirmed in human chronic viral infections such as HIV, HBV, and HCV (9, 17, 19). Unlike normal memory CD8 T cells generated after acute infection, exhausted CD8 T cells express extremely low levels of CD127 and CD122, which are the receptors for the homeostatic cytokines IL-7 and IL-15, and thus have major defects in cytokine-mediated homeostatic proliferation (19, 20). Tumor antigen-specific T cells with high tumor antigen load have been shown to respond similar to virus-specific T cells upon chronic infection. Furthermore, CD8<sup>+</sup> tumor infiltrating lymphocytes (TILs) show low levels of CD25 and CD127 expression and thus are refractory to IL-2 and IL-7 signaling, indicating that they are unable to proliferate, produce effector cytokines, and differentiate into functional memory cells (21). For example, CML-specific CD8 T cells exhibit decreased production of effector cytokines such as IFN- $\gamma$ , TNF- $\alpha$ , and IL-2 in a retroviral-induced murine CML model (21). Cancer-germline antigen NY-ESO-1-specific CD8 T cells represent a highly dysfunctional population of tumor-induced T cells in patients with advanced melanoma (22). TILs from human metastatic melanoma lesions also exhibit similar functional impairment (23). Defective cytokine signaling due to down-regulation of cytokine receptor probably impedes the maintenance of antigen-specific T cells as well as their functional integrity, thereby leading to failed immunological con-



**Fig. 1.** Chronic environment negatively regulating immune responses during chronic viral infection and tumor growth. Dysfunctional or suppressive APCs can negatively modulate T cell responses. Increase of multiple inhibitory receptors on T cells can limit T cell functions and control of disease. Tregs including traditional Foxp3<sup>+</sup> CD4 T cells can modulate T cell responses. Immunosuppressive cytokines such as IL-10 and TGF- $\beta$  produced in a chronic environment can inhibit virus- or tumor-specific T cell responses.



**Fig. 2.** Sequential and progressive changes of T cells in phenotype and functionality lead to T cell exhaustion in a chronic environment, in which antigen and/or inflammation persist. During persistent antigen stimulation, such as chronic viral infections and the tumor-bearing state, exhausted T cells undergo sequential phenotypic changes and a progressive loss of effector functions as follows: impaired functionality including effector cytokines production, cytotoxicity, proliferation, and cytokine-mediated self-renewal; overexpression of inhibitory receptors; down-regulation of cytokine receptors; changes in transcription factors; alteration of intracellular signaling.

trol of persisting pathogens and tumors.

### Overexpression of inhibitory receptors on exhausted T cells

There has been considerable interest in T cell exhaustion since a better understanding of the mechanisms responsible for progressive T cell dysfunction could provide novel therapeutic targets for the treatment of persisting infections and tumor growth. Recent studies have focused on the crucial role of inhibitory receptors overexpressed on exhausted T cells in the regulation of T cell function (Fig. 2). Gene expression profiling studies have suggested the presence of a number of potential inhibitory receptors on exhausted CD8 T cells (18). A functional role for inhibitory receptors was initially demonstrated in chronically infected mice with LCMV following blockade of the programmed death 1 (PD-1) pathway (24). PD-1, an inhibitory receptor of the CD28 superfamily (25, 26), is highly expressed on exhausted CD8 T cells during chronic LCMV infection, and *in vivo* blockade of this pathway can rejuvenate CD8 T cell function and enhance viral control (24, 27). Subsequently, involvement of the PD-1 pathway has also been observed during various human chronic viral infections, including HIV, HBV, and HCV in humans (28-34), and during non-human primate simian immunodeficiency virus (SIV) infection (35). The level of PD-1 expression per cell is important in regulating T cell exhaustion during chronic viral infection (27, 30). The frequency of HIV-specific CD8 T cells expressing PD-1 is correlated with viral load, declining CD4 counts, and decreased proliferation capacity (29, 36). Moreover, HCV-specific CD8 T cells with an impaired ability to proliferate and produce cytokines are closely associated with PD-1 expression (37). Accumulating evidence has indicated that tumors also exploit PD-1-dependent immune suppression. Expression of PD-1 ligands, such as PD-L1 and PD-L2, has been observed on a wide variety of tumors and hematologic malignancies. A strong correlation between PD-1 ligand expression on tumor cells and unfavorable prognosis has been demonstrated for various cancers (38-44). Importantly, high expression of PD-1 on TILs has been reported (21, 23, 45), and

PD-1 blockade has been shown to enhance the frequency of cytokine-producing cells (45). Currently, two humanized monoclonal antibodies against PD-1, ONO- 4538/MDX-1106 and CT011, are in clinical trials for treatment of cancer and HCV infection. Phase I clinical trial with ONO- 4538/MDX-1106 has been performed on 39 patients with non-smallcell lung cancer, renal cell carcinoma, colorectal cancer, melanoma, and prostate cancer (46). One patient with colorectal cancer displayed a complete response, and two patients with renal cell carcinoma and melanoma had partial responses. In a single-dose phase I study of CT-011, clinical benefits were observed in 33% of the patients, including one complete remission (47).

Recent observations revealed that inhibitory receptors other than PD-1, such as lymphocyte activation gene 3 (LAG-3), T cell immunoglobulin mucin 3 (TIM-3), cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), natural killer cell receptor 2B4 (2B4, CD244), leukocyte immunoglobulin-like receptor superfamily B member 3 (LILRB3, PIRB) and 4 (LILRB4, GP49), and CD160, are co-expressed on exhausted T cells during chronic viral infection and tumor progression (22, 48-52). There is considerable diversity in the number and type of inhibitory receptors expressed on T cells, and different inhibitory receptors may regulate distinct aspects of functional exhaustion during chronic viral infection (16, 48). Of note, many of these inhibitory receptors are co-expressed by the same exhausted CD8 T cells and appear to parallel the exhausted state of the cells as well as the severity of infection during chronic LCMV infection (48). Blockade of PD-1 and LAG-3 inhibitory receptor pathways together synergistically improve T cell responses and diminish viral load during chronic viral infection (48). We also found that co-expression of PD-1 and TIM-3 is associated with more severe CD8 T cell exhaustion, and that combined blockade PD-1 and TIM-3 pathways *in vivo* leads to much greater reversal of T cell exhaustion and viral control than blockade of either pathway alone (50). Similarly, a fraction of TILs co-expressing PD-1 and TIM-3 exhibit a more severe exhausted phenotype in mice bearing solid tumors and in

patients with advanced melanomas (22, 52). Furthermore, combined targeting of the PD-1 and TIM-3 pathways is more effective in controlling tumor growth than targeting either pathway alone (52). Collectively, these results indicate that multiple inhibitory receptors cooperate and independently contribute to T cell exhaustion during chronic infection and tumor growth, suggesting that reversal of T cell exhaustion could be improved by therapeutic targeting of multiple inhibitory receptor pathways simultaneously.

### Alteration of intracellular signaling pathways in exhausted T cells

In addition to the elevated expression of multiple inhibitory receptors, the molecular signature of T cell exhaustion reveals pervasive changes in transcription in comparison to functional T cells, including altered expression of transcription factors, changes in signal transduction, and down-regulation of key metabolic genes (Fig. 1) (2, 14, 18). For example, it was recently found that expression of Blimp-1, while initially induced following either acute or chronic LCMV infection, is dramatically up-regulated in virus-specific CD8 T cells as the chronic infection progresses and CD8 T cells become exhausted (53). Studies using mice with a conditional deficiency in Blimp-1 demonstrated that Blimp-1 promotes the over-expression of inhibitory receptors and also represses key molecules involved in normal memory CD8 T cell differentiation, such as IL-7 receptor and CD62L (53). Agnellini *et al.* showed that defective translocation of nuclear factor of activated T cells (NFAT) into the nucleus is responsible for defective cytokine production by exhausted CD8 T cells during chronic viral infection (54). Recently, basic leucine zipper transcription factor ATF-like (BATF) was identified as another transcription factor contributing to T cell exhaustion (55). BATF is known as a negative regulator of the transcription factor activator protein 1 (AP-1). PD-1 ligation up-regulates the basic leucine zipper transcription factor ATF-like (BATF) gene in both HIV-specific CD8 T cells from individuals with active HIV disease and LCMV-specific CD8 T cells from chronically infected mice (55). BATF is not only up-regulated in exhausted T cells but also inhibits T cell function, which demonstrates that PD-1 ligation impairs chronic virus-specific T cells through up-regulation of BATF. Besides transcription factors, suppressor of cytokine signaling 3 (Socs3), a protein known to modulate IL-6 responsiveness, seems to be involved in T cell exhaustion (56). Socs3 level is higher in T cells derived from mice chronically infected with LCMV, and T cell-specific deficiency of Socs3 leads to enhanced function of T cells, thereby controlling viremia. Of interest, IL-7 treatment decreases the level of Socs3 and reinvigorates the immune response to chronic virus infection, suggesting the possibility of IL-7 as a possible regimen for treating chronic viral infection. However, a number of questions remain to be answered regarding the fundamental control mechanism by which CD8 T cells undergo an altered pattern of differentiation and become exhausted during chron-

ic viral infection and tumor growth. In this context, understanding the transcriptional orchestration of T cell exhaustion could provide not only a novel mechanistic insight into T cell fate and differentiation decisions but could also reveal therapeutic opportunities.

## EXTRINSIC FACTORS REGULATING T CELL IMMUNITY IN A CHRONIC ANTIGEN ENVIRONMENT

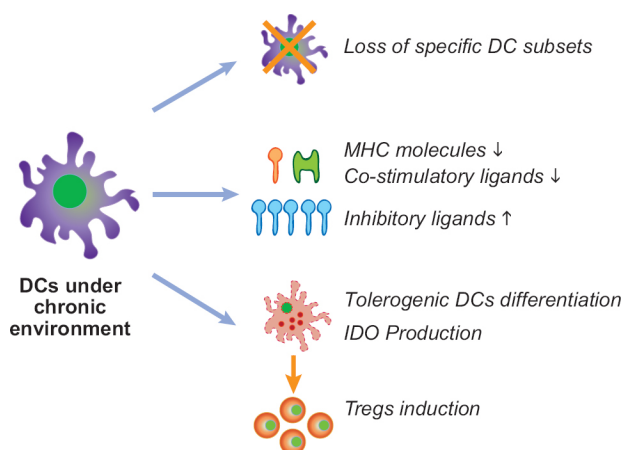
### Alteration of antigen-presenting cells during chronic pathogen infection

Individuals chronically infected with pathogens might contain certain levels of burden in either multiple or specific tissues depending on their cell tropism. Even though newly generated T cells from bone marrow can be primed by APCs, pathogen-specific T cells with cytotoxic or helper functionality are rarely observed during chronic pathogen infection. This ironic observation could raise the possibility that antigen presentation is probably not efficient due to improper T cell priming by non-professional APCs or by functionally altered APCs (Fig. 1). Indeed, it has been reported that some viral and bacterial proteins expressed inside cells directly interfere with T cell priming by modulating the expression of MHC or co-stimulatory molecules (57-60). Consequently, dominant and subdominant epitopes are not efficiently presented to T cells, which means that T cells are not optimally activated or often tolerated in some cases. Additionally, the prolonged presentation of epitopes on APCs occasionally leads to deletion as well as exhaustion of corresponding T cells (15, 17, 61, 62). In this session, we will introduce some recent works to explain T cell malfunction caused by improper antigen presentation and T cell priming.

Many chronic pathogen infections are represented by high levels of persisting pathogen burden. The amount of antigen seen by responding T cells may be one of the factors influencing T cell exhaustion (Fig. 1) (17, 63). Lymphoid stromal cells are often infected with chronic viruses such as HIV and LCMV (64, 65). Chronic pathogen infections of such non-professional APCs probably cause a variety of immunosuppression events, since they do not sufficiently express co-stimulatory molecules and produce immuno-stimulatory cytokines, unlike professional APCs, rendering responding T cells functionally tolerogenic or anergic. Further, stromal cells can drive mature DCs to differentiate into regulatory DCs under certain conditions (66, 67). Therefore, targeting and infection of non-professional APCs such as stromal cells and endothelial cells by chronic pathogens have been proposed as mechanisms that contribute to pathogen persistence (65, 68).

Chronic human viral infection often leads to the loss of dendritic cell populations (Fig. 3). In the blood of HIV-1-infected donors, the absolute numbers of myeloid DCs (mDCs) and plasmacytoid DCs (pDCs) were shown to be decreased and inversely correlated with plasma viral load (69, 70). A very similar finding was reported in patients infected chronically with

HCV (71). The loss of DCs could be due to preferential infection by chronic viruses. Indeed, several studies have demonstrated that both mDC and pDCs are susceptible to infections by HIV (72-77). The virus could be transferred to CD4 T cells through T-DC interaction, followed by the preferential deletion of responding CD4 T cells (73). It has been also demonstrated that chronic LCMV preferentially and persistently infects mDCs among DC populations (78). Therefore, the enhanced infection of DCs, followed by reduced T cell stimulatory capacity, is probably one of the mechanisms by which chronic pathogens initiate immunosuppression within the host (79, 80). More interestingly, independent of preferential DC infection by chronic pathogens, it has been reported that the functional impairment of DCs is associated with the exhaustion of T cell function and progression of disease during HIV, HBV, HCV, and LCMV infections (81-86). However, the molecular mechanisms underlying impaired T cell function mediated by DCs during chronic pathogen infection have to be fully elucidated. Recent studies suggested that dendritic cells induce T cell exhaustion or tolerance through signaling by inhibitory receptors such as PD-1 and CTLA-4 (83, 87). Indeed, PD-L1 is up-regulated on mDCs, whereas MHC molecules and co-stimulatory molecules such as B7-1, B7-2, and CD40 are down-regulated (83, 85, 88). Of interest, up-regulated PD-L1 seems to impair DC function and correlate with disease progression during HIV and HBV infections as well as tumor development (83, 85, 88). On the other hand, how the expression of MHC molecules, co-stimulatory molecules, and inhibitory molecules is regulated during antigen persistence still remains elucidated. Taken together, the continual integration of inhibitory receptors on T cells with their ligands



**Fig. 3.** Functional modulation of DCs during chronic pathogen infections and cancer development. DCs are modulated by different mechanisms as follows: Loss of DC subset, down-regulation of MHC and co-stimulatory molecules, up-regulation of inhibitory molecules, differentiation into tolerogenic DCs followed by induction of Tregs, and inhibition of DC development by altered production of IFN-I.

on DCs or other cells might play a critical role in generating and sustaining T cell exhaustion.

Recently, it was demonstrated that pDCs generated during HIV (89), HCV (90), and cancer development (91) can induce Tregs via an indoleamine 2,3-dioxygenase (IDO)-dependent mechanism (Fig. 3). In addition, Tregs induced by pDCs were shown to secrete IL-10 and markedly up-regulate PD-L1 on bystander conventional DCs, followed by inhibition of DC maturation. These data indicate that pDCs limit the induction of overly intense immune responses through the generation of Tregs. In addition to pDCs, mDCs seem to be involved in the proliferation of Tregs during chronic HCV infection (90). Dolganiuc *et al.* demonstrated that mDCs from HCV-infected patients limit T cell proliferation in an IL-2/IL-10-dependent manner and induce Treg expansion. Therefore, it can be proposed that DCs themselves are not only phenotypically and functionally modulated under antigen persistence but also induce other regulatory cells such as Tregs.

Chronic pathogen infections are also often associated with brief or long-lasting suppression of host immunity via a variety of mechanisms. Such virus-induced immunosuppression can lead to increased susceptibility to opportunistic infections, which represent a global threat to human health. One of the mechanisms underlying enhanced susceptibility is the impairment of type I interferon production (IFN-I) (Fig. 3). pDCs are known to be a major cell population producing IFN-I through toll-like receptor (TLR) signaling (92, 93). Generally, pDCs produce most IFN-I within the first 2 days after pathogen infection and then differentiate into mature DCs with enhanced antigen-presenting capacity, after which they become refractory upon secondary stimulation and lose their IFN-I production ability (94). LCMV infection of DCs within bone marrow results in increased local production of IFN-I, which inhibits DC development of precursor cells and later suppresses the IFN-I signaling pathway in DCs (95, 96). Measles virus (MV) was reported to block signaling for IFN-I production via TLR on pDCs (97). Such modulation can subsequently serve to impair natural killer cell responses, maturation of other subtypes of DCs, and expansion/differentiation of pathogen-specific T and B cells (98). Presumably, active negative feedback circuits that suppress IFN-I production are maintained during chronic pathogen infection, since the pattern of persistent pathogens can still be recognized by TLRs. However, the underlying mechanism should be further investigated.

### Suppression of T cell immunity by Tregs

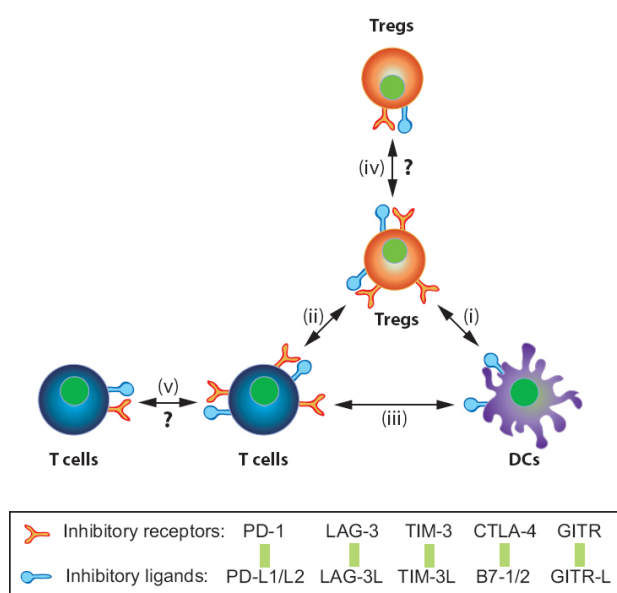
In general, T cells showing canonical expression of forkhead box P3 (Foxp3) and CD25 molecules can be classified as 'natural Tregs' (99, 100). Foxp3 is required for their development, maintenance, and function, thereby helping Tregs regulate the immune system and prevent autoimmunity (101, 102). Further investigations demonstrated that natural Tregs express a variety of inhibitory molecules such as LAG-3, glucocorticoid-induced tumor necrosis factor receptor (GITR), CTLA-4, and PD-1 either

on the cell surface or in the intracellular region (Fig. 4). General perception to date of immunosuppressive mechanisms by natural Tregs includes both direct and indirect suppression. Direct suppression is based on the observation that, for instance, CTLA-4, a representative inhibitory molecule expressed on Tregs, can bind to B7-1 or B7-2 on APCs, inhibiting their maturation and function and resulting in aberrant priming of antigen-specific T cells (103). Besides the interaction between Tregs and APCs, it was recently hypothesized that Tregs can sense recently activated effector T cells through a receptor-ligand interaction, and this in turn might potentiate Treg function such as increased production of the regulatory mediators IL-35 (104) and granzyme (105, 106). As an indirect mechanism, Tregs might suppress effector T cells by consuming local IL-2, which is required for the survival of actively dividing effector T cells, since the high expression level of CD25 empow-

ers Tregs to bind efficiently to IL-2 (107, 108). Indirect suppression might also occur through the secretion of immunosuppressive cytokines such as IL-10 or transforming growth factor- $\beta$  (TGF- $\beta$ ), which suppresses the function of effector T cells and maturation of APCs (109), but their contribution to Treg function is still a matter of debate (110-113).

The immunosuppressive functions of natural Tregs are essential in preventing organisms from exhibiting severe pathological responses upon pathogen infection. Under certain circumstances, conventional T cells have been known to differentiate into Tregs, called 'induced Tregs', resulting in the acquisition of Foxp3 expression and suppressive functionality (114, 115). There are several studies showing enhancement in the frequency of natural and induced Tregs during helminthic infections (116, 117) and bacterial infections such as *Leshimania guyanensis* (118), *Listeria monocytogens* (119), and *Mycobacterium tuberculosis* (120). Upon chronic viral infection, Tregs contribute to an increase in the viral load or the inhibition of antiviral T cell responses. Interestingly, increased frequency of Tregs was previously observed during chronic viral infections with HBV (109, 121, 122), HCV (13, 90, 123-127), and HIV (89, 128-130). Other than in human, enhancement of Tregs frequency has been observed in mice models upon chronic infections with coronavirus (131), retrovirus (132-136), and arenavirus (137). Increasing evidence also indicates that Tregs within the tumor microenvironment might play a substantial role in the suppression of T cell immune responses against cancer cells. Many groups reported elevated frequency of Tregs within the tumors or peripheral blood of a variety of cancers (88, 138-146).

To date, it is still not well understood how Tregs influence chronic pathogen infections and tumor growth (Fig. 4). As previously described, Tregs can act through direct cell-to-cell contact and/or an indirect manner via immunosuppressive cytokine production. Although it is still not precisely clear whether or not Tregs elevated under antigen persistent environment can directly exhaust T cells, accumulating data indicate that, at the very least, Tregs potentially contribute to the inhibition of T cell effectiveness and promote prolonged antigen persistence and secondary T cell exhaustion (14). A more interesting point is that chronic microenvironment contributes to the enhancement of the immunosuppressive function and proliferation capacity of Tregs. Therefore, many researchers are trying to elucidate which factors are strongly associated with the functional enhancement of Tregs during chronic pathogen infection and tumor growth. One set of candidates are a variety of inhibitory molecules up-regulated on Tregs. Indeed, Johanns *et al.* demonstrated that Treg expression of CTLA-4 directly increases the suppressive potency of Tregs during persistent *Salmonella* infection by showing that the effects of Treg ablation are recapitulated after CTLA-4 *in vivo* blockade (118). In non-small cell lung cancer patients, Tregs present in TILs were reported to express very high level of CTLA-4 and directly inhibit T cell proliferation via CTLA-4 signaling (145). Furthermore, a trial to



**Fig. 4.** Regulatory network composed of Tregs, T cells, and DCs to suppress T cell immunity via inhibitory receptor-ligand interaction. Effector T cells might be exhausted within the network. Various interactions such as Treg:DC, Treg:T cell, Treg:Treg, or T cell:T cell can eventually lead to the suppression of effector T cells. These mechanisms are mediated by reciprocal interactions between inhibitory receptors and ligands as follows: (i) Tregs directly interact with DCs to enhance their own suppressive function of Tregs or to decrease the capacity of DCs to prime T cells, (ii) Tregs probably alter T cell activation signaling to diminish the proliferation and function of antigen-specific T cells, (iii) DCs facilitate inhibitory receptor signaling in effector T cells, leading to T cell functional exhaustion, (iv) inhibitory receptor-ligand interactions occurring between Tregs probably potentiate suppression of effector T cell immune responses, (v) a similar interaction between effector T cell and exhausted T cells might affect their own function, leading to exhaustion. The interactions for (iv) and (v) are not proved yet.

block CTLA-4 on both Tregs and effector T cells led to a successful antitumor response (147). In melanoma patients, LAG-3 on Tregs was reported to mediate the reduction of CD8 T cell proliferation as well as the production of the immunosuppressive cytokines such as IL-10 and TGF- $\beta$ 1 (148). Some other studies reported that in ovarian cancer patients, Tregs with a high expression level of GITR in ascites or blood suppressed TAA-specific immune responses (88). On the other hand, PD-1 is expressed on the surface of Tregs as well as exhausted T cells (137, 149, 150), and its signaling on Tregs plays a role in induced-Treg (iTreg) production, maintenance, and function (151). However, whether or not the PD-1 pathway plays a role in expansion or function of Tregs in a chronic antigen environment is not yet clear. Recently, it was reported that PD-1 blockade of Tregs alleviated the suppressive potency of Treg in melanoma patients (152).

It now seems obvious that inhibitory receptors expressed on Tregs have profound effects on the suppressive function of induced Tregs in a chronic antigen environment. However, it is still necessary to investigate the mechanism by which the ligation of inhibitory receptors enhances suppressive function. Based on previous publications, we can speculate on the mechanisms of inhibitory molecules on Tregs during chronic infection and cancer development as follows (Fig. 4): (i) inhibitory receptors on Tregs directly interact with their ligands expressed on DCs, thereby facilitating the expansion of Tregs, increasing the potential of Tregs to suppress T cell responses, or decreasing the capacity of DCs to prime naïve T cells, (ii) inhibitory receptor ligation between Tregs and effector or exhausted T cells probably alters T cell activation signaling to diminish the proliferation and function of antigen-specific T cells, and (iii) inhibitory ligands on DCs activate inhibitory receptor signaling in effector T cells, resulting in functional exhaustion. Even though there is no supporting evidence, (iv) inhibitory receptor-ligand interactions occurring between Tregs probably potentiate the suppression of effector T cell immune responses, and (v) a similar interaction between effector or exhausted T cells might affect T cell function, leading to exhaustion. Based on the possibility that inhibitory receptors expressed on exhausted T cells are shared on Tregs, targeting of inhibitory receptors such as PD-1, CTLA-4, and GITR could be a promising strategy for treating chronic pathogen infections and cancers (46, 47, 153-157).

#### Increase of immunosuppressive cytokines

IL-10 is an immuno-regulatory cytokine that can attenuate inflammatory responses (158). IL-10 has multiple effects and has been shown to reduce pro-inflammatory cytokine production, impede the function of APCs, dampen T cell responses, and also affect B cell dysregulation (158, 159). IL-10 is produced by CD4 T cells such as Tregs, as well as many other cell types such as DCs, macrophages, B cells, and CD8 T cells (158, 159). Chronic infection by EBV, HBV, HCV, HIV, or LCMV is associated with the increased production of IL-10 (160-167).

Recently, two studies identified IL-10 as a key regulator of viral persistence during chronic LCMV infection (161, 163). In a previous study, mice lacking IL-10 or treated with an IL-10R blocking antibody rapidly controlled replication of a chronic LCMV strain and developed functional T cell responses (161, 163). These important observations demonstrate that the levels of IL-10 can have a profound influence on the outcome of chronic infection as well as the quality of the cellular immune response. Another immunosuppressive cytokine, TGF- $\beta$ , can impact on immune responses during persisting infections (168, 169). TGF- $\beta$  controls immune responses and maintains immune homeostasis by affecting proliferation, differentiation, and survival of multiple immune cell lineages (169-171). The significance of the TGF- $\beta$  pathway on the development of T cell exhaustion has been further dissected following chronic LCMV infection. Interestingly, the level of TGF- $\beta$  is elevated under these conditions, and thus the TGF- $\beta$  pathway appears to regulate the size and function of T cells (172).

The tumor microenvironment has also been shown to contain the soluble factors, IL-10 and TGF- $\beta$ , which can inhibit T cell function (173). These cytokines can be produced by tumor cells themselves or non-tumor stromal cells (174). IL-10 has been shown to inhibit DC-mediated CD8 T cell priming *in vitro* (175). In addition, IL-10 blocks DC recruitment in response to a granulocyte-macrophage colony-stimulating factor (GM-CSF)-based cancer vaccine, indicating that the priming phase of an anti-tumor immune response also might be antagonized by IL-10 (176). The inhibitory action of TGF- $\beta$  on anti-tumor T cells was demonstrated through the use of a dominant-negative TGF- $\beta$  receptor expressed in T cells. Adoptive transfer of T cells with a TGF- $\beta$  signaling defect results in improved tumor control *in vivo* (177). Importantly, in a study using metastatic melanoma gene array, most tumors contained cells expressing IL-10 and/or TGF- $\beta$  (178), suggesting that these cytokines could be utilized to establish an immunosuppressive microenvironment for tumors.

#### CONCLUSION

Immunological dysfunction after chronic pathogen infections and cancer development is the strongest threat to human health. During recent several years, researchers found that there is a common phenotype imprinted on T cells in a chronic antigen environment. Chronic pathogen- or tumor-specific T cells themselves are functionally altered, followed by failure to control diseases. Nowadays, we have accumulated tremendous knowledge about the phenotypic and functional characteristics of exhausted T cells, but we still do not know the exact mechanisms causing T cell exhaustion. In this review, we tried to elucidate the factors to date contributing to the suppression of T cell immune responses during chronic pathogen infections and tumor growth. There are a variety of unknown environmental factors leading to T cell exhaustion. Understanding the chronic niche is definitely required to design a

strategy to combat chronic pathogen infections and cancer progression. In this regard, combination therapy to block multiple inhibitory molecules and neutralize regulatory cytokines along with optimal antigenic stimulation should be considered as a treatment for chronic pathogen infections and cancers.

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