A Comprehensive Review on T Cells, B Cells, Natural Killer Cells, and Dendritic Cells Exhaustion: From Main Concepts to Clinical Use

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Abstract- During chronic infections, a distinct physiological condition named "exhaustion" arises that is associated with the dysfunctionality in immune cells and their eventual removal from the environment, which evidently help the progress of infection or tumors in human or animal bodies. This state of immune cells could be under the control of different elements such as the antigen load, help from inhibitory cells, lack of costimulatory signals and etc. Exhaustion that has been found in different immune system cells, is usually accompanied by impaired effector function and proliferation of immune cells, poor memory recall, upregulation of inhibitory molecules, compromised metabolism and altered transcription program, and is considered a reversible process, unlike other physiological states like anergy or senescence, organized through the blockage of several factors. Although the emergence of these cells in viral infections and cancer is an undesirable event, the importance of the presence of exhausted cells in autoimmune diseases and organ transplantation is highlighted as a positive change. In this review, we aimed to determine the occurrence of this process in different immune cells, the characteristics obtained by these cells, effective and primitive factors on exhaustion, metabolic and transcriptional cell changes, and the use of these cells in autoimmune diseases and organ transplantation.

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Introduction

Despite the differences in the nature of infectious agents, immune system responses usually start with the reactions of innate immunity, from taking up the antigens to processing and presenting them to more specified immune cells belonging to adaptive immunity. In other words, this acute response requires the establishment of a collaboration between immune cells, which begins with antigen-presenting cells in charge of picking up the antigens and delivering them to T cells, usually lasts for 1-2 weeks, proceeds with clonal proliferation and differentiation of B and T cells, and finally ends up in the clearance of infectious agents deletion of 95% of effector cells and the formation of memory responses by T and B cells (1). However, following chronic infection, an almost stable and dynamic phase is formed in the body; in other words, the immune system and the infectious agent

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balance each other. During this phase of the infection, the failure of immune cells in this battle and the persistence of the infection cause continuous stimulation by the antigens, the immune cells become exhausted and are unable to remove the agent; in other words, they become addicted to the infectious agent (2). The appearance of this status shares some properties with anergy and senescence, which means immune cells that have gone through exhaustion lose their functions, and it eventually leads to their removal and apoptosis (3). More interestingly, the footprints of this phenomenon have been sighted in tumors. In the microenvironment of cancer, due to low immunogenicity and low expression of tumor antigens and co-stimulatory molecules, high expression of inhibitory and inflammatory cytokines, and acidic environment of the tumor, immune effector cells are affected and differentiated into exhausted cells (4).

Although it seems that the development of this physiological state in cells delays the response to the growth of the infection and cancer cells, but prolonged response causes pathological reactions that ultimately lead to damage to tissue. Therefore, the production of these cells during prolonged response can prevent tissue damage (5). So far, this phenomenon has been discovered and well-studied in T cells; however, recent studies have shown this process is not only limited to T cells, but also it can occur in B cells (6), natural killer (NK) cells (2), as well as innate lymphoid-like cells (ILC) (7), and dendritic cells(DC) (8), suggesting that exhaustion might be a strategy coming from infectious agents or cancer cells to guarantee their persistence. More notably, exhaustion has been sighted in CAR T cells (9). Therefore, considering the extension of exhaustion in immune cells and its impacts on the efficiency of the responses, in this review we will focus on immune cells exhaustion, underlying reasons and the process of its progress, its influence on the function of immune cells, features of exhausted cells, and the present methods to preserve and reverse this phenomenon and the answer to this question that how exhaustion can be advantageous.

Exhaustion of T cells

The history of the discovery of exhaustion dates back to the 1960s when the experiments conducted on the exposure to high doses of antigen, led to the description of depletion and exhaustion (10). For the first time, this process was detected in CD8+T cells of mice infected with lymphocytic choriomeningitis virus (LCMV) as, not only were these cells not able to remove infected cells but also they did not cause any immunopathological death (11). After the discovery of this phenomenon in mice, studies were carried out to identify this phenomenon in primates such as humans, which led to the discovery of this phenomenon in human infections with Simian immunodeficiency virus (SIV), Human Immunodeficiency Virus (HIV) (12), Hepatitis B virus (HBV) (13), Hepatitis C virus (HCV) (14), and human T-cell lymphotropic virus (HTLV) (15), and bacterial infection with Mycobacterium tuberculosis (16), and fungal infections such as Candida albicans and Metastatic cancers (17). Also, in a recent study, the presence of a group of T cells with the exhaustion phenotype was detected in patients with common variable immune deficiency (CVID) (18).

Exhaustion of CD8+T cell as the first and major group affected by exhaustion, is defined by the stepwise and progressive loss of CD8+T cell functions, starting with Interleukin-2 (IL-2) production, followed by impairing tumor necrosis factor-α (TNF-α) production, and in severe levels of exhaustion, CD8+T cells either entirely or partially lose the ability to produce interferon-gamma (IFN-γ), beta-chemokines, or degranulation of them, which eventually leads to their physical elimination. Moreover, Up-regulation of inhibitory receptors such as programmed cell death protein 1 (PD-1/PD-L1), lymphocyte-activation gene (LAG-3), T cell immunoglobulin and mucin domain-containing protein 3 (Tim-3), 2B4/CD244, CD160, T cell immunoreceptor with Ig and immunoreceptor tyrosine-based inhibition motif (ITIM) domains (TIGIT), GP49B, and Cytotoxic T-lymphocyte protein 4 (CTLA4) has been sighted as the most prominent features of exhausted T cells (19). Other features that have been found out in these cells include changes in response to hemostatic cytokines and metabolic pathways, lack of antigen-independent homeostasis, and changes in chemokine receptors, signaling, migration, and implantation (20). Concerning the factors involved in the establishment of Exhaustion in CD8+T cells, along with the duration of infection, the type of epitope delivered, and the load of the virus, other factors such as the level of cytokines produced, the presence of CD8+T cells, NK cells, and Antigen-presenting cells(APC) cells can be at the root of this phenomenon (21). The type of infection can also determine the characteristics of exhausted cells (5), which will be discussed in the following sections.

$CD4^+$T cells, like $CD8^+$T cells, undergo exhaustion following prolonged, chronic infection, and cancer (22). Exhaustion in CD4+T cells usually occurs following continuous stimulation by antigen, meanwhile, it seems that stimulatory signals play a role in inducing this condition in $CD4^+$T cells. Like CD8+T cells in these

cells, increased expression of CD200, B And T Lymphocyte Associated (BTLA), CTLA4, TIM3, LAG-3, and PD 1, and decreased production of functional cytokines such as IL-2, TNF-α, and INFγ (Figure 1) (23) can be detected in CD4+T cells and, unlike exhausted CTLs these cells can produce IL-10 or IL-21. Although CD4+T cells possess some common transcription factors with $CD8^+$ T cells, they specifically express GATA-Binding Protein 3 (GATA3), T-Box Transcription Factor 21 (Tbx21), RAR Related Orphan Receptor A (Rora), B-Cell Lymphoma 6 Protein (Bcl6), and B-Lymphocyte-Induced Maturation Protein 1 (Blimp-1). Having said that, the expression of these factors is not observed in all $CD4^+$T cells presented in infection, which indicates the presence of different subgroups of $CD4^+$ T cells in exhaustion during chronic infection (24). Recent studies have also shown a decrease in response to T helper type 2 (Th2) cells following chronic infection with filarial nematode Litomosoides sigmodontis in the infected mice. This phenotype was associated with loss of functional capacity to produce interleukins 2, 5, and 4 and increased expression of PD-1which could be restored by blocking PD-1/PDL-2 (25). Although this phenotype had been previously considered to be related to the exhaustion phenotype, recent studies have shown that the transcriptional profile of these cells is different from exhausted $CD4^+$T cells and has some similarities with the cellular anergy (26).

Exhaustion of NK cells

The same as T cell, prolonged exposure to the microenvironment of a tumor or a chronic infection usually results in a state of dysfunctionality in NK cells. This state in NK cells, which was first evidenced in the 1985s, and has been earlier sighted in other adaptive immune cells, is suspected to be the exhaustion phase accompanied by suppressing the anti-tumor/infection potential of NK cells (27).

Under normal circumstances, the elimination of infected cells by NK cells is typically carried out through three mechanisms, which are categorized into two models of direct and indirect. In the direct model, mediated by the release of granules containing vital factors in perforation of cell membranes, caspase-dependent or independent mechanisms like perforin/granzyme; antibody-dependent cell-mediated cytotoxicity (ADCC), tumor necrosis factor receptor superfamily member 6 (TNFRSF6) (Fas ligand (FasL)) and TNF-related apoptosis-inducing ligand (TRAIL) play the leading roles. The indirect model is also based on the function of NK cells in the production of IL-10, TNF-α, INF-γ, chemokine ligands-4 (CCL-4), CCL-3, and-5, which could recruit, activate, and promote differentiation of innate and adaptive inflammatory immune cells (28). However, NK cells functions can be impaired and become targets of exhaustion, probably due to the presence of inhibitory factors like regulatory cells or soluble factors in the microenvironment of tumors or infections, which are naturally programmed to activate some specific pathways to control NK cells activity and prevent these cells from attacking normal cells (29). The exhausted phenotype of NK cells has been observed in several cancers including, acute myeloid leukemia, breast, pancreas, colorectal, and stomach cancer (30) and viral infections with HIV (31), HCV (32), and HBV (33) viruses.

The status of exhaustion in NK cells could probably stem from impaired signaling, the presence of regulatory cells such as myeloid-derived suppressor cells (MDSC) that inhibit natural killer group 2D (NKG2D) expression by producing suppressive cytokines, recruitment of melanoma-derived fibroblasts that produce prostaglandin E2 (PGE2) and Indoleamine 2, 3-dioxygenases (IDO), inhibitory cytokines such as transforming growth factor-beta (TGF-β), exosomes produced by tumor cells, and increased expression of co-stimulatory receptors and hypoxia in the tumor microenvironment (Figure 1) (34). Moreover, in the experiment performed by Felices *et al*., continuous exposure to IL-15 has been shown to lead to a decline in viability, function and signaling of NK cells, which was accompanied by reduced fatty acid oxidation (FAO). The permanent exposure to IL-15 exerted dysfunctional NK cells, which was reckoned to be exhausted NK cells, providing evidence that prolonged cytokine exposure might result in detrimental impacts and dysfunctionality in NK cells, which can be associated with the exhaustion phase (35). Like exhausted T cells, once that NK cells have gone through exhaustion, several changes occur in the phenotype of these cells, which affect their activity. Some of these changes are including metabolic changes, changes in gene transcription, increased expression of inhibitory receptors such as TIGIT, as one of the vital NK cells exhaustion markers, Tim-3, PD-1, and NKG2A, and decreased expression of NKG2D, CD16, natural cytotoxicity receptors (NCRs) NK lineage-restricted progenitor (NKp30, NKp44, NKp46), CD226, and 2B4 receptors, reduced expression of molecules involved in cytotoxicity such as perforin and granzyme, increased proliferation and decreased expression of INFγ (2). As these changes come with the expression of inhibitory molecules, it seems that blocking them could reverse and return functions of NK cells as TGF-β blocking in a study was associated with restoring

dysfunctional NK cells in human hepatocellular carcinoma or in another experiment, TIM-3 blockade was accompanied by the improved phenotype of NK cells (36).

Exhaustion of B cells

B cells, as the most vital member of the humoral immune system, are considered another target of exhaustion, which could possibly limit their responses to infections. While the features of exhausted T cells and factors influencing this phenomenon in T cells have been well defined, studies about this condition in B cells in different infections are incomplete. However, it seems continued exposure to bacterial and viral antigens, for example, in chronic HIV infection, leads to exhaustion of memory B cells. Specifically, exhaustion of B cells in HIV infection was demonstrated by the presence of a specific group of B cells called Tissue-like memory B cells mainly recognized by the high expression of CD20, low expression of CD21, other markers like Fc receptor-like protein 4(FCRL-4), and inhibitory molecules (37).

In an experiment on patients with GVHD, dysfunctional B cells were presented by $CD21^{-}$, $CD27^{-}$, $CD10^{-}$, $CD19^{+}$, decreased proliferation, increased expression of inhibitory receptors, and changed implantation receptors (38). The most important similarity of these cells with exhausted T cells is the expression of inhibitory receptors such as PD-1, CD72, CD22 receptors, and Leukocyte-associated immunoglobulin-like receptor 1 (Lair-1)(Although these receptors are expressed in naive cells too, their expression is increased in exhausted B cells.), CD32b, and Sialic acid-binding Ig-like lectin 6 (Siglec-6) receptors, which are specific to exhausted B cells, and CD85d, CD85j, and CD85k, which are commonly found in NK cells, as well as implant-related receptors such as CD11c and C-X-C Motif Chemokine Receptor 3 (CXCR3). Characteristics such as decreased proliferation and immunoglobulin diversity in B cells following exhaustion have also been observed (6). Although similar groups of dysfunctional B cells have been seen in other chronic infectious and non-infectious diseases such as HCV, Mycobacterium tuberculosis, and Plasmodium falciparum, not all of them were nominated as exhausted cells as in a recent study on Plasmodium falciparum, the presence of atypical B cells defined by $CD19^{+}$, $CD27^{-}$, $CD10^{-}$, $CD21^{-}$was observed alongside the persistence of Plasmodium falciparum, but these cells have not been defined as exhausted cells (39).

Exhaustion of DCs

DCs are a subset of bone marrow-derived cells located in various tissues and organs to act as initiators of the immune reactions. This function of DC's is performed by tacking up the antigen, processing and presenting it to specialized acquired immune cells. Three subsets are defined for dendritic cells, including pDC, mDC type one and two, and monocyte-derived DCs, which play essential roles in infection and tumors as one of the most important suppliers of antigens that can also be influenced by exhaustion (40). Previously the role of IL-10 in the exhaustion of DCs, and the role of CD40 in restoring IL-10 dependent exhaustion has been discovered (41). In the following studies, LCMV CL-13 and HIV infections were shown to be capable of targeting plasmacytoid dendritic cells (pDC) and use these cells to spread and cause a chronic condition (42). In HIV specifically, the viruses can interfere with the function of DCs by delaying the production of INFα, which are considered vital for the initiation of an immune response. In infections with HBV and HIV decreased numbers of pDCs with impaired ability to produce INF-α, reduced ability of myeloid dendritic cells (mDCs) to stimulate T cells, have been observed (43). In CL-13 infection, the reduction in the production of INF-α, and decrease in expression of co-stimulatory molecules and major histocompatibility complex (MHC) class I and II, may interfere with the ability of DCs to deliver antigen and stimulate T cells, which eventually could prevent the differentiation and maturation of DCs (44). Not surprising that, in people with chronic infection with HCV, the number of mDCs and pDCs, their ability to stimulate T cells, and produce INF-α was lower than healthy people (45). Exhaustion of pDCs in the tumor microenvironment has also been associated with a defect in the production of INF-α along with a defect in the production of IL-6 and TNF-α, and induction of the production of IL-10 by $CD4^{+}$ T cells (Figure 1) (46). Finally, a recent study found that the continuous stimulation of DCs after LCMV infection by TLR-7 signaling may reduce the production of INF-α and sustain the population of exhausted pDCs (47). As a result, it can be assumed that exhausted pDCs have a deficiency in the production of INF-α in response to stimulation with TLR, chronic infection, or stimulation by other viruses, and this deficiency can cause the disruption of responses by the innate immune system and control of infection.

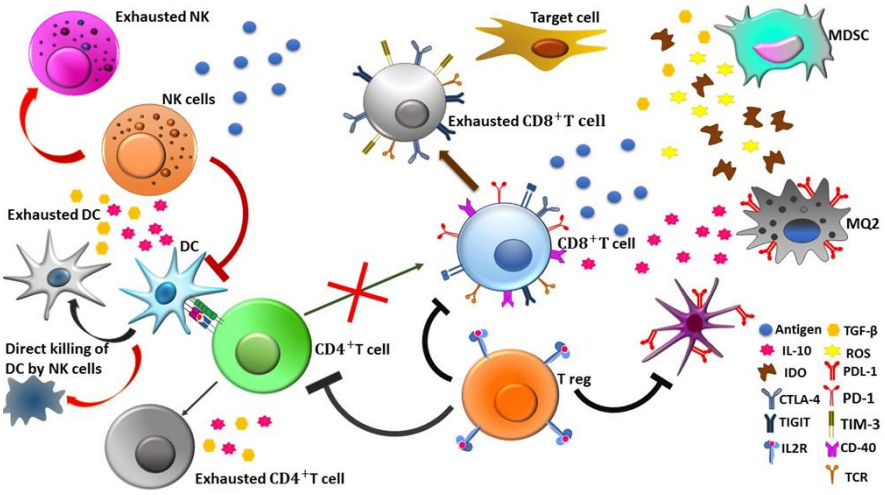

Figure 1. During the persistence of an antigen, a number of factors could help establish exhaustion of immune cells like 〖CD8〗^+T cells, 〖"CD4"〗^"+" T cells, NK cells, and dendritic cells, which is followed by the increase of the expression of inhibitory receptors. T regs by consuming IL-2 and production of IL-10 and TGF-β suppress 〖"CD4"〗^"+" and 〖CD8〗^+T cell responses. The formation of M2 macrophages and MDSC, which produce IL-10, TGF-β, and ROS, contribute to T regs formation and inhibiting T cells and NK cells functions. On the one hand, the presence of NK cells could limit the other immune cells' functions by a range of mechanisms like direct killing and the production of inhibitory factors. On the other hand, the deletion of the help of 〖"CD4"〗^"+" T cells could restrict 〖CD8〗^+T cells function and help the survival of infectious agents

Supportive factors of exhaustion

Antigen load

The common feature in the chronic infections and cancer, besides the other involved factors in exhaustion seems to be long term exposure of immune cells with antigens, and the high load of antigen (21). While several studies have shown that this phenomenon mostly takes place at higher doses of chronic infections with viruses such as LCMV, HBV, HCV, and HIV infection (48), infections with yellow fever and the flu can lead to the formation of memory and effector cell responses (49). Therefore, it can be concluded that the magnitude, duration, and repeated T cell activation by the provided antigens exert a differentiating phase which is followed by the phenotypic and functional quality of the response.

Furthermore, unstable viral epitope mutations by creating plasticity status in exhausted subsets can change the phenotypic and functional states of anti-vital T cells (21). In viral diseases such as LCMV, HIV, HCV, and SIV, the emergence of an escape mutation prevents T cell exhaustion. However, this mutation does not prevent loss of function, increased PD-1 expression, and progression to exhaustion in non-mutated epitope-specific cells, specifically in LCMV. In HIV, HCV, and SIV infections with mutations in the presented epitope, a decrease in PD-1 expression was observed in T cells specific for non-mutant epitopes (50). Recent studies indicated the role of the type of epitope presented and the type of infection in the severity of exhaustion. The first stage of exhaustion, which is accompanied by a lack of IL-2 production, loss of cytotoxicity, and poor cell proliferation, is seen in a group of viral infections, including LCMV, HIV, and HCV. The second stage of exhaustion, which is associated with the non-production of TNF-α and incomplete production of INFγ, has occurred in LCMV, HIV, HCV, HBV, and adenovirus. The Full Exhaustion phase occurs only in LCMV, HIV, HCV, and HTLV, and eventually, the removal of exhausted cells occurs in LCM (51).

Cellular factors affecting exhaustion

Presence of NK cells and their inhibitory effects

NK cells, as a group of innate lymphoid-like cells, originating from the common lymphoid progenitor (CLP), have the potential to differentiate into ILC2, ILC3 (including CCR6+LTi-like cells). These cells are able to attack and lysis infected cells during viral and tumor infections (as it was previously mentioned) (52). However, these cells could play a different role during chronic infection, and unexpectedly, limit the antiviral responses of $CD8^+$T cells, which can eventually lead to the exhaustion of $CD8^+$T cells. However, this reaction might be against what these cells are programmed to act, this response seems to protect the body from pathological damage caused by the activity of $CD8^+$T cells (53).

This activity of NK cells has been observed during chronic infection with LCMV, HBV, HCV, HIV, and a group of bacterial infections such as Listeria

monocytogenes, Toxoplasma gondii, and Yersinia pestis (54). This reaction of NK cells is usually determined by the production of IL10 and TGF-β cytokines (55), a direct killing of T cells by NKGD2 signaling, expression of UL16 binding protein 1(ULBP1-3) and MHC Class I Chain-Related gene A (MICA) ligands in T cells, and restricting the functions of APC cells by the release of perforin (56), and limiting their ability to deliver antigens. Moreover, the depletion of these cells following the chronic infection is also associated with an increase in the number of $CD8^+$ T cells, preserving cytotoxic T lymphocyte (CTL) responses and protecting them from exhaustion (57).

Depletion of $CD4^+$ T cells help

Although $CD8^+$T cell activation is triggered by MHC class I signaling and DC stimulatory signals, the help from $CD4^+$ T cells seems to play an important role in the activation and response of these cells, since the helping signals promote maintenance of central memory T cells and seem to be vital for the regulation of the function and size of effector memory T cells (58). This help is often provided by cross-talking with DCs and activating them by CD40-CD40L signaling (59). It has also been reported that this cross-talk can be established directly by CD40-CD40L signaling between $CD4^+$ T and $CD8^+$ T cells (60). Moreover, the production capacity of IL2 and IL21 by these cells is considered an important factor for the activation and proliferation of $CD8^+$T cells (61). This means that, the lack of $CD4^+$T help can accelerate and increase the intensity of exhaustion since the main resource of IL-2 and IL-21 production, which are vital for the maintenance of CTLs responses, has been impaired (62). However it should be noted that , in some infections, stimulation of toll-like receptors (TLRs) stimulates the maturation of DCs and the production of factors needed to activate $CD8^+$T, such as INF type I, which could eliminate the need for $CD4^+$ T cells presence (63). During chronic LCMV infection, the need for IL2 and IL21 plays a vital role in maintaining $CD8^+$T responses. As In the absence of IL21 probably because of the lack of CD4 T cells help, the virus remains at a higher dose in the body, and the inability to express the IL2 receptor is associated with the removal of $CD8^+$T cells (64). The importance of the presence of $CD4^+$T cells and IL21 production can be specifically seen in HIV, HBV, and HCV infections as the higher IL21 levels gets, the better response of $CD8^+$T cells and control of the virus can be formed. More specifically the impact of the presence CD4+T cell can be observed in HIV infection, where CD4+T cells are the main targets of the virus, and the exhaustion of CD8+T cells occurs earlier (65).

Regulatory T cells (T regs) and their synergistic effect on exhaustion

$CD4^+$, $CD127^-$, and $CD25^+$ T regs that eventually appear in the environment at the last stage of response to infections have a role in subsiding the immune responses and forming tolerance. Furthermore, they prevent the immune system from overreaction resulting in pathologic damage and autoimmunity (66). Considering their role in the modulating immune reactions it seems the presence of T regs can be effective in inducing exhaustion in chronic infections and cancer, as the depletion of these cells following the infection with LCMV in mice has improved the function of $CD8^+$ T cells and their proliferation (67). However, it should be noted that the depletion of these cells was not associated with a reduction in viral load. In addition to chronic infection with LCMV, the presence of T regs has been observed in HCV, HBV, HIV, and tumor microenvironment (68).

The function of T regs to suppress responses is normally accompanied by the high expression of CD25, which enables T regs to consume IL-2 in the environment, and subsequently leads to the deficiency of this vital cytokine for effector T cells, meaning that it has inhibitory effects on $CD8^+$T proliferation and activity (69). Furthermore, the production of IL10, TGF-β, and IL35 by these cells can limit the responses of $CD8^+$T cells and help to induce this phenomenon in them (70). T regs by inhibiting $CD4^+$T cells activity and impending CD4 +T cells help, increasing the expression of inhibitory receptors such as PD-1 and CTLA4 in DCs, help to the growth of exhaustion (67). Besides the expression of CTLA4 in T regs suppresses the functions of $CD8^+$T cells (71). Finally, T regs, by affecting antigen-presenting cells make these cells produce IDO, which has a suppressive effect on effector cells (72). The presence of $CD8^+$T regs, which are involved in limiting immune responses during autoimmune diseases (73), has also been demonstrated during chronic infections such as LCMV and this group of cells can impact the function of other groups of T cells by the production of CCL4 and perforin. Therefore, it is not far-fetched to expect that targeting $CD8^+$T regs can be associated with restoring the function of CD8 $CD8^+$T cells (74).

The effect of the presence of APCs on exhaustion
DCs

DCs that act like detonators in the initiation of responses and activation of adaptive immune cells, during chronic infections become the main targets of the

infectious agents. For example, the function of pDCs, which play a key role in producing INFα/β and activation of CD4+T cells , CD8+T cells , macrophages (MQ), and mDCs get impaired (75),as in HBV and HIV infection, the ability of pDCs to produce INFα decreases, which in turn affects the function of the dendritic cells and T cells (76). During HIV and HCV infections, the virus uses pDCs to spread and survive in the body for a long time, meaning that these cells become the depository of the infection. In addition, preventing the maturation of DCs, the reduction of the first and second stimulatory signals required for T cell activation following DC infection can play a role in the endurance of the infection (77). Furthermore, by expressing PDL-1 and IL-10, these cells can participate in the survival of the infection as well as inhibit the antiviral responses of T cells and cause the spread of exhaustion (78).

In the tumor microenvironment, these cells also acquire immunosuppressive properties and take part in exhaustion through various mechanisms. Studies have shown that in the microenvironment of the tumor, the differentiation and maturation of DCs are usually impaired, which subsequently affects the function of these cells, and could cause these cells to fail in the presenting of antigens. This phenomenon can be along with an increase in the expression of PDL-1, and factors such as IL-6, TGF-B, OX40L, Inducible T Cell costimulatory ligand (ICOSL)(that helps the induction of T regs and production of IL-10 by them), Galactin-1, and IDO (79).

Regulatory B cells (B regs)

This group of B cells that contribute to less than 11% of the population of B cells, with a similar phenotype like plasma cells (expression of CD1d, CD27, and CD24) and several subgroups are believed to be responsible for maintaining 383 tolerance and homeostasis by the production of IL-10, TGF-β, and IL-35 (80) and subsequently suppressing the immune system meaning that they can play a role in a range of diseases 385 including HIV, HBV, bacterial diseases, and cancer (81) and favor them. For example, in HBV infection, these cells, by producing IL-10, can play a role in suppressing CD8+T responses of producing INF-γ and TNF-α, and CD4+T cell responses (82). IL-10 production by B regs can have substantial impacts as it is associated with the induction of T regs that can suppress the production of antiviral cytokines from pDCs (83). In HIV infection, IL-10 expression and increased PD-L1 expression in these cells are associated with 391 impaired immune function, inhibition of the function of other APCs, and proliferation of CD4+T cells; moreover, depleting these cells could help the recovery of CD8+T cells (84). Studies in mouse models have also shown that these cells could increase T regs formation by expressing TGF-β. Besides, these cells have been shown exhibit phenotypic expression of TGF-β, IL-10, and PDL-1, which suppress the responses of DC, MQ, B, and T cells in the tumor (81). It has also been illustrated that the expression of CTLA4 396 in CD4 +T cells forms and differentiates the iBreg group, which can regulate immune responses by producing TGF-β 397 and IDO and stimulate the appearance of T regs (85).

Macrophages (MQ)

MQs are the other group of effector cells, belonging to the innate immune system, with the ability phagocytose pathogens and release both pro-inflammatory and antimicrobial mediators that origins from hematopoietic progenitor cells and differentiate to monocytes. After entering the blood system, monocytes, in exposure to infectious agents, differentiates to macrophages. Macrophages, which have a significant part in eliminating diseased and damaged cells through their programmed cell death, are considered to have the plasticity to two groups, based on environmental factors and cytokines, classified as M1 (classically activated macrophages) and the M2 (alternatively activated macrophages), in other words, the function of these cells can be influenced by the plasticity (86).

M2 macrophages, with some subtypes, known to have repair and suppressive function, appearing at the final stages of infection, can also be recruited in chronic infections and cancer. For instance, following HCV, HBV, and HIV infections, and M2s can produce IL10 and TGF-β, which suppresses the immune system (87). In the tumor microenvironment, macrophages called Tumor-associated macrophages (TAM) can repress the immune system and promote exhaustion by various mechanisms. These cells express PDL-1, PDL-2, B7-1, B7-2, and expressing L-arginine could reduce TCR expression and promote T cell exhaustion. Moreover, TAMs can produce ROS, IL-10, TGF-β, which could stimulate T regs, suppress T cells cytotoxicity and induce exhaustion. They are also capable of expressing human leukocyte antigen (HLA-E) for NK, and non-classical HLA-G for T cells that inhibits these cells. Finally, by evacuating the environment from vital amino acids like tryptophan and L-arginine, TAMs can speed the process of exhaustion (88).

MDSCs

These cells with origination from myeloid cells and heterogenicity have been observed to take part in reduction

and suppressing the immune responses. These cells, especially in tumors, generally use a variety of mechanisms to attenuate immune responses. They impede responses through the production of reactive oxygen species (ROS), nitrogen oxide (NO), which mediates induction of oxidative stress, the inhibition of the differentiation of other myeloid cells, and STAT-5 and JAK3 phosphorylation, plus stimulation the apoptosis of T cells (89). MDSCs can also take advantage of two factors of inducible nitric oxide synthase (iNOS) and arginase (ARG)-1 to impair CD8 +T cell functions and reduce CD3ζ expression (90). Production of IDO by MDSCs which leads to formation of kynurenine could hamper the expansion of effector T cells. Moreover, depletion of amino acids such as L-arginine, L-tryptophan, and L-cysteine, could hugely impact T cells, impair T cells metabolism. More interestingly MDSCs are seemed to contribute to suppress NK cells responses by the production of TGF-β and ROS, and inducing T regs by IL-10 and TGF-β production (91).

In chronic infections such as HCV and HIV, this cell group also plays an essential role in suppressing immunity. During HCV infection, this cell group can modulate cellular responses by depleting L-arginine and the ROS-dependent pathway (92). In HIV infection, the presence of these cells and the production of arginase type 1 is associated with a decrease in the clonal proliferation of T cells and suppression of their function. Receiving highly active antiretroviral therapy (HAART) treatments has been incited to cause a reduction in the number of these cells and is associated with the restoration of $CD8^+$T cellular responses (93).

The effect of inhibitory and stimulatory cytokines on the exhaustion

Exhaustion promoting cytokines

TGF-β

TGF-β, as one of the cytokines that play an important role in the homeostasis of cells and organs, takes a role in suppressing and reducing immune responses. This cytokine, which can be secreted by various sources such as T regs, TAMs, MDSCs and cancerous cells, binds to its receptor and initiates the SMAD pathway inside the target cells (94). This cytokine can inhibit T cell proliferation by inhibiting IL-2 production, as well as inhibiting c-Myc involved in the cell cycle along with increasing cyclin-dependent kinase inhibitors p21 and p27 (95). TGF-β via the production of SMAD and ATF1, the suppression of INF-γ and β and granzyme, could suppress the cytotoxicity of $CD8^+$T cells (96). TGF-β which is produced by TAMs stimulate the expression of CTLA-4, TIM-3, and PD-1in T cells in a dose dependent manner. Moreover, TGF-β seems to affect NK cells and inhibit the various functions of these cells. This effect is mediated through three pathways: silencing of T-bet expression and transcription, followed by decreased INF-γ expression (97), decreased DNAX-activating protein of 12 kDa (DAP12) levels following the impact of miR-183 produced by TGF-β (98) and decreased NKP30 and NKG2D receptor expressions (99). Finally, in B cells, this cytokine inhibits activation, proliferation through the inhibition of cyclins involved in the cell cycle, increases p27 and p21 expression, decreases c-Myc expression (100) and switching class of antibodies (exception for IgA) (101). TGF-β can also exert the exhaustion of other immune cells via several processes, including the increase in the production of arginase and IDO in dendritic cells, reducing MHC class II expression in DCs, reducing the presentation of antigen by DCs, and inducing the phenotype of undifferentiated myeloid cells, which limits the function of APC (102). Therefore, regarding the role of this soluble factor in the exhaustion treatment with blocking TGF-β antibodies have resulted in improved function of CD8+T cells and restored their population in malignant pleural effusion (MPE) (103).

IL10

IL-10, with anti-inflammatory characteristics, and secreted by a range of different cells, has an essential function in infections by confining the immune reactions to antigens and thereby preventing damage to the host. Moreover, depending on the type of cytokines, IL-10 is released which can exert vital functions (104). This cytokine induces its effects through signal transducer and activator of transcription-3 (STAT3) (105). More notably the polymorphism of this cytokine or its receptor may be responsible for differences in the growth, establishment or immediate clearance of infections (106). IL10 could reduce the production of pro-inflammatory cytokines such as TNF-α, INF-γ, and IL-2, the clonal proliferation, and differentiating of $CD4^+$T cells (106). In other words, it could cause the ablation of the cross-talk of $CD4^+$ and $CD8^+$T cells. In NK cells, IL10 has been shown to be capable of limiting the production of inflammatory cytokines, although it can also induce cytotoxic effects in NK cells (107). It can also cause a reduction in the expression and the presence of antigen by MHC class II and the inhibition of the production of inflammatory chemokines and cytokines such as monocyte chemo-attractant protein (MCP)-5, MCP-1, TNF-α, IL-18, and IL-6 (108). High expression of IL-10 has been detected in chronic viral infections with HIV, HCV, HBV, LCMV (109), tumors (104). In an experiment on patients with

ovarian cancer, presence dysfunctional CD8 +T cells were correlated with the population of infiltrating follicular regulatory T (Tfr) cells with upregulated IL-10 expression (110). Sawant *et al*., has recently found that in the microenvironment of the tumor IL-10 and IL-35 released by T regs could cause the exhaustion of intratumoral T cells in a BLIMP1 dependent pathway (70). In a study immunotherapy and treatment of chronic LCMV infection has been indicated to inhibit IL-10 production and increase T cell responses (111) proposing this fact that IL-10 is closely related to the induction of exhaustion. Another experiment in LCMV infection revealed usage of PD-1 blocking antibodies is conversely correlated with the expression of IL-10 by CD8 +T cells on the other hand, blocking IL-10 expression is associated with strengthening the impacts of blocking PD-1 (112).

IL-2

IL-2, produced by T cells whilst an immune response, is necessary for the growth, proliferation, and differentiation of naïve T cells into effector T cells, and its activity begins with the phosphorylation of a group of STATs such as STAT5A, STAT5B, STAT1, and STAT3 (113). Of note because the importance of IL2 in the activation and maintenance of T cell responses, it is the most studied of this group of molecules for the treatment of cancer (114). IL2 performs critical functions for the elimination of cancer cells and virus-infected cells. In one such function, IL2 promotes $CD8^+$ T and NK cells cytotoxic activities. Thus, the signaling pathways contributing to T cell exhaustion could be linked to the signaling pathways contributing to IL2 loss, and it seems that providing this cytokine in the environment can save and recover exhausted T cells responses and deactivate the PD-1 signaling pathway (64). The use of this interleukin in the treatment of chronic LCMV, HIV, and HCV infection by increasing the number of infectious T cells, controlling infection, reducing virus loading. Moreover, its usage along with PD-1 signaling blocker showed a synergistic effect on the increase of $CD8^+$T cells numbers (64). IL-2 therapy in patients with malignant pleural effusion (MPE) of lung cancer have been illustrated to cause a reduction in PD-1 expression and improved INF-γ, granzyme B expression, and CD8+T cells proliferation (115). Moreover, an experiment on murine models infected with Mycobacterium tuberculosis showed that treated mice with IL-2 had restored antigen specific T cell responses (116).

However it should be noted that the use of this cytokine can have contrary effects as T regs, have an abundant expression of the IL-2R, and are reliant on IL-2 produced by activated T cells, therefore the usage of IL-2 might end up in the rise of T regs which eventually benefit the expansion of exhaustion (117). Also, IL-2 induces antigen-induced cell death (ACID), which stimulates cells to reach the final stages of exhaustion, inhibits the growth of memory cells, and controls the expression of inhibitory receptors that taken together might influence the survival and functions of transferred cells in In-vivo (118).

IL-7

IL-7, as another required cytokine for T-cell development as well as for the survival and homeostasis of mature T-cells, can be released by Epithelial and stromal cells of primary lymphatic organs and fibroblasts of secondary lymphatic organs (119). When the binding between IL-7 and the receptor which consists of two chains: IL-7Rα and the γc chain occurs, it induces the phosphorylation of the tyrosine Y449 on the IL-7Rα chain as a result of transphosphorylation of Jak1 and Jak3 which turns Y449 to the docking site for STAT5. In response, phosphorylated and dimerized STAT5, is translocated to the nucleus where it regulates gene expression implicated in differentiation. Finally, important metabolic pathways in the cell undergo changes, which in turn regulate migration, metabolism, survival, and cell cycle progression (120). These features and the ability of this cytokine to protect T cells' responses have made it a candidate for reversing exhausted T cells responses and limit the occurrence of this phenomenon (121). IL7-R expression in exhausted cells in mice infected with chronic infection has been inversely related to the intensity of exhaustion (122). The use of this cytokine in the treatment of HIV has also been associated with an increase in the number of $CD4^+$T and $CD8^+$T naïve and memory cells (123). In vaccination against the tumor, the use of this cytokine as an adjuvant has been associated with an increase in the population of effector and memory T cells (124). In prostate cancer, NKG2D-CAR T cells that were also carrying IL-7 gene, besides enhanced potential of IL-7 production, had a better anti-tumor activity, declined apoptosis and exhaustion rate improved survival, and proliferation (125). Finally, using IL-7 therapy in infectious disease and tumor models has been indicated to limit the expression of PD-1 in $CD8^+$T cells (126).

IL-15

IL-15, as a cytokine with a similar function to IL-2, binds to the IL2R-β receptor and is released by a wide range of non-hematopoietic and hematopoietic cells. Although the main source for the production of this cytokine is monocyte, macrophage, and dendritic cell cells, the production of this cytokine in low dose has also been

observed by T and B cells (127). However, IL2 production is one of the first functions to get defective in the exhaustion process, and compared to IL-15, has a higher ability to affect the response of cells (5), like interleukin 2, it can stimulate $CD8^+$T cell proliferation, maintain the cytotoxic properties of $CD8^+$ T cells, and direct the differentiation of these cells during acute infection. It has also been revealed to plays a role in maintaining and growing homeostasis and functions of NK and natural killer T cells (NKT) (128). Unlike IL-2, IL-15 does not stimulate and induce T regs and, by inducing B-cell lymphoma 2 (BCL-2), increases T cell survival (129). IL-15 in TCR transgenic mouse models has been shown to cause an increase in the activity of $CD8^+$T cells that have recognized a specific melanoma antigen (130). CAR-T/IL15 cells, in a study had better proliferation ability, preserved less differentiated stem cell memory (Tscm) phenotype, and decreased expression of exhausted related markers, which was accompanied with declined mTOR activity and improved metabolic function, suggesting that IL-15 through hampering mTOR activity could help maintaining cells in Tscm phenotype (131). In mouse models of repeated sepsis IL-15 was capable of increasing survival through its improving impact on sepsis-induced T exhaustion which was followed by expansion of the populations of NK and MQs (132).

Having said that, IL-15 has some limitations, including a short half-life, cytotoxicity, and its ability to induce the expression of inhibitory cytokines in chronic infections that could limit its use in the clinic (118). Furthermore, IL15, like interleukin-2, can stimulate T cells to express inhibitory receptors such as Tim3 and B4, not surprising that targeting the receptor of these two cytokines in chronically infected mice reversed the induction of these two inhibitory receptors (117).

IL-21

IL-21 with a γ-chain, and similarities with IL-2, is released by NKT, $CD8^+$T cells, and some subsets of $CD4^+$ T cells like TFH and TH17 with pleiotropic properties have multiple impacts on a broad range of cell types, including, but not limited to, B cells, monocytes, CD4+and CD8+T cells, macrophages and DCs. In T and B cells, IL-21 activates STAT1, STAT3, and STAT5, with STAT3 activation being the most potent and sustained (133). This cytokine, which is capable of influencing the expression of several transcription factors, can determine the differentiation and the fate of $CD4^+$T cells and $CD8^+$T and inhibit Tregs (133). In $CD8^+$ T cells specifically, it is accompanied by the increase of survival, cytokine production, proliferation, and cytotoxicity of these cells (134). In other words, with the help of BCL6 and transcription factor T-cell factor 1 (TCF1) transcription factors, IL-21 can preserve these cells in less differentiated forms and memory-like phase (135). In studies on models of SIV infection, the essential role of IL-21 in anti-viral immunity has been proven. IL-21 produced by $CD4^+$ T cells During chronic SIV infection sustain $CD8^+$ T cell expansion and their potency of the production of IFN-γ, TNF-α, and IL-2, which could mean that IL-21 directly influence $CD8^+$ T cells to limit chronic viral infections (136). In murine models infected with LCMV that were lacking IL21 production or its receptor, CD8+T cells reached the final stage of exhaustion sooner and were not capable of infection clearance (137). An experiment found that IL-21 was able to reprogram the mitochondrial metabolism of $CD8^+$T cells, induce phenotypes of memory-like cells and alter metabolism toward fatty acid oxidation (138). Moreover, it was shown that the proliferation of $CD8^+$ T cells in the presence of IL21 reduced the expression of PD-1 and CD57, which indicates the importance of this cytokine in the exhaustion (138). More recently it has been illustrated that lactate dehydrogenase (LDH) inhibition which was combined with IL-21 treatment causes an increase in the generation of TSCM cells and subsequently an improvement in the antitumor and survival rate (139). Furthermore, early studies provided compelling evidence that IL-21 is a promising immunotherapeutic agent for tumors. IL-21 promotes the maturation, enhances cytotoxicity, and induces production of IFN-γ, and perforin by $CD8^+$T cells (140). Nevertheless, in some tumors such as breast (141), T cell leukemia (142), Hodgkin's lymphoma (143), and multiple myeloma (144), this interleukin has been indicated to be pro-tumorigenic, suggesting that better understanding of the role of IL-21 in these malignancies is required.

IFNα/β: cytokines with complexed nature

Type 1 interferon which consist of different subtypes including INFα and INFβ have been shown to be vital not only for innate but also for adaptive immunity against viral infections. The signaling of INF-Is happens through signal heterodimeric transmembrane receptor, comprised of IFNAR1 and IFNAR2, which results in receptor dimerizing, activation of Janus kinase 1 (JAK1) and tyrosine kinase 2 (TYK2), docking of STAT-1and STAT-2, which finally results in the translocation of STAT1, STAT2 and IRF9 to the nucleus to target and stimulate expression of interferon-stimulated genes (ISG) (145). The release of INFα/β as critical proinflammatory cytokines from DCs can help other immune system with activation

antiviral mechanisms and attenuate the viral expansion and replication during acute infections (146). However, it seems once a chronic viral infection is established in a host, secretion of INFα/β remains high and could stimulate expression of exhaustion promoting factors like PD-1, IL-10, and IDO, besides in an experiment on LCMV, it was found out that T-Cell-Specific Transcription Factor 1(TCF-1) by stimulation of B-Cell Lymphoma 6 Protein (BCL-6) could antagonize the impact of INF-I, and suppress exhaustion related factors (147). On the contrary, in a study it was shown that lacking the negative regulator of INF-I, 2'-5' oligoadenylate synthetase-like 1 (OASL1) during the infection of mouse models with LCMV signaling of INF-I resulted in the induction of antiviral CD8+T cells responses and elimination of viremia (148). Taken together it seems in the early stages of the viral infections INFα/β could have defining roles in the quality of response and repressing the expansion of infections, but this role might backfire by the lasting of period of the presence of infection. However contradictory data on the area of INF-I and exhaustion requires more investigation.

Influence of inhibitory receptors on the progression of exhaustion

Inhibitory receptors are among the most important regulatory pathways for self-tolerance, control of autoreactivity of immune cells, and prevention of immunopathogenic responses (21). These inhibitory receptors often have an ITIM sequence (except for a few inhibitory receptors such as LAG3) that can inhibit cells in three ways: 1. Interference in the downstream signaling pathway of activator receptors and TCR (149). Compete with co-stimulatory pathways for binding to common ligands or interfering with the formation of lipid rafts or micro clusters increased expression of genes that disrupt T cell function (150).

PD-1

PD-1, classified as one of the negative immunoregulatory molecules, is one of the receptors that have been mostly studied regarding exhaustion of immune cells, which promote immune evasion of tumor cells, which is usually up-regulated in chronic viral infections such as HSV, HIV, HCV, and HBV (151). Two ligands of PDL-1, with the ability to be expressed by immune and non-immune cells, and PDL-2 with the ability to be represented by B cells of the germinal center and DCs and MQ cells have been discover for PD-1 (152). PD-L1 and PD-L2 are believed to have different roles in the process of the regulation of the immune system. While PD-L1 can restrict T-cells functions located in peripheral tissues, PD-L2 suppresses immune T-cell activation based on lymphoid organs. Once the PD-1: PDL-1 binding has occurred, PD-1 acquire the capability to form micro clusters with TCR (153). In the following step, tyrosine sequences are phosphorylated in the second cytoplasmic receptor of this receptor, which by recruiting SHP-2, can dephosphorylate CD28 and weaken the PI3K-Akt pathway (154). However, it has recently been reported that Src homology region 2 domain-containing phosphatase-2 (SHP-2) is not critical for T cell exhaustion, suggesting that other mechanisms must be involved in the induction of exhaustion by PD-1 (155). Upon launching the signaling path way of PD-1, the production of the Basic Leucine Zipper ATF-Like Transcription Factor (BATF), INF-γ production, and consequently repress clonal proliferation and INF-γ expression in T cells (149). Moreover, PD-1 seems to be able to limit immune responses by shortening the duration of APC-T cell interaction and increasing the threshold of TCR signals needed to activate T cell functions (156). Additionally, arresting lymphocyte at G0-G1 phase by the inhibition of cyclin-dependent kinases (CDKs) can be one of the other impacts of PD-L1-engaged PD1 (157).

Studies have shown that blocking this receptor increases the potential for T cell function in cytokine production, cytokine production capacity, and cytotoxic properties (158). Blocking PD-1 is also a new therapeutic target in treating various tumors, especially high-mutation tumor (159). However, the genetic deletion of PD-1 could lead to further accumulation of fully exhausting $CD8^+$T cells, which indicates a new role for this receptor and suggest further studies on the role of PD-1 in immune exhaustion (155).

LAG-3

LAG-3-CD223 is a ligand to MHC class II, like CD4, belonging to non-ITIM receptors, which is normally expressed on activated T cells, B cells, and pDCs. It has been shown that this receptor can inhibit cell function and cell cycle growth through the KIEELE motif in the intracellular tail, which is required for exerting these effects (21). Moreover, LAG-3 is able to deter the calcium flow of TCR signaling as well as disrupt cell proliferation and cytokine production (160). Although, Recent preclinical studies have shown a role for LAG-3 in $CD8^+$ T cell exhaustion and the expression of LAG-3 has been demonstrated in both tumor (161) and chronic infection (19), blocking it in LCMV infection has not shown a significant change in inhibiting T cell exhaustion. Although, the precise mechanisms by which LAG-3 negatively modulates T cell functions have not been

discovered yet It seems the inhibition of LAG3 in combination with other inhibitory receptors could be effective on the occurrence and growth of the exhaustion (162). As in a study simultaneous blocking of PD-1 and LAG-3 has been associated with a synergistic effect in restoring T ex-cell function (19). In another experiment on HBV exhausted CD4+T cells was shown to express high levels of PD-1 and LAG-3 and the functions of CD4+T cells was partially restored after the use of antibody against PD-1 and LAG-3 (163). Moreover, exhausted T cells in lymphoma was illustrated to have high expression of PD-1 and LAG-3 and expression of LAG-3 was found to be associated to poor prognosis in patients with lymphoma, more interestingly it was revealed that population of T cells with PD-1+LAG-3- markers were more activated comparing to the PD-1+LAG-3- cells, suggesting the vital role of LAG-3 in establishing exhaustion in cells (164).

CTLA-4

CTLA-4, a CD28 homolog with high affinity for B7-1/2, as an Immunoglobulin superfamily member (IgSF), is highly expressed by exhausted T, Activated T cells, and T regs. The expression of this receptor can be seen following an infection like LCMV (though blocking this receptor, unlike tumor, does not have an effect on viral load control and reversing exhaustion of cells), HIV (high expression of CTLA-4 is observed in $CD4^+$T cells unlike $CD8^+$T cells), HCV and HBV (165). This inhibitory receptor which lacks ITIM, and is thought to exhibit its inhibitory functions through the Tyr-Xaa-Xaa-Met domain, can affect T cell proliferation and function via two models; 1. increase in the threshold of T cell activation or 2. Interfere with TCR intracellular signaling (166). While the CD28: B7-1/2 interaction serves as a co-stimulatory signal for T cell proliferation and activation, the CTLA-4: B7-1/2 binding acts as a co-inhibitory signal thwart early T cell activation. CTLA-4 seems to compete with CD28, and by forming a network, can inhibit CD28: B7-1/2 binding. Besides that, CTLA-4 has a higher affinity for B7-1/2 (166), which strengthen the receptor- ligand engagement. The blockade of this receptor has been widely used in the treatment of tumors and, through the ADCC mechanism, which prepares the T regs for cell death by MQs (167). In an experiment on murine models of breast cancer using antibodies to block CTLA-4 resulted in an increase in the motility of tumor infiltrating lymphocytes (168). More recently in a study it has been cleared that unlike the use of PD-1 blocking antibodies which could result in restoring the functions of CD4 +T cells, using ipilimumab which blocks CTLA-4 could repress CD4+ effector T-cell responses (169). Taken together it seems so much is unknown about the role of this molecule in the cellular exhaustion.

TIM-3

TIM-3, a co-inhibitory receptor that is expressed on IFN-γ-producing T cells, NK cells(the higher expression of TIM-3 is associated with facing serious defects their killing function), FoxP3+T regs, and innate immune cells (macrophages and dendritic cells) where it has been shown to suppress their responses upon interaction with its ligand (170). Expression of this receptor, along with PD-1, has been observed in viral infections such as LCMV, HIV, HCV as well as tumors such as melanoma and non-Hodgkin's lymphoma (171). However it is not yet clear under what circumstances Tim-3 exhibits inhibitory effects or shows co-stimulatory results, It seems that its inhibitory effects are dependent on the co-expression of other molecules like carcinoembryonic antigen adhesion molecule1 (CEACAM-1), meaning that under the condition of the presence of CEACAM-1 as a heterophilic ligand, TIM-3 can act as an inhibitory receptor (172). Moreover, Galectin-9, phosphatidyl serine (PtdSer) and high mobility group protein B1 (HMGB1), has also been introduced to be the ligands of TIM-3, each of which through different mechanisms could promote inhibitory functions for instance Galectin-9 has been shown to be capable of triggering cell death in Tc ells, or Tim-3-HMGB1 engagement could be responsible for hampering transport of nucleic acid to endosomes and interfering in DCs signaling (173).

Regarding effects of blocking TIM-3, simultaneous blockade of TIM-3 and PD-1, compared with blockade of PD-1 alone, increased survival in patients with tumor. Additionally, simultaneously targeting CEACAM-1 and TIM-3 has shown to cause an increase the inhibitory effect on tumor growth (172). Moreover, in murine models of lung cancer, it was indicated that blocking PD-1 could result in an increase in the expression of TIM-3, suggesting that TIM-3 could act like marker for PD-1 antibody resistance, and perhaps blocking antibodies against multiple inhibitory molecules would be more effective as in hepatocellular carcinoma and gastric cancer blockade of PD-1 and TIM-3 could improve functions of existing T cells in the tumor (174).

TIGIT

TIGIT is one of the most recent immune checkpoints investigated as an immunotherapeutic target. It is expressed on activated and memory T cells, NK cells, and T regs (170). Binding to either of its two ligands on APCs, CD155 (PVR: poliovirus receptor) and CD112 (PVRL2,

nectin-2) expressed on turmeric cells, can induce inhibitory effects by two main ways:1. They are competing with CD266 (as CD266 has the ability to stimulate functions like INF-γ production) for binding to CD155 (175) 2. The ITIM sequence of this inhibitory receptor restricts responses in NK cells and further promotes IL-10 production and decreases IL-12 production by DCs, thereby suppressing immune responses (176).

Inhibition of this inhibitory receptor in combination with inhibition of PD-1 controlled tumor and viral infections, which makes TIGIT a potential target for cancer treatment (177). In NK cells, it was found out TIGIT expression, and no other inhibitory molecules was directly associated with the exhaustion of these cells, and the blockade of TIGIT could result in the prevention of NK cells exhaustion and improvement of NK cells-based immunotherapy (178). Yang *et al*., in a recent experiment showed that TIGIT had a high expression in T cells in follicular lymphoma which was correlated exhaustion phenotype in these cells, moreover poor prognosis and survival of patients were linked to the enhanced population of TIGIT+T cells and inhibition of TIGIT signaling could be an appropriate way for prevention of exhaustion of T cells (179). In liver cancer it was illustrated TIGIT could be hallmark of T cell exhaustion in this type of cancer as it was identified on exhausted T cells of different stages and combined inhibition of PD-1 and TIGIT could lead to better results in suppressing tumor growth (180). In the mice model of multiple myeloma (MM) with a relapse also increased expression of PD-1, TIGIT, and IL-10 in CD8+T cells was observed, and target the expression of PD-1 or TIGIT combined with autologous stem cell transplantation had better outcomes (181). Therefore, according to the mentioned studies it seems TIGIT might be a promising target in the limiting cellular exhaustion.

Metabolic changes during exhaustion

One of the most important changes of exhausted T cells is the shift in the metabolic cycle of these cells. Cell metabolism flexibility is vital for T cells because it allows them to acquire the characteristics needed to respond maximally to infection, such as establishing the necessary signals, clonal proliferation, cytokine production, and cytotoxicity (182). Achieving the mentioned functions requires alternation in the process of gaining energy. The reached signals account for these changes. In the naive state, where the cell is waiting for the antigen to be delivered, the cell needs for low levels of energy, are mainly acquired by receiving ATP and using glucose in the mitochondrial oxidative phosphorylation (OXPHOS) pathway (183). Receiving signals from TCR and secondary signals from APC cells, which is followed with activation of T cells, is associated with increased expression of mTOR and Myc, metabolic change to glycolysis (Warburg effect), increased expression of GLUT1, lactate production, and glutaminase (184). Though it is thought to be an inappropriate way of using glycose, this metabolic change is required to respond to the growth of cellular effector activity. It should be mentioned that the Glyceraldehyde 3-phosphate dehydrogenase (GADPH) glycolysis enzyme limits the translation of interferon-gamma mRNA (184). Also, in the aerobic glycolysis pathway, the cells can produce the intermediate metabolites required for the pentose phosphate pathway, which induces the synthesis of nucleotides and amino acids (185). After clearance of the infection and deletion of 90% of the effector cells, memory cells that have a native-like phenotype with increased mitochondrial biomass and spare respiratory capacity (SRC), in which a balance between three pathways in cells including aerobic glycolysis, OXPHOS, and fatty acid oxidation (FAO)is stuck (186). Moreover, through the effect of IL15 on cells and the positive regulation of the enzyme carnitine palmitoyl transferase (CPT1a), their aerobic capacity increases (187). Changes in the expression of genes involved in the metabolism of T cells and inhibition of the two main processes: Glycolysis and OXPHOS, providing the needed energy of cells, following the expression of PD-1 and CTLA-4, is a proof that exhaustion is capable of changing this part of cells (Figure 2) (20). With the onset of exhaustion, glucose transport, especially transport by the glutaminase type 1, is limited. Although the appearance of swollen mitochondria and an increase in mitochondrial biomass, which is expected to come with increased function, has been associated with depolarization, decreased function, decreased oxygen consumption rates, and SRCs, and increased ROS production (20). in mouse tumor models reduced oxygen consumption, mitochondrial dysfunction, and glucose deficiency were observed in exhausted T cells. Furthermore, environmental conditions and the availability of required materials can determine the severity of exhaustion. For example, the presence and availability of amino acids such as L-arginine, which mediate metabolic changes and shift to oxidative phosphorylation, is associated with increased survival and cytotoxicity of T cells at the tumor site (188).

Moreover, glutamine and leucine, which play a role in TCR signaling, may be involved in causing or preventing this phenomenon (189). One of the other defining factors in exhaustion is hypoxia in the tumor environment, which can induce angiogenesis, invasion and the growth of tumor cells. During the onset of hypoxia, Von Hippel-Lindau

Tumor Suppressor (Vhl), as one of the tumor suppressors does not interfere with the subunits of hypoxia inducible factor (Hif) transcription factor and causes the expression of Hif. By depleting the Vhl gene, the pressure on the Hif is removed, which can lead to continuous T cell responses and an increase in their survival without undergoing exhaustion (190). In tumors and chronic infections, when lack of glucose occurs, a decrease in the expression of GLUT-1, along with changes in the levels of phosphoenolpyruvate (PEP),which is involved in preserving Nuclear factor of activated T-cells (NFAT)-Ca2+ and effector functions by impending sarco/ER Ca(2+)-ATPase (SERCA) activity, happen (191). PD-1 is one of the other debated sections, which is highly expressed following continuous stimulation of TCR causes negative feedback on TCR and desensitization. Since the stimulation of TCR is associated with two incidences: activation of PI3k-Akt-mTOR pathway and deactivation of FOXO1, necessary in the survival of T cells, so PD-1 signaling is then thought to be associated with long-term, limited responses and adequate bioenergetics balance for cells (20). Studies on mTOR Factor, which is recruited following the stimulation of TCR and its blocking, is accompanied by the recovery of mitochondria in exhausted T cells, proves the important role of this factor in metabolic changes during exhaustion. Furthermore, the use of Rapamycin as an inhibitor of mTOR is associated with metabolic shifting to FAO and stimulation of memory differentiation (192). Taken together, according to the mentioned results, it seems that metabolic changes can play a potential role in being targeted in the treatment.

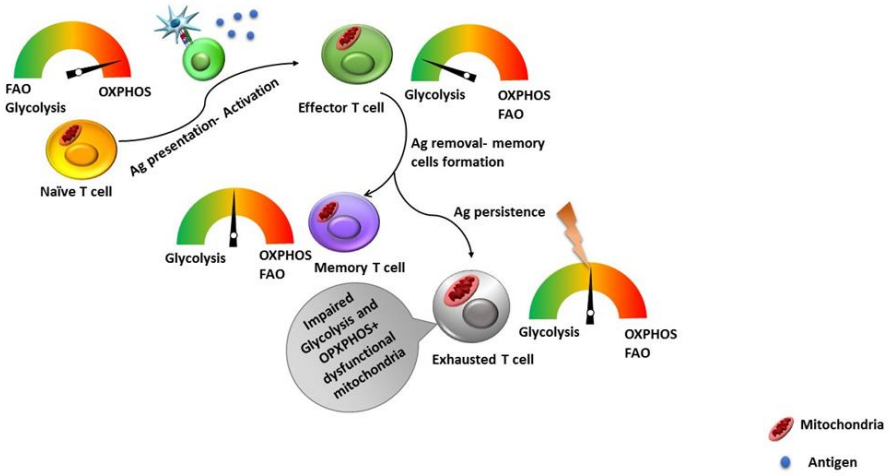

Figure 2. The metabolic status of different cell states. Naive T cells, when they have not received antigen presented by APC, use OXPHOS to acquire energy. Once T cells have been activated, metabolic pathways are shifted to Glycolysis to which is set to answer properly to the growth changes happening in effector cells. After removing infectious agents, memory cells with a balanced metabolism between three main pathways guard the body. However, with the establishment of exhaustion, this equilibrium is disrupted, and inflated and dysfunctional mitochondria, increased ROS production, and reduced oxygen uptake can be expected

Changes in profiles of transcription on exhaustion

Numerous studies have shown that as cells undergo exhaustion, the transcriptional profile of them get influenced by changes that are distinct from the effector and memory states (24). Although specific transcription factors have not been introduced for exhaustion, Transcription factors such as EOMES, T-bet, BATF, BLIMP1, NFAT, FOXO1, VHL, and FOXP1 are considered to play a role in T cell exhaustion (193). The mentioned transcription factors are also expressed in other T cell populations, but the pathway in which these factors are influenced, the expression and target genes in the exhausted cells are different (Figure 3). For example, T-bet in acute infection in $CD8^+$ T cells is responsible for controlling the differentiation and growth of T cell subsets (194). While in the exhausted cell population, it holds the population of non-terminal progenitor cells in chronic infection (195). Another factor EOMES in acute infection, which is involved in the growth of central T cells and homeostasis, takes part in the formation of terminally differentiated exhausted T cells (196). NFAT also, along with the activation of T cells and releasing calcium into the cell, starts to form heterodimers with AP-1, which ultimately induces transcriptional activation of genes

involved in effector cell function (197). During chronic infection such as LCMV, the formation of this heterodimer is disrupted, and this balance shifts to unpaired NFAT, which binds only to the PD-1 promoter and results in the expression of Pdcd1 and other inhibitory receptor genes (198). Other transcription factors specifically involved in the transcription fork, such as FOXO-1, IRF-4, and BLIMP-1, can act as positive exhaustion regulators and increase in exhausted cells (193). In addition, the three transcription factors IRF-4, NFATc1, and BATF play a key role in the establishment of this phenomenon in T cells and the expression of inhibitory factors. For example, IRF-4 plays a role in impairing cytokine production and suppressing anabolic metabolism (199). Therefore, according to the studies done so far, the control of transcription factors during exhaustion can be more complicated than memory, and effector phases since they can be used in different differential processes, and future studies can map the function and regulation of these transcription factors.

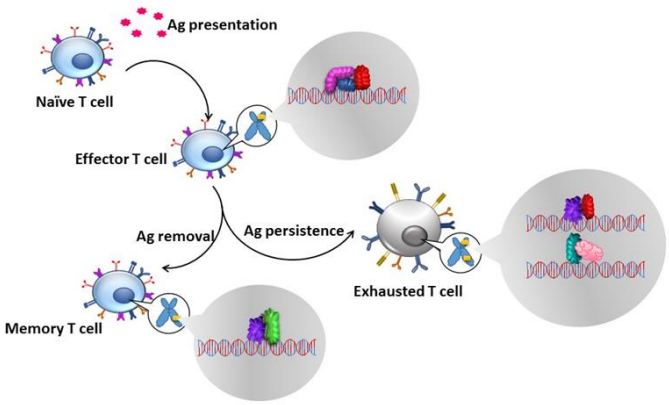

Figure 3. The transcriptional differences between the different modes in the $CD8^+$T cell. In the effector mode, a group of transcription factors is used, while in the memory mode, other points on the genome are targeted. But in the exhaustion, the expression of genes undergoes major changes, so that the expression of some genes is done in the absence of a set of transcription factors

The other side of the coin

In recent years, a new approach has been developed to the phenomenon of exhaustion in a group of diseases, which means that although the presence of these cells during chronic infection and tumor microenvironment is associated with poor prognosis of the disease, during autoimmune diseases, when eruptions of immune responses and tissue damage are observed, the presence of these cells seems to be beneficial through choosing the appropriate method (Figure 4). The presence of exhaustion in $CD8^+$ T cells in systemic lupus erythematous, hemorrhagic fever, type 1 diabetes, idiopathic pulmonary fibrosis, and anti-antibody-associated vasculitis were found to be associated with a reduced risk of recurrence and better prognosis (200). Also, after using a monoclonal antibody (Teplizumab) in the treatment of type 1 diabetes, the presence and the proliferation of exhausted $CD8^+$ T cells have been associated with the better disease control (201). The use of these cells is not limited to autoimmunity, and the presence of these cells seems to be considered in organ transplantation. In patients with myeloid leukemia, it was reported that the continued presence of the exhausted recipient $CD8^+$ T cells were associated with better therapeutic effects and better expansion of haplo-NK cells (202). In the cardiac allograft vasculopathy mouse model, better survival was observed in mice with exhausted $CD4^+$ T cells. These cells were associated with features such as overexpression of inhibitory receptors, defects in cytokine production, and decreased proliferation (203). In a trial clinical trial performed as withdrawal immunosuppression in HCV-infected liver receptors, this procedure was successful in half of the patients with an increase in the number of exhausted T cells (204). Another study that investigated the role of exhausted T cells in the efficacy and induction of treatment on the renal recipients showed a significant increase in these cells in recipients that were treated with anti-thymocyte globulin. Also, an expected increase in these cells was observed following treatment (lymphocyte depletion) that was associated with better connective tissue function, suggesting the use of these cells in graft follow-up (205).

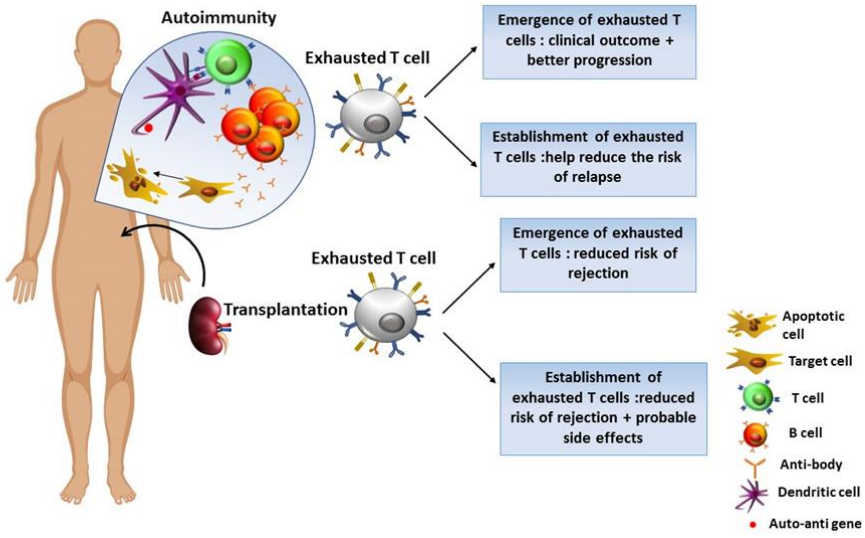

Figure 4. The use of exhausted cells in clinical opportunities. In autoimmune diseases and organ transplantation, the presence of these cells can lead to improvement (in organ transplantation via increasing tissue survival and in autoimmunity via reducing the likelihood of disease recurrence), which makes them a possible treatment option, although possible side effects should be considered

Immune exhaustion, a new phase occurring in a range of immune cells, is one of the most important known cellular events in numerous pathological events. Although improvements over the past decade have shed considerable light on the mechanisms of exhaustion and have introduced novel therapeutic opportunities, there are also still many unanswered questions that require studying different aspects of this phenomena in various immune cells and the network developing following this event between cells leading to the severity or suppressing cause not only can a better understanding of this network lead to choosing proper targets for immunotherapy in cancer and improve the efficiency of existing methods but also they can be used in autoimmune diseases where the uncontrolled responses cause pathological damage. The identification of various and vigorous negative regulatory pathways that have the capacity to be targeted experimentally and clinically could be considered a major step forward since it can guide to revolutionize immunotherapy of chronic infections and cancer. Future mechanistic and clinical studies are required to foster cutting-edge immune-based interventions for chronic infections and cancer.

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References

1. Chaplin DD. Overview of the immune response. J Allergy Clin Immunol 2010;125:S3-23.
2. Bi J, Tian Z. NK Cell Exhaustion. Frontiers in immunology 2017;8:760.
3. Crespo J, Sun H, Welling TH, Tian Z, Zou W. T cell anergy, exhaustion, senescence, and stemness in the tumor microenvironment. Curr Opin Immunol 2013;25:214-21.
4. Jiang Y, Li Y, Zhu B. T-cell exhaustion in the tumor microenvironment. Cell Death Dis 2015;6:e1792.
5. Wherry EJ, Blattman JN, Murali-Krishna K, van der Most R, Ahmed R. Viral persistence alters CD8 T-cell immunodominance and tissue distribution and results in distinct stages of functional impairment. J Virol 2003;77:4911-27.
6. Moir S, Fauci AS. B-cell exhaustion in HIV infection: the role of immune activation. Curr Opin HIV AIDS 2014;9:472-7.
7. Ebihara T, Taniuchi I. Exhausted-like Group 2 Innate Lymphoid Cells in Chronic Allergic Inflammation. Trends Immunol 2019;40:1095-104.
8. Macal M, Lewis GM, Kunz S, Flavell R, Harker JA, Zuñiga EI. Plasmacytoid dendritic cells are productively infected and activated through TLR-7 early after arenavirus infection. Cell Host Microbe 2012;11:617-30.
9. Kasakovski D, Xu L, Li Y. T cell senescence and CAR-T cell exhaustion in hematological malignancies. J Hematol

Oncol 2018;11:91.
10. Sterzl J, Silverstein AM. Developmental Aspects of Immunity. Adv Immunol 1967;6:337-459.
11. Moskophidis D, Lechner H, Pircher H, Rolf M, Zinkernagel RM. Virus persistence in acutely infected immunocompetent mice by exhaustion of antiviral cytotoxic effector T cells. Nature 1993;362:758-61.
12. Goepfert PA, Bansal A, Edwards BH, Ritter Jr GD, Tellez I, McPherson SA, et al. A significant number of human immunodeficiency virus epitope-specific cytotoxic T lymphocytes detected by tetramer binding do not produce gamma interferon. J Virol 2000;74:10249-55.
13. Schlaak JF, Tully G, Löhr HF, Gerken G, Meyer zum Büschenfelde KH. The presence of high amounts of HBV-DNA in serum is associated with suppressed costimulatory effects of interleukin 12 on HBV-induced immune response. J Hepatol 1999;30:353-8.
14. Gruener NH, Lechner F, Jung MC, Diepolder H, Gerlach T, Lauer G, et al. Sustained dysfunction of antiviral CD8+ T lymphocytes after infection with hepatitis C virus. J Virol 2001;75:5550-8.
15. Greten TF, Slansky JE, Kubota R, Soldan SS, Jaffee EM, Leist TP, et al. Direct visualization of antigen-specific T cells: HTLV-1 Tax11-19- specific CD8(+) T cells are activated in peripheral blood and accumulate in cerebrospinal fluid from HAM/TSP patients. Proc Natl Acad Sci U S A 1998;95:7568-73.
16. Day CL, Abrahams DA, Lerumo L, Janse van Rensburg E, Stone L, O'rie T, et al. Functional capacity of Mycobacterium tuberculosis-specific T cell responses in humans is associated with mycobacterial load. J Immunol 2011;187:2222-32.
17. Davoodzadeh Gholami M, Kardar GA, Saeedi Y, Heydari S, Garssen J, Falak R. Exhaustion of T lymphocytes in the tumor microenvironment: Significance and effective mechanisms. Cell Immunol 2017;322:1-14.
18. Klocperk A, Unger S, Friedmann D, Seidl M, Zoldan K, Pfeiffer J, et al. Exhausted phenotype of follicular CD8 T cells in CVID. J Allergy Clin Immunol 2020;146:912-15.e13.
19. Blackburn SD, Shin H, Haining WN, Zou T, Workman CJ, Polley A, et al. Coregulation of CD8+ T cell exhaustion by multiple inhibitory receptors during chronic viral infection. Nat Immunol 2009;10:29-37.
20. Bengsch B, Johnson AL, Kurachi M, Odorizzi PM, Pauken KE, Attanasio J, et al. Bioenergetic Insufficiencies Due to Metabolic Alterations Regulated by the Inhibitory Receptor PD-1 Are an Early Driver of CD8(+) T Cell Exhaustion. Immunity 2016;45:358-73.
21. Kahan SM, Wherry EJ, Zajac AJ. T cell exhaustion during persistent viral infections. Virology 2015;479-80:180-93.
22. Brooks DG, Teyton L, Oldstone MB, McGavern DB. Intrinsic functional dysregulation of CD4 T cells occurs rapidly following persistent viral infection. J Virol 2005;79:10514-27.
23. Wherry EJ, Kurachi M. Molecular and cellular insights into T cell exhaustion. Nature reviews. Nat Rev Immunol 2015;15:486-99.
24. Crawford A, Angelosanto JM, Kao C, Doering TA, Odorizzi PM, Barnett BE, et al. Molecular and transcriptional basis of CD4(+) T cell dysfunction during chronic infection. Immunity 2014;40:289-302.
25. van der Werf N, Redpath SA, Azuma M, Yagita H, Taylor MD. Th2 cell-intrinsic hypo-responsiveness determines susceptibility to helminth infection. PLoS pathogens 9, e1003215.(2013)
26. .26 J. A. Knipper, A. Ivens, M. D. Taylor, Helminth-induced Th2 cell dysfunction is distinct from exhaustion and is maintained in the absence of antigen. PLoS Pathog 2013;9:e1003215.
27. Brahmi Z, Bray RA, Abrams SI. Evidence for an early calcium-independent event in the activation of the human natural killer cell cytolytic mechanism. J Immunol 1985;135:4108-13.
28. Smyth MJ, Cretney E, Takeda K, Wiltrout RH, Sedger LM, Kayagaki N, et al. Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) contributes to interferon gamma-dependent natural killer cell protection from tumor metastasis. J Exp Med 2001;193:661-70.
29. Hasmim M, Messai Y, Ziani L, Thiery J, Bouhris JH, Noman MZ, et al. Critical Role of Tumor Microenvironment in Shaping NK Cell Functions: Implication of Hypoxic Stress. Front Immunol 2015;6:482.
30. Peng YP, Zhu Y, Zhang JJ, Xu ZK, Qian ZY, Dai CC, et al. Comprehensive analysis of the percentage of surface receptors and cytotoxic granules positive natural killer cells in patients with pancreatic cancer, gastric cancer, and colorectal cancer. J Transl Med 2013;11:262.
31. De Maria A, Fogli M, Costa P, Murdaca G, Puppo F, Mavilio D, et al. The impaired NK cell cytolytic function in viremic HIV-1 infection is associated with a reduced surface expression of natural cytotoxicity receptors (NKp46, NKp30 and NKp44). Eur J Immunol 2003;33:2410-8.
32. Meier UC, Owen RE, Taylor E, Worth A, Naoumov N, Willberg C, et al., Shared alterations in NK cell frequency, phenotype, and function in chronic human immunodeficiency virus and hepatitis C virus infections. J Virol 2005;79:12365-74.
33. Sun C, Fu B, Gao Y, Liao X, Sun R, Tian Z, et al. TGF-beta1 down-regulation of NKG2D/DAP10 and 2B4/SAP expression on human NK cells contributes to HBV

persistence. PLoS Pathog 2012;8:e1002594.

34. Shifrin N, Raulet DH, Ardolino M. NK cell self-tolerance, responsiveness and missing self-recognition. Semin Immunol 2014;26:138-44.
35. Felices M, Lenvik AJ, McElmurry R, Chu S, Hinderlie P, Bendzick L, et al. Continuous treatment with IL-15 exhausts human NK cells via a metabolic defect. JCI Insight 2018;3:e96219.
36. Sun H, Huang Q, Huang M, Wen H, Lin R, Zheng M, et al. Human CD96 Correlates to Natural Killer Cell Exhaustion and Predicts the Prognosis of Human Hepatocellular Carcinoma. Hepatology 2019;70:168-83.
37. Moir S, Ho J, Malaspina A, Wang W, DiPoto AC, O'Shea MA, et al. Evidence for HIV-associated B cell exhaustion in a dysfunctional memory B cell compartment in HIV-infected viremic individuals J Exp Med 2008;205:1797-805.
38. Khoder A, Alsuliman A, Basar R, Sobieski C, Kondo K, Alousi AM, et al. Evidence for B Cell Exhaustion in Chronic Graft-versus-Host Disease. Front Immunol 2017;8:1937.
39. Holla P, Ambegaonkar A, Sohn H, Pierce SK. Exhaustion may not be in the human B cell vocabulary, at least not in malaria. Immunol Rev 2019;292:139-48.
40. Collin M, Bigley V. Human dendritic cell subsets: an update. Immunology 2018;154:3-20.
41. Kajino K, Nakamura I, Bamba H, Sawai T, Ogasawara K. Involvement of IL-10 in exhaustion of myeloid dendritic cells and rescue by CD40 stimulation. Immunology 2007;120:28-37.
42. Bergthaler A, Flatz L, Hegazy AN, Johnson S, Horvath E, Löhning M, et al. Viral replicative capacity is the primary determinant of lymphocytic choriomeningitis virus persistence and immunosuppression. Proc Natl Acad Sci U S A. 2010;107:21641-6.
43. Duan XZ, Wang M, Li HW, Zhuang H, Xu D, Wang FS. Decreased frequency and function of circulating plasmocytoid dendritic cells (pDC) in hepatitis B virus infected humans. J Clin Immunol 2004;24:637-46.
44. Lee LN, Burke S, Montoya M, Borrow P. Multiple mechanisms contribute to impairment of type 1 interferon production during chronic lymphocytic choriomeningitis virus infection of mice. J Immunol 2009;182:7178-89.
45. Kanto T, Inoue M, Miyatake H, Sato A, Sakakibara M, Yakushijin T, et al. Reduced numbers and impaired ability of myeloid and plasmacytoid dendritic cells to polarize T helper cells in chronic hepatitis C virus infection. J Infect Dis 2004;190:1919-26.
46. Labidi-Galy SI, Sisirak V, Meeus P, Gobert M, Treilleux I, Bajard A, et al. Quantitative and functional alterations of plasmacytoid dendritic cells contribute to immune tolerance in ovarian cancer. Cancer Res 2011;71:5423-34.
47. Macal M, Jo Y, Dallari S, Chang AY, Dai J, Swaminathan S, et al., Self-Renewal and Toll-like Receptor Signaling Sustain Exhausted Plasmacytoid Dendritic Cells during Chronic Viral Infection. Immunity 2018;48:730-44.e5.
48. Boni C, Fisicaro P, Valdatta C, Amadei B, Di Vincenzo P, Giuberti T, et al. Characterization of hepatitis B virus (HBV)-specific T-cell dysfunction in chronic HBV infection. J Virol 2007;81:4215-25.
49. Belz GT, Xie W, Altman JD, Doherty PC. A previously unrecognized H-2D(b)-restricted peptide prominent in the primary influenza A virus-specific CD8(+) T-cell response is much less apparent following secondary challenge. J Virol 2000;74:3486-93.
50 .Blattman JN, Wherry EJ, Ha SJ, van der Most RG, Ahmed R. Impact of epitope escape on PD-1 expression and CD8 T-cell exhaustion during chronic infection. J Virol 2009;83:4386-94.
51. Shin H, Wherry EJ. CD8 T cell dysfunction during chronic viral infection. Curr Opin Immunol. 2007;19:408-15.
52. Abel AM, Yang C, Thakar MS, Malarkannan S. Natural Killer Cells: Development, Maturation, and Clinical Utilization. Front Immunol 2018;9:1869.
53. Cook KD, Whitmire JK. The depletion of NK cells prevents T cell exhaustion to efficiently control disseminating virus infection. 53. Cook KD, Whitmire JK. The depletion of NK cells prevents T cell exhaustion to efficiently control disseminating virus infection. J Immunol 2013;190:641-9.
54. Cook KD, Waggoner SN, Whitmire JK. NK cells and their ability to modulate T cells during virus infections. Crit Rev Immunol 2014;34:359-88.
55 .Gray JD HM, Ohtsuka K, Horwitz DA. Generation of an inhibitory circuit involving CD8+ T cells, IL-2, and NK cell-derived TGF-beta: contrasting effects of anti-CD2 and anti-CD3. J Immunol 1998;160:2248-54.
56 .Cerboni C, Zingoni A, Cippitelli M, Piccoli M, Frati L, Santoni A. Antigen-activated human T lymphocytes express cell-surface NKG2D ligands via an ATM/ATR-dependent mechanism and become susceptible to autologous NK- cell lysis. Blood 2007;110:606-15.
57. Barber MA, Zhang T, Gagne BA, Sentman CL. NK cells negatively regulate antigen presentation and tumor-specific CTLs in a syngeneic lymphoma model. J Immunol 2007;178:6140-7.
58. Ahrends T, Busselaar J, Severson TM, Babala N, de Vries E, Bovens A, et al. CD4(+) T cell help creates memory CD8(+) T cells with innate and help-independent recall capacities. Nat Commun 2019;10:5531.
59. Bedoui S, Heath WR, Mueller SN. CD4(+) T-cell help amplifies innate signals for primary CD8(+) T-cell

immunity. Immunol Rev 2016;272:52-64.
60. Bourgeois C, Rocha B, Tanchot C. A role for CD40 expression on CD8+ T cells in the generation of CD8+ T cell memory. Science 2002;297:2060-3.
61. Cox MA, Kahan SM, Zajac AJ. Anti-viral CD8 T cells and the cytokines that they love. Virology 2013;435:157-69.
62. Matloubian M, Concepcion RJ, Ahmed R. CD4+ T cells are required to sustain CD8+ cytotoxic T-cell responses during chronic viral infection. J Virol 1994;68:8056-63.
63. Laidlaw BJ, Craft JE, Kaech SM. The multifaceted role of CD4(+) T cells in CD8(+) T cell memory. Nat Rev Immunol 2016;16:102-11.
64. Bachmann MF, Wolint P, Walton S, Schwarz K, Oxenius A. Differential role of IL-2R signaling for CD8+ T cell responses in acute and chronic viral infections. Eur J Immunol 2007;37:1502-12.
65. Chevalier MF, Julg B, Pyo A, Flanders M, Ranasinghe S, Soghoian DZ, et al. HIV-1-specific interleukin-21+ CD4+ T cell responses contribute to durable viral control through the modulation of HIV-specific CD8+ T cell function. J Virol 2011;85:733-41.
66. Romano M, Fanelli G, Albany CJ, Giganti G, Lombardi G. Past, Present, and Future of Regulatory T Cell Therapy in Transplantation and Autoimmunity. Front Immunol 2019;10:43.
67. Penaloza-MacMaster P, Kamphorst AO, Wieland A, Araki K, Iyer SS, West EE, et al. Interplay between regulatory T cells and PD-1 in modulating T cell exhaustion and viral control during chronic LCMV infection. J Exp Med 2014;211:1905-18.
68. Borst J, Ahrends T, Babala N, Melief CJM, Kastenmuller W. CD4(+) T cell help in cancer immunology and immunotherapy. Nat Rev Immunol 2018;18:635-47.
69. Fontenot JD, Rasmussen JP, Gavin MA, Rudensky AY. A function for interleukin 2 in Foxp3-expressing regulatory T cells. Nat Immunol 2005;6:1142-51.
70. Sawant DV, Yano H, Chikina M, Zhang Q, Liao M, Liu C, et al. Adaptive plasticity of IL-10(+) and IL-35(+) Treg cells cooperatively promotes tumor T cell exhaustion. Nat Immunol 2019;20:724-35.
71. Topalian SL, Taube JM, Anders RA, Pardoll DM. Mechanism-driven biomarkers to guide immune checkpoint blockade in cancer therapy. Nat Rev Cancer 2016;16:275-87.
72. Mellor AL, Munn DH. IDO expression by dendritic cells: tolerance and tryptophan catabolism. Nat Rev Immunol 2004;4:762-742.
73. Kim HJ, Wang X, Radfar S, Sproule TJ, Roopenian DC, Cantor H. CD8+ T regulatory cells express the Ly49 Class I MHC receptor and are defective in autoimmune prone B6-Yaa mice. Proc Natl Acad Sci U S A 2011;108:2010-5.
74. Holderried TA, Lang PA, Kim HJ, Cantor H. Genetic disruption of CD8+ Treg activity enhances the immune response to viral infection. Proc Natl Acad Sci U S A 2013;110:21089-94.
75. Ng CT, Snell LM, Brooks DG, Oldstone MB. Networking at the level of host immunity: immune cell interactions during persistent viral infections. Cell Host Microbe 2013;13:652-64.
76. Ladell K, Hashimoto M, Iglesias MC, Wilmann PG, McLaren JE, Gras S, et al. A molecular basis for the control of preimmune escape variants by HIV-specific CD8+ T cells. Immunity 2013;38:425-36.
77. Fitzgerald-Bocarsly P, Jacobs ES. Plasmacytoid dendritic cells in HIV infection: striking a delicate balance. J Leukoc Biol 2010;87:609-20.
78. Wilson EB, Kidani Y, Elsaesser H, Barnard J, Raff L, Karp CL, et al. Emergence of distinct multiarmed immunoregulatory antigen-presenting cells during persistent viral infection. Cell Host Microbe 2012;11:481-91.
79. Veglia F, Gabrilovich DI. Dendritic cells in cancer: the role revisited. Curr Opin Immunol 2017;45:43-51.
80. Blair PA, Norena LY, Flores-Borja F, Rawlings DJ, Isenberg DA, Ehrenstein MR, et al. CD19(+)CD24(hi)CD38(hi) B cells exhibit regulatory capacity in healthy individuals but are functionally impaired in systemic Lupus Erythematosus patients. Immunity 2010;32:129-40.
81. Olkhanud PB, Damdinsuren B, Bodogai M, Gress RE, Sen R, Wejksza K, et al. Tumor-evoked regulatory B cells promote breast cancer metastasis by converting resting CD4(+) T cells to T-regulatory cells. Cancer Res 2011;71:3505-15.
82. Das A, Ellis G, Pallant C, Lopes AR, Khanna P, Peppa D, et al. IL-10-producing regulatory B cells in the pathogenesis of chronic hepatitis B virus infection. J Immunol 2012;189:3925-35.
83. Menon M, Blair PA, Isenberg DA, Mauri C. A Regulatory Feedback between Plasmacytoid Dendritic Cells and Regulatory B Cells Is Aberrant in Systemic Lupus Erythematosus. Immunity 2016;44:683-97.
84. Siewe B, Stapleton JT, Martinson J, Keshavarzian A, Kazmi N, Demarais PM, et al. Regulatory B cell frequency correlates with markers of HIV disease progression and attenuates anti-HIV CD8(+) T cell function in vitro. JJ Leukoc Biol 2013;93:811-8.
85. Nouel A, Pochard P, Simon Q, Segalen I, Le Meur Y, Pers JO, et al. B-Cells induce regulatory T cells through TGF-beta/IDO production in A CTLA-4 dependent manner. J Autoimmun 2015;59:53-60.

86. Ruytinx P, Proost P, Van Damme J, Struyf S. Chemokine-Induced Macrophage Polarization in Inflammatory Conditions. Front Immunol 2018;9:1930.
87. Brockman MA, Kwon DS, Tighe DP, Pavlik DF, Rosato PC, Sela J, et al. IL-10 is up-regulated in multiple cell types during viremic HIV infection and reversibly inhibits virus-specific T cells. Blood 2009;114:346-56.
88. Noy R, Pollard JW. Tumor-associated macrophages: from mechanisms to therapy. Immunity 2014;41:49-61.
89. Wu AA, Drake V, Huang HS, Chiu S, Zheng L. Reprogramming the tumor microenvironment: tumor-induced immunosuppressive factors paralyze T cells. Oncoimmunology 2015;4:e1016700.
90. Rodriguez PC, Zea AH, Culotta KS, Zabaleta J, Ochoa JB, Ochoa AC. Regulation of T cell receptor CD3zeta chain expression by L-arginine. J Biol Chem 2002;277:21123-9.
91. Goh C, Narayanan S, Hahn YS. Myeloid-derived suppressor cells: the dark knight or the joker in viral infections? Immunol Rev 2013;255:210-21.
92. Cai W, Qin A, Guo P, Yan D, Hu F, Yang Q, et al. Clinical significance and functional studies of myeloid-derived suppressor cells in chronic hepatitis C patients. J Clin Immunol 2013;33:798-808.
93. Qin A, Cai W, Pan T, Wu K, Yang Q, Wang N, et al. Expansion of monocytic myeloid-derived suppressor cells dampens T cell function in HIV-1-seropositive individuals. J Virol 2013;87:1477-90.
94. Batlle E, Massague J. Transforming Growth Factor-beta Signaling in Immunity and Cancer. Immunity 2019;50:924-40.
95. Brabletz T, et al. Transforming growth factor beta and cyclosporin A inhibit the inducible activity of the interleukin-2 gene in T cells through a noncanonical octamer-binding site. Mol Cell Biol 1993;13:1155-62.
96. Thomas DA, Massague J. TGF-beta directly targets cytotoxic T cell functions during tumor evasion of immune surveillance. Cancer Cell 2005;8:369-80.
97. Yu J, Wei M, Becknell B, Trotta R, Liu S, Boyd Z, et al. Pro- and antiinflammatory cytokine signaling: reciprocal antagonism regulates interferon-gamma production by human natural killer cells. Immunity 2006;24:575-90.
98. Castriconi R, Cantoni C, Della Chiesa M, Vitale M, Marcenaro E, Conte R, et al. Transforming growth factor beta 1 inhibits expression of NKp30 and NKG2D receptors: consequences for the NK-mediated killing of dendritic cells. Proc Natl Acad Sci U S A 2003;100:4120-5.
99. Donatelli SS, Zhou JM, Gilvary DL, Eksioglu EA, Chen X, Cress WD, et al. TGF-beta-inducible microRNA-183 silences tumor-associated natural killer cells. Proc Natl Acad Sci U S A 2014;111:4203-8.
100. Petit-Koskas E, Genot E, Lawrence D, Kolb JP. Inhibition of the proliferative response of human B lymphocytes to B cell growth factor by transforming growth factor-beta. Eur J Immunol 1988;18:111-6.
101. Kehrl JH RA, Wakefield LM, Jakowlew S, Sporn MB, Fauci AS. Transforming growth factor beta is an important immunomodulatory protein for human B lymphocytes. J Immunol 1986;137:3855-60.
102. Papaspyridonos M, Matei I, Huang Y, do Rosario Andre M, Brazier-Mitouart H, Waite JC, et al. Id1 suppresses anti-tumour immune responses and promotes tumour progression by impairing myeloid cell maturation. Nat Commun 2015;6:6840.
103. Wang X, He Q, Shen H, Xia A, Tian W, Yu W, et al. TOX promotes the exhaustion of antitumor CD8(+) T cells by preventing PD1 degradation in hepatocellular carcinoma. J Hepatol 2019;71:731-41.
104. Mannino MH, Zhu Z, Xiao H, Bai Q, Wakefield MR, Fang Y. The paradoxical role of IL-10 in immunity and cancer. Cancer lett 2015;367:103-7.
105. Couper KN, Blount DG, Riley EM. IL-10: the master regulator of immunity to infection. J Immunol 2008;180:5771-7.
106. Blackburn SD, Wherry EJ. IL-10, T cell exhaustion and viral persistence. Trends Microbiol 2007;15:143-6.
107. Lauw FN, Pajkrt D, Hack CE, Kurimoto M, van Deventer SJ, van der Poll T. Proinflammatory effects of IL-10 during human endotoxemia. J Immunol 2000;165:2783-9.
108. Moore KW, de Waal Malefyt R, Coffman RL, O'Garra A. Interleukin-10 and the interleukin-10 receptor. Annu Rev Immunol 2001;19:683-765.
109. Parish IA, Marshall HD, Staron MM, Lang PA, Brustle A, Chen JH, et al. Chronic viral infection promotes sustained Th1-derived immunoregulatory IL-10 via BLIMP-1. J Clin Invest 2014;124:3455-68.
110. Li L, Ma Y, Xu Y. Follicular regulatory T cells infiltrated the ovarian carcinoma and resulted in CD8 T cell dysfunction dependent on IL-10 pathway. Int Immunopharmacol 2019;68:81-7.
111. Ni G, Wang T, Walton S, Zhu B, Chen S, Wu X, et al. Manipulating IL-10 signalling blockade for better immunotherapy. Cell Immunol 2015;293:126-9.
112. Brooks DG, Ha SJ, Elsaesser H, Sharpe AH, Freeman GJ, Oldstone MB. IL-10 and PD-L1 operate through distinct pathways to suppress T-cell activity during persistent viral infection. Proc Natl Acad Sci U S A 2008;105:20428-33.
113. Dhupkar P, Gordon N. Interleukin-2: Old and New Approaches to Enhance Immune-Therapeutic Efficacy. Adv Exp Med Biol 2017;995:33-51.
114. Rosenberg SA, Mule JJ, Spiess PJ, Reichert CM, Schwarz SL. Regression of established pulmonary metastases and

subcutaneous tumor mediated by the systemic administration of high-dose recombinant interleukin 2. J Exp Med 1985;161:1169-88.

115. Hu CY, Zhang YH, Wang T, Chen L, Gong ZH, Wan YS, et al. Interleukin-2 reverses CD8(+) T cell exhaustion in clinical malignant pleural effusion of lung cancer. Clin Exp Immunol 2016;186:106-14.
116. Liu X, Li F, Niu H, Ma L, Chen J, Zhang Y, et al. IL-2 Restores T-Cell Dysfunction Induced by Persistent Mycobacterium tuberculosis Antigen Stimulation. Front Immunol 2019;10:2350.
117. Beltra JC, Bourbonnais S, Bedard N, Charpentier T, Boulange M, Michaud E, et al. IL2Rbeta-dependent signals drive terminal exhaustion and suppress memory development during chronic viral infection. PProc Natl Acad Sci U S A 2016;113:E5444-53.
118. Shourian M, Beltra JC, Bourdin B, Decaluwe H. Common gamma chain cytokines and CD8 T cells in cancer. Semin Immunol 2019;42:101307.
119. Bradley LM, Haynes L, Swain SL. IL-7: maintaining T-cell memory and achieving homeostasis. Trends Immunol 2005;26:172-6.
120. Takada K, Jameson SC. Naive T cell homeostasis: from awareness of space to a sense of place. Nat Rev Immunol 2009;9:823-32.
121. Jiang Q, Li WQ, Aiello FB, Mazzucchelli R, Asefa B, Khaled AR, et al. Cell biology of IL-7, a key lymphotrophin. Cytokine Growth Factor Rev 2005;16:513-33.
122. Lang KS, Recher M, Navarini AA, Harris NL, Lohning M, Junt T, et al. Inverse correlation between IL-7 receptor expression and CD8 T cell exhaustion during persistent antigen stimulation. Eur J Immunol 2005;35:738-45.
123. Levy Y, Lacabaratz C, Weiss L, Viard JP, Goujard C, Lelievre JD, et al. Enhanced T cell recovery in HIV-1-infected adults through IL-7 treatment. J Clin Invest 2009;119:997-1007.
124. Colombetti S, Levy F, Chapatte L. IL-7 adjuvant treatment enhances long-term tumor-antigen-specific CD8+ T-cell responses after immunization with recombinant lentivector. Blood 2009;113:6629-37.
125. He C, Zhou Y, Li Z, Farooq MA, Ajmal I, Zhang H, et al. Co-Expression of IL-7 Improves NKG2D-Based CAR T Cell Therapy on Prostate Cancer by Enhancing the Expansion and Inhibiting the Apoptosis and Exhaustion. Cancers (Basel) 2020;12:1969.
126. Pellegrini M, Calzascia T, Elford AR, Shahinian A, Lin AE, Dissanayake D, et al. Adjuvant IL-7 antagonizes multiple cellular and molecular inhibitory networks to enhance immunotherapies. Nat Med 2009;15:528-36.
127. Mishra A, Sullivan L, Caligiuri MA. Molecular pathways: interleukin-15 signaling in health and in cancer. Clin Cancer Res 2014;20:2044-50.
128. Kennedy MK, Glaccum M, Brown SN, Butz EA, Viney JL, Embers M, et al. Reversible defects in natural killer and memory CD8 T cell lineages in interleukin 15-deficient mice. J Exp Med 2000;191:771-80.
129. Shenoy AR, Kirschnek S, Hacker G. IL-15 regulates Bcl-2 family members Bim and Mcl-1 through JAK/STAT and PI3K/AKT pathways in T cells. Eur J Immunol 2014;44:2500-7.
130. Klebanoff CA, Finkelstein SE, Surman DR, Lichtman MK, Gattinoni L, Theoret MR, et al. IL-15 enhances the in vivo antitumor activity of tumor-reactive CD8+ T cells. Proc Natl Acad Sci U S A 2004;101:1969-74.
131. Alizadeh D, Wong RA, Yang X, Wang D, Pecoraro JR, Kuo CF, et al. IL15 Enhances CAR-T Cell Antitumor Activity by Reducing mTORC1 Activity and Preserving Their Stem Cell Memory Phenotype. Cancer Immunol Res 2019;7:759-72.
132. Saito M, Inoue S, Yamashita K, Kakeji Y, Fukumoto T, Kotani J. IL-15 Improves Aging-Induced Persistent T Cell Exhaustion in Mouse Models of Repeated Sepsis. Shock 2020;53:228-35.
133. Spolski R, Leonard WJ. Interleukin-21: a double-edged sword with therapeutic potential. Nat Rev Drug Discov 2014;13:379-95.
134. Liu S, Lizee G, Lou Y, Liu C, Overwijk WW, Wang G, et al. IL-21 synergizes with IL-7 to augment expansion and anti-tumor function of cytotoxic T cells. Int Immunol 2007;19:1213-21.
135. Cui W, Liu Y, Weinstein JS, Craft J, Kaech SM. An interleukin-21-interleukin-10-STAT3 pathway is critical for functional maturation of memory CD8+ T cells. Immunity 2011;35:792-805.
136. Elsaesser H, Sauer K, Brooks DG. IL-21 is required to control chronic viral infection. Science 2009;324:1569-72.
137. Frohlich A, Kisielow J, Schmitz I, Freigang S, Shamshiev AT, Weber J, et al. IL-21R on T cells is critical for sustained functionality and control of chronic viral infection. Science 2009;324:1576-80.
138. Loschinski R, Bottcher M, Stoll A, Bruns H, Mackensen A, Mougiakakos D. IL-21 modulates memory and exhaustion phenotype of T-cells in a fatty acid oxidation-dependent manner. Oncotarget 2018;9:13125-38.
139. Hermans D, Gautam S, Garcia-Canaveras JC, Gromer D, Mitra S, Spolski R, et al. Lactate dehydrogenase inhibition synergizes with IL-21 to promote CD8(+) T cell stemness and antitumor immunity. Proc Natl Acad Sci U S A 2020;117:6047-55.
140. Moroz A, Eppolito C, Li Q, Tao J, Clegg CH, Shrikant PA. IL-21 enhances and sustains CD8+ T cell responses to

achieve durable tumor immunity: comparative evaluation of IL-2, IL-15, and IL-21. J Immunol 2004;173:900-9.

141. Wang LN CY, Ruge F, Jiang WG. Interleukin 21 and Its Receptor Play a Role in Proliferation, Migration and Invasion of Breast Cancer Cells. Cancer Genomics Proteomics 2015;12:211-21.
142. Ueda M, Imada K, Imura A, Koga H, Hishizawa M, Uchiyama T. Expression of functional interleukin-21 receptor on adult T-cell leukaemia cells. Br J Haematol 2005;128:169-76.
143. Lamprecht B, Kreher S, Anagnostopoulos I, Johrens K, Monteleone G, Jundt F, et al. Aberrant expression of the Th2 cytokine IL-21 in Hodgkin lymphoma cells regulates STAT3 signaling and attracts Treg cells via regulation of MIP-3alpha. Blood 2008;112:3339-47.
144. Brenne AT, Ro TB, Waage A, Sundan A, Borset M, Hjorth-Hansen H. Interleukin-21 is a growth and survival factor for human myeloma cells. Blood 2002;99:3756-62.
145. Suprunenko T, Hofer MJ. Complexities of Type I Interferon Biology: Lessons from LCMV. Viruses 2019;11:172.
146. McNab F, Mayer-Barber K, Sher A, Wack A, O'Garra A. Type I interferons in infectious disease. Nat Rev Immunol 2015;15:87-103.
147. Wu T, Ji Y, Moseman EA, Xu HC, Manglani M, Kirby M, et al. The TCF1-Bcl6 axis counteracts type I interferon to repress exhaustion and maintain T cell stemness. Sci Immunol 2016;1:eaai8593.
148. Lee MS, Park CH, Jeong YH, Kim YJ, Ha SJ. Negative regulation of type I IFN expression by OASL1 permits chronic viral infection and CD8(+) T-cell exhaustion. PLoS Pathog 2013;9:e1003478.
149. Quigley M, Pereyra F, Nilsson B, Porichis F, Fonseca C, Eichbaum Q, et al. Transcriptional analysis of HIV-specific CD8+ T cells shows that PD-1 inhibits T cell function by upregulating BATF. Nat Med 2010;16:1147-51.
150. Butte MJ, Keir ME, Phamduy TB, Sharpe AH, Freeman GJ. Programmed death-1 ligand 1 interacts specifically with the B7-1 costimulatory molecule to inhibit T cell responses. Immunity 2007;27:111-22.
151. Barber DL, Wherry EJ, Masopust D, Zhu B, Allison JP, Sharpe AH, et al. Restoring function in exhausted CD8 T cells during chronic viral infection. Nature 2006;439:682-7.
152. Keir ME, Butte MJ, Freeman GJ, Sharpe AH. PD-1 and its ligands in tolerance and immunity. Annu Rev Immunol 2008;26:677-704.
153. Yokosuka T, Takamatsu M, Kobayashi-Imanishi W, Hashimoto-Tane A, Azuma M, Saito T. Programmed cell death 1 forms negative costimulatory microclusters that directly inhibit T cell receptor signaling by recruiting phosphatase SHP2. J Exp Med 2012;209:1201-17.
154. Chemnitz JM, Parry RV, Nichols KE, June CH, Riley JL. SHP-1 and SHP-2 associate with immunoreceptor tyrosine-based switch motif of programmed death 1 upon primary human T cell stimulation, but only receptor ligation prevents T cell activation. J Immunol 2004;173:945-54.
155. Odorizzi PM, Pauken KE, Paley MA, Sharpe A, Wherry EJ. Genetic absence of PD-1 promotes accumulation of terminally differentiated exhausted CD8+ T cells. J Exp Med 2015;212:1125-37.
156. Fife BT, Pauken KE, Eagar TN, Obu T, Wu J, Tang Q, et al. Interactions between PD-1 and PD-L1 promote tolerance by blocking the TCR-induced stop signal. Nat Immunol 2009;10:1185-92.
157. Arasanz H, Gato-Canas M, Zuazo M, Ibanez-Vea M, Breckpot K, Kochan G, et al. PD1 signal transduction pathways in T cells. Oncotarget 2017;8:51936-45.
158. Fourcade J, Sun Z, Pagliano O, Guillaume P, Luescher IF, Sander C, et al. CD8(+) T cells specific for tumor antigens can be rendered dysfunctional by the tumor microenvironment through upregulation of the inhibitory receptors BTLA and PD-1. Cancer Res 2012;72:887-96.
159. Curran MA, Montalvo W, Yagita H, Allison JP. PD-1 and CTLA-4 combination blockade expands infiltrating T cells and reduces regulatory T and myeloid cells within B16 melanoma tumors. Proc Natl Acad Sci U S A 2010;107:4275-80.
160. Hannier S TM, Bismuth G, Triebel F. CD3/TCR complex-associated lymphocyte activation gene-3 molecules inhibit CD3/TCR signaling. J Immunol 1998;161:4058-65.
161. Matsuzaki J, Gnjatic S, Mhawech-Fauceglia P, Beck A, Miller A, Tsuji T, et al. Tumor-infiltrating NY-ESO-1-specific CD8+ T cells are negatively regulated by LAG-3 and PD-1 in human ovarian cancer. Proc Natl Acad Sci U S A 2010;107:7875-80.
162. Richter K, Agnellini P, Oxenius A. On the role of the inhibitory receptor LAG-3 in acute and chronic LCMV infection. Int Immunol 2010;22:13-23.
163. Dong Y, Li X, Zhang L, Zhu Q, Chen C, Bao J, et al. CD4(+) T cell exhaustion revealed by high PD-1 and LAG-3 expression and the loss of helper T cell function in chronic hepatitis B. BMC Immunol 2019;20:27.
164. Yang ZZ, Kim HJ, Villasboas JC, Chen YP, Price-Troska T, Jalali S, et al. Expression of LAG-3 defines exhaustion of intratumoral PD-1(+) T cells and correlates with poor outcome in follicular lymphoma. Oncotarget. 2017;8:61425-39.
165. Cao H, Zhang R, Zhang W. CTLA4 interferes with the HBVspecific T cell immune response (Review). Int J Mol

Med 2018;42:703-12.

166. Egen JG, Kuhns MS, Allison JP. CTLA-4: new insights into its biological function and use in tumor immunotherapy. Nat Immunol 2002;3:611-8.
167. Romano E, Kusio-Kobialka M, Foukas PG, Baumgaertner P, Meyer C, Ballabeni P, et al. Ipilimumab-dependent cell-mediated cytotoxicity of regulatory T cells ex vivo by nonclassical monocytes in melanoma patients. 167. Romano E, Kusio-Kobialka M, Foukas PG, Baumgaertner P, Meyer C, Ballabeni P, et al. Ipilimumab-dependent cell-mediated cytotoxicity of regulatory T cells ex vivo by nonclassical monocytes in melanoma patients. Proc Natl Acad Sci U S A 2015;112:6140-5 .
168. Ruocco MG, Pilones KA, Kawashima N, Cammer M, Huang J, Babb JS, et al. Suppressing T cell motility induced by anti-CTLA-4 monotherapy improves antitumor effects. J Clin Invest 2012;122:3718-30.
169. Rosskopf S, Leitner J, Zlabinger GJ, Steinberger P. CTLA-4 antibody ipilimumab negatively affects CD4(+) T-cell responses in vitro. Cancer Immunol Immunother 2019;68:1359-68.
170. Turnis ME, Andrews LP, Vignali DA. Inhibitory receptors as targets for cancer immunotherapy. Eur J Immunol 2015;45:1892-905.
171. Anderson AC. Tim-3: an emerging target in the cancer immunotherapy landscape. Cancer Immunol Res 2014;2:393-8.
172. Huang YH, Zhu C, Kondo Y, Anderson AC, Gandhi A, Russell A, et al. CEACAM1 regulates TIM-3-mediated tolerance and exhaustion. Nature 2015;517:386-90.
173. Joller N, Kuchroo VK. Tim-3, Lag-3, and TIGIT. Curr Top Microbiol Immunol 2017;410:127-56.
174. Zhou G, Sprengers D, Boor PPC, Doukas M, Schutz H, Mancham S, et al. Antibodies Against Immune Checkpoint Molecules Restore Functions of Tumor-Infiltrating T Cells in Hepatocellular Carcinomas. Gastroenterology 2017;153:1107-19 e10.
175. Lozano E, Dominguez-Villar M, Kuchroo V, Hafler DA. The TIGIT/CD226 axis regulates human T cell function. J Immunol 2012;188:3869-75.
176. Attanasio J, Wherry EJ. Costimulatory and Coinhibitory Receptor Pathways in Infectious Disease. Immunity 2016;44:1052-68.
177. Chauvin JM, Pagliano O, Fourcade J, Sun Z, Wang H, Sander C, et al. TIGIT and PD-1 impair tumor antigen-specific CD8(+) T cells in melanoma patients. J Clin Invest 2015;125:2046-58.
178. Zhang Q, Bi J, Zheng X, Chen Y, Wang H, Wu W, et al. Blockade of the checkpoint receptor TIGIT prevents NK cell exhaustion and elicits potent anti-tumor immunity. Nat Immunol 2018;19:723-32.
179. Yang ZZ, Kim HJ, Wu H, Jalali S, Tang X, Krull JE, et al. TIGIT Expression Is Associated with T-cell Suppression and Exhaustion and Predicts Clinical Outcome and Anti-PD-1 Response in Follicular Lymphoma. Clin Cancer Res 2020;26:5217-31.
180. Ostroumov D, Duong S, Wingerath J, Woller N, Manns MP, Timrott K, et al. Transcriptome profiling identifies TIGIT as a marker of T cell exhaustion in liver cancer. Hepatology 2021;73:1399-418.
181. Minnie SA, Kuns RD, Gartlan KH, Zhang P, Wilkinson AN, Samson L, et al. Myeloma escape after stem cell transplantation is a consequence of T-cell exhaustion and is prevented by TIGIT blockade. Blood 2018;132:1675-88.
182. Johnson MO, Siska PJ, Contreras DC, Rathmell JC. Nutrients and the microenvironment to feed a T cell army. Semin Immunol 2016;28:505-13.
183. Jones RG, Thompson CB. Revving the engine: signal transduction fuels T cell activation. Immunity 2007;27:173-8.
184. Chang CH, Curtis JD, Maggi LB, Jr., Faubert B, Villarino AV, O'Sullivan D, et al. Posttranscriptional control of T cell effector function by aerobic glycolysis. Cell 2013;153:1239-51.
185. Pearce EL, Pearce EJ. Metabolic pathways in immune cell activation and quiescence. Immunity 2013;38:633-43.
186. O'Sullivan D, van der Windt GJ, Huang SC, Curtis JD, Chang CH, Buck MD, et al. Memory CD8(+) T cells use cell-intrinsic lipolysis to support the metabolic programming necessary for development. Immunity 2014;41:75-88.
187. van der Windt GJ, Everts B, Chang CH, Curtis JD, Freitas TC, Amiel E, et al. Mitochondrial respiratory capacity is a critical regulator of CD8+ T cell memory development. Immunity 2012;36:68-78.
188. Geiger R, Rieckmann JC, Wolf T, Basso C, Feng Y, Fuhrer T, et al. L-Arginine Modulates T Cell Metabolism and Enhances Survival and Anti-tumor Activity. Cell 2016;167:829-42 e13.
189. Nakaya M, Xiao Y, Zhou X, Chang JH, Chang M, Cheng X, et al. Inflammatory T cell responses rely on amino acid transporter ASCT2 facilitation of glutamine uptake and mTORC1 kinase activation. Immunity 2014;40:692-705.
190. Doedens AL, Phan AT, Stradner MH, Fujimoto JK, Nguyen JV, Yang E, et al. Hypoxia-inducible factors enhance the effector responses of CD8(+) T cells to persistent antigen. Nat Immunol 2013;14:1173-82.
191. Ho PC, Bihuniak JD, Macintyre AN, Staron M, Liu X, Amezquita R, et al. Phosphoenolpyruvate Is a Metabolic Checkpoint of Anti-tumor T Cell Responses. Cell 2015;162:1217-28.
192. Mineharu Y, Kamran N, Lowenstein PR, Castro MG.

Blockade of mTOR signaling via rapamycin combined with immunotherapy augments antiglioma cytotoxic and memory T-cell functions. Mol Cancer Ther 2014;13:3024-36.

193. Agnellini P, Wolint P, Rehr M, Cahenzli J, Karrer U, Oxenius A. Impaired NFAT nuclear translocation results in split exhaustion of virus-specific CD8+ T cell functions during chronic viral infection. Proc Natl Acad Sci U S A 2007;104:4565-70.
194. Intlekofer AM, Takemoto N, Kao C, Banerjee A, Schambach F, Northrop JK, et al. Requirement for T-bet in the aberrant differentiation of unhelped memory CD8+ T cells. J Exp Med 2007;204:2015-21.
195. Kaech SM, Cui W. Transcriptional control of effector and memory CD8+ T cell differentiation. Nat Rev Immunol 2012;12:749-61.
196. Banerjee A, Gordon SM, Intlekofer AM, Paley MA, Mooney EC, Lindsten T, et al. Cutting edge: The transcription factor eomesodermin enables CD8+ T cells to compete for the memory cell niche. J Immunol 2010;185:4988-92.
197. Macian F, Garcia-Rodriguez C, Rao A. Gene expression elicited by NFAT in the presence or absence of cooperative recruitment of Fos and Jun. EMBO J 2000;19:4783-95.
198. Martinez GJ, Pereira RM, Aijo T, Kim EY, Marangoni F, Pipkin ME, et al. The transcription factor NFAT promotes exhaustion of activated CD8(+) T cells. Immunity 2015;42:265-78.
199. Man K, Gabriel SS, Liao Y, Gloury R, Preston S, Henstridge DC, et al. Transcription Factor IRF4 Promotes CD8(+) T Cell Exhaustion and Limits the Development of Memory-like T Cells during Chronic Infection. Immunity 2017;47:1129-41 e5.
200. McKinney EF, Lee JC, Jayne DR, Lyons PA, Smith KG. T-cell exhaustion, co-stimulation and clinical outcome in autoimmunity and infection. Nature 2015;523:612-6.
201. Long SA, Thorpe J, DeBerg HA, Gersuk V, Eddy J, Harris KM, et al. Partial exhaustion of CD8 T cells and clinical response to teplizumab in new-onset type 1 diabetes. Sci Immunol 2016;1:eaai7793.
202. Williams RL, Cooley S, Bachanova V, Blazar BR, Weisdorf DJ, Miller JS, et al. Recipient T Cell Exhaustion and Successful Adoptive Transfer of Haploidentical Natural Killer Cells. Biol Blood Marrow Transplant 2018;24:618-22.
203. Sarraj B, Ye J, Akl AI, Chen G, Wang JJ, Zhang Z, et al. Impaired selectin-dependent leukocyte recruitment induces T-cell exhaustion and prevents chronic allograft vasculopathy and rejection. Proc Natl Acad Sci U S A 2014;111:12145-50.
204. Bohne F, Londono MC, Benitez C, Miquel R, Martinez-Llordella M, Russo C, et al. HCV-induced immune responses influence the development of operational tolerance after liver transplantation in humans. Sci Transl Med 2014;6:242ra81.
205. Fribourg M, Anderson L, Fischman C, Cantarelli C, Perin L, La Manna G, et al. T-cell exhaustion correlates with improved outcomes in kidney transplant recipients. Kidney Int 2019;96:436-49.