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CD8+T Cell Exhaustion and Immune Checkpoint Blockade Treatment

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Abstract. T cells play an important role in anti-tumor immunity. Its subset that contains the surface receptor CD8, also called cytotoxic T cells, functions to facilitate tumor cell elimination. Chronic exposure of CD8+ T cells to antigens and inflammation can cause the T cells to lose their functions as well as changes in their surface expressions, named T cell exhaustion (Tex). This article reviews the molecular mechanisms of this cellular process and the influence of the tumor microenvironment (TME) on it. This article focuses on the mechanism of action of the four major upregulated immune checkpoint proteins (ICPs) and introduces ICB therapies targeting each ICP. Based on these mechanisms, scientists have suggested various interventions to reverse T-cell exhaustion. Recent clinical data show that although ICB therapy has significant efficacy in some patients, its limitations in patient selection limit the popularization of the treatment effect. ICB which curbs Tex by blocking the signal pathways that suppress T cell functions, summarizes the current understandings and progress, and identifies the gaps on this topic.

Keywords: Tumor microenvironment (TME); CD8+ T cell exhaustion; cancer immunotherapy; intervention method; immune checkpoint blockade (ICB).

1. Introduction

The tumor microenvironment (TME) is an integrated heterogeneous environment surrounding the tumor cells that can help with tumor cell activation and functioning. The components of TME vary by type and the location of the tumor, but they all contain the same set of cell groups and immune systems. Most significant of which is CD8+ T cells, that can actively react to tumor cells and other antigens. In general, a CD8+ T cell will react immediately against the antigen, and after the clearance of the antigen in the area, some of them will die, while a minor group will turn into memory T cells and get prepared for future reactions against the same antigen.1 In the case of cancer, antigens cannot be completely erased, causing the signal leading to T cell death cannot be activated, so the "exhausted T cells" that cannot die replace memory T cells' functions and fight against the antigens chronically, because of which the T cell receptors (TCR) receive continuous stimulation and show functional loss [1,2]. In addition to the increased expression of cell surface inhibitory receptors, alterations in epigenetics, cell metabolism, cytokine signaling, and gene transcription are also commonly found in exhausted T cells [3, 4]. These functional changes may adversely raise problems in the effectiveness of cancer immunological interventions [5]. ICB, on the other hand, is a therapeutic approach to reverse CD8+ Tex by inhibiting the negative signaling pathways between the inhibitory receptors on the exhausted CD8+ T cells (TEX) and their corresponding ligands. Recent studies have revealed the great importance of Tex and ICB in cancer intervention, and have focused on the ICB interventions of four major receptors that attenuate the functioning of CD8+ T cells, namely cytotoxic T-lumphocyte associated protein 4 (CTLA-4), T-cell immunoglobulin and mucin structural domain 3 (TIM-3), lymphocyte activation gene 3 (LAG-3), and programmed death protein 1/programmed death ligand 1 (PD-1/PD-L1).1 This article therefore aims to summarize the current knowledge on Tex and ICB, review recent clinical progress in the field, as well as discuss the up- and downsides of existing interventions to inform future research in cancer therapy and drug development.

In this article, we discuss the relationship between TME and CD8+ Tex and the molecular mechanism of CD8+ Tex, along with introducing the mechanisms of the four common immune checkpoint molecules mentioned above. We then describe how present ICB techniques can reverse Tex and thus assist cancer therapy, and discuss the current research obstacles and future research directions in the related fields.

2. CD8+ Tex

T cells, particularly CD8+ T cells, that are subjected to chronic inflammation and prolonged antigen exposure demonstrate an increase in inhibitory receptors (CTLA-4, PD-1, TIM-3, LAG-3, etc.) while exhibiting a loss of other effector molecules. For example, alterations in cytokine production (e.g. IL-2, and TNF-α), modifications in gene transcription, and attenuation of proliferation have been observed. Furthermore, regulatory T cells (Tregs), by definition, can up- or downregulate the expression of T cells, in which case it can further contributes to the loss of CD8+ T cell effector functions by suppressing their proliferation. This state of "exhaustion" makes it challenging for the CD8+ T cells to control persistent infections and tumor progression. However, TEX are potentially malleable and can be restored to immunity by certain means.

2.1. Comparison of TEX with Normally Functioning T Cell

There are notable distinctions between TEX and normally functioning T cells at the cellular, molecular, and functional levels in TME. The phenotypical characteristics indicate that TEX typically exhibit elevated levels of inhibitory receptors, including PD-1, TIM-3, and LAG-3. These receptors interact with their ligands to exert an inhibitory effect on T-cell activity.

TEX also exhibits distinct transcription factor expression patterns, such as the increased presence of thymocyte selection-associated high mobility group box protein (TOX), which may be associated with increased inhibitory receptor expression and decreased T cell functionality. Conversely, transcription factors (TFs) of normal T cells, including T-bet, GATA3, and RORγt, typically promote T cell activation and effector functions while contributing to the development of effector T cell subsets. In addition, at the epigenetic level, DNA methylation and histone modifications in TEX can impede gene expression, whereas they are known to facilitate gene expression and functions in normal T cell [6].

In terms of metabolic profiling, TEX tends to rely on glycolysis rather than aerobic metabolism for energy production, a metabolic mode that, although less efficient, provides cells with the necessary ATP under hypoxic conditions. Compares to which normal T cells and effector T cells are more reliant on oxidative phosphorylation to satisfy their high-energy requirements in the antitumor immune response. Surprisingly, TEX may be able to more efficiently utilize metabolic wastes accumulated in the TME, such as lactate, for repurposing as an energy source or for maintaining the reduced state of the cell, which contributes to tumor angiogenesis and immune escape of tumor cells.

2.2. Effect of TME on Tex

TME is a sophisticated ecosystem composed of tumor cells and various infiltrating immune cells (including T lymphocytes, B lymphocytes, TAM, NK, DC, etc.). Tumor cells promote their own growth and evade immune attack by establishing a pro-tumor and immunosuppressive microenvironment. In this environment, immune cells such as DCs can be functionally depleted by non-productive interactions with tumor cells and by exposure to immunosuppressive cytokines and other immunosuppressive cells. Meanwhile, tumor cells activate the PD-1/PD-L1 signaling pathway by expressing immune checkpoint molecules such as PD-L1. PD-L1 binds to PD-1 on the surface of T cells, inhibiting T-cell activity and proliferation and promoting T-cell depletion.

T-cell depletion is a key feature of TME, which is closely linked to the establishment and maintenance of TME, and together they promote the malignant development of tumors [7].

3. Molecular Mechanisms of CD8+ Tex

3.1. Regulatory Role of TFs

TOX is a major regulatory molecule of TEX differentiation and development, which is consistently expressed at high levels in the state of Tex. Its expression in TEX is also positively correlated with PD-1 expression, and participates in epigenetic remodeling processes such as openness to chromatin. TEX can initiate and dominate the development of TEX exhibits differentiation at the transcriptional level. Likewise, during chronic infections, TOX contributes to Tex by increasing the expression of genes associated with depletion while suppressing those related to effector cells [8]. The levels of TOX and its regulated molecular processes can act as indicators for more precise detection and evaluation of TEX;secondly, this series of studies identified TOX as a key molecule in the regulation of TEX, and the modulation of TOX can also alter the phenotype of TEX cells, or may lead to a change in the phenotype of TEX cells. TEX cell phenotype, which may be useful for future tumor immunotherapy.

In addition, Id2 molecules may be involved in regulating T cell proliferation and differentiation, and interact with other TFs (e.g.TOX) to influence T cell depletion status.

3.2. Alterations in Metabolic Processes

In the TME, CD8+ T cell depletion is manifested by a deficiency in metabolic processes, mitochondrial dysfunction, and an elevated endoplasmic reticulum stress response. This mitochondrial dysfunction is defined as damage to the mitochondrial respiratory chain resulting from prolonged T cell stimulation. Furthermore, metabolic pathway reprogramming results in metabolite alterations due to the TME, including hypoxia-induced metabolic pathway modifications. At the same time, reprogramming of cholesterol metabolism within TME can modulate the anti-tumor activity of immune cells. A major structural element of cell membranes, cholesterol plays an important role in maintaining cell structure and function. By altering cholesterol metabolic pathways, tumor cells can affect T-cell function and thus contribute to T-cell depletion [9].

3.3. Upregulation of Immune Checkpoint Proteins (ICPs)

ICPs represent a class of proteins that regulate the activity of the immune system. They play a crucial role in maintaining autoimmune tolerance and preventing excessive immune responses. However, within the TME, tumor cells frequently exploit these proteins to evade immune surveillance and attack. Tumor cells diminish the functionality of immune cells by enhancing the expression of ICPs, including PD-1/PD-L1, and CTLA-4, in order to evade the immune system and circumvent recognition and clearance, which the mechanism is introduced in the next section.

3.4. Mechanisms of ICPs in CD8+ Tex

Immune cell activities are usually regulated by their surface proteins ICPs (e.g. PD-1, CTLA-4, etc.). These proteins help prevent the immune system from overreacting under normal conditions and protect their own tissues from attack. However, many immune cells similar to CD8+ T cells are highly aggressive towards antigens, in which case the ICPs will operate to suppress the immune response [10]. Normally, ICPs on CD8+ T cells are activated only when the corresponding external antigen is completely eliminated, causing CD8+ T cells to cease their work thereby ensuring that the immune system is not harmed; whereas CD8+ TEX in the TME are further exacerbated by the increased number of these ICPs leading to an early termination of their work prior to the disappearance of the antigen.

3.5. CTLA-4

Te cell surface of Treg cells there exists a class of co-receptors called CD28, which connects B7 ligands B7-1 and B7-2 on Antigen Presenting Cell (APC) and promotes the activation of Treg cells, differentiation, and co-stimulates CD8+ T cells' activation; whereas CTLA-4 presents on the surface of activated Treg cells and acts to exert an inhibitory effect on these functions when necessary. Normally, activated CTLA-4 is rapidly endocytosed to ensure a rapid response to T cell activation,

and is immediately removed when inhibition is not necessary. However, the high affinity of CTLA-4 for the B7 ligand can cause the B7 ligand to preferentially connect to the CTLA-4 receptor on CD8+ T cells while ignoring signals from the CD28 receptor, resulting in the inability of CD8+ T cells to be activated by this method (Figure 1) [11].

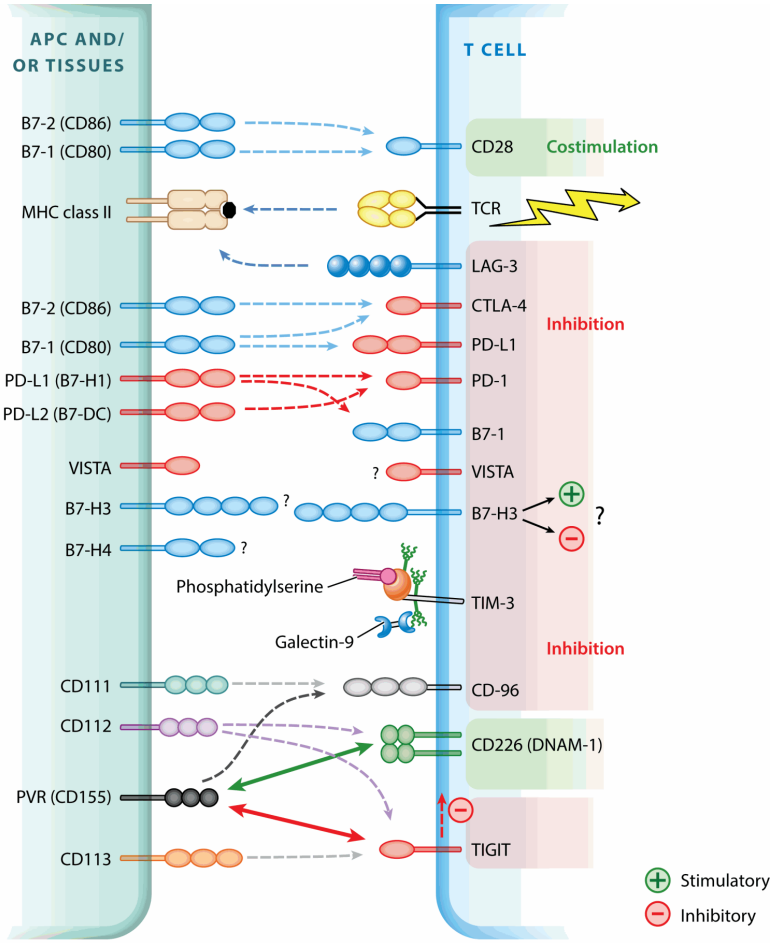

Fig. 1 Different ICPs and their role in T cell activation or suppression [11].

The concentration of Treg cells is, in turn, negatively correlated with the level of granzyme B (GzmB) secretion by CD8+ T cells, meaning that CD8+ T cells without Treg cells have higher levels of Gzmb secretion [1,12]. Since GzmB is known as a pathway used by CD8+ T cells to promote tumor cell apoptosis, the down-regulation of GzmB secretion also leads to a decreased efficiency of tumor cell clearance [13].

3.6. TIM-3

TIM-3 is a transmembrane protein expressed primarily on T helper cells type 1 (Th1) and T cytotoxic cells type 1 (Tc1). In contrast to the two major B7 ligands of CTLA-4, TIM-3 has four common ligands: galectin-9, high-mobility group protein B1 (HMGB1), phosphatidylserine (PtdSer), and Carcinoembryonic antigen-related cell adhesion molecule-1 (CEACAM1), and because these ligands can be present on different cells, they have different roles in the management of immune cells [14]. TIM-3/Galectin-9 interaction and the binding of TIM-3 and the extracellular domain of CEACAM1 can give rise to the release of BAT3, which inhibits TCR signaling [15]. HMGB1 is an alarmin to send danger signals to the immune system, therefore binding it with TIM-3 can inactivate the normal functioning of part of the immunity including CD8+ T cells; while contrastingly, the interaction between TIM-3 and PtdSer promotes clear the apoptotic vesicles from the TME, although the exact physiological effects of such interactions are not yet fully understood [14,15].

3.7. LAG-3

LAG-3 and CTLA-4 have similar mechanisms, appropriating the MHC class II molecules from the CD4 receptor through higher affinity, leading to inhibitory signaling [16]. Unlike CTLA-4, the binding between LAG-3 and MHC class II molecules does not directly turn off the activation of CD8+ T cells, instead, it selectively connects to the peptide-MHCII complex (pMHCII), which is a stabilized structure of MHC class II molecules on APCs. Since LAG-3 can directly convert the internal inhibitory signals of CD4+ T cells, the inhibitory function of LAG-3 on CD4+ T cells can be carried out without interrupting the binding between CD4 receptors and stable pMHCII.17 And if CD4+ T cells, the key factors initiating CD8+ T cells, are inhibited, CD8+ T cells will also be affected in terms of their responses and functions. Similar to TIM-3/PtdSer, the mechanism behind LAG-3/MHC class II molecules still requires more effort to be put into learning [17].

3.8. PD-1/PD-L1

PD-1 is an important ICPs on CD8+ T cells, and its binding to its ligand, PD-L1, stops the T cells from continuous functioning and thus prevents the immune system from over-functioning after antigens and inflammation have been cleared. PD-1+ CD8+ T cells can be further categorized into high-PD-1 expression (PD-1hi) T cells and low-PD-1 expression (PD-1lo) T cells, where PD-1lo T cells have better cytotoxicity, can kill tumor cells more effectively, and are also generally desired CD8+ T cells; however, PD-1lo T cells will develop into PD-1hi T cells during the exhaustion, and over-binding with their ligands together with the function of other co-receptors will inhibit the functions of regularly acting CD8+ T cells, reduce their proliferations, and leads to the exhaustion of CD8+ T cells [1, 18].

4. ICBs

Scientists and clinicians have developed various of strategies to restore CD8+ T cells' functions, wherein monoclonal antibody (mAb)therapy is the most widely used one that applies to and functions on different ICP, followed by some other therapies like small molecule inhibitors, and bispecific antibody therapy [19].

4.1. CTLA-4 Blockade

The primary methods for blocking CTLA-4 include mAb therapy, small molecule inhibitors, and combined therapy with PD-1/PD-L1 inhibitors. MAb therapy targets CTLA-4 on T cells in the TME through engineered antibodies, blocking the immune checkpoint interaction and ensuring that T cells function properly. A notable example is ipilimumab, which inhibits the binding of CTLA-4 to B7 molecules, thereby lifting the suppression of T-cell activation and maintaining their active state [20].

The combined therapy of CTLA-4 and PD-1/PD-L1 inhibitors can enhance T cell activation and proliferation at different stages, boosting the immune response against tumors. PD-1/PD-L1 inhibitors work by interrupting the binding between PD-1 and PD-L1, reactivating suppressed CD8+ T cells and enhancing their ability to eliminate tumors. This combination therapy operates through two main pathways: first, CTLA-4 inhibitors promote the activation of initial T cells in the lymph nodes, while PD-1/PD-L1 inhibitors improve the function of effector T cells in the TME; second, CTLA-4 inhibitors reduce the number and activity of Tregs in the TME, while PD-1/PD-L1 inhibitors help overcome tumor-induced T cell exhaustion. Additionally, this combination therapy decreases the number and suppressive function of Treg cells, increasing the proportion of CD4+ and CD8+ T cells in tumor tissues, thereby producing a stronger anti-tumor effect.

4.2. TIM-3 Blockade

mAbs also constitute a principal strategy to interfere with TIM-3 binding. Examples like TSR-022 can impede TIM-3's interaction with its ligand Gal-9, mitigating T cell inhibition. Studies highlight TIM-3 expression and binding on dendritic cells (DCs) as crucial factors linked to anti-tumor immune responses [21]. By knocking off the TIME-3 proteins expressed on DCs with the mAb specificity,

tumor growth can be significantly inhibited and thus upregulates CD8+ T cell infiltration, particularly of early activation, effector, memory, and memory precursor T cells pivotal in anti-tumor immunity [22].

4.3. LAG-3 Blockade

Anti-LAG-3 mAbs can compete with the ligands to bind LAG-3 proteins, thereby diminishing their inhibitory impact on T cells. Blocking LAG-3's ligand interaction reduces the inhibitory signals T cells receive, aiding in maintaining their activated state and enhancing their responsiveness to tumor antigens, thus fostering T cell proliferation and differentiation [23]

In addition to mAbs, bispecific antibody therapy is also extensively employed to block LAG-3 pathways. Unlike mAbs, bispecific antibodies can simultaneously bind two distinct proteins; for instance, one arm binds LAG-3, while the other binds PD-1 or PD-L1. This double blockade eliminates T cell suppression by blocking both immune checkpoints concurrently, thereby activating T cells more effectively, as well as enabling enhanced tumor cell recognition and attack [24].

4.4. PD-1/PD-L1 Blockade

Although mAb therapy is the prevalent method for inhibiting the PD-1/PD-L1 pathway, these antibodies can be classified as anti-PD-1 (e.g., Nivolumab, Pembrolizumab) or anti-PD-L1 (e.g., Atezolizumab), which bind to PD-1 and PD-L1, respectively, preventing their mutual interaction [25,26].

Several key proteins play important roles in controlling immune responses, particularly those related to T cells. CTLA-4, for instance, helps regulate the activity of T cells by binding to specific molecules, preventing their overactivation. TIM-3, which has several different partners, can either suppress or assist immune responses depending on its interactions. LAG-3, similar to CTLA-4, also limits the activation of certain immune cells, ensuring they don’t overreact. PD-1, commonly found on T cells, helps stop these cells from working too hard, but if this control becomes too strong, it can weaken the body’s ability to fight off threats. All of these proteins are crucial in balancing immune activity to prevent it from harming healthy tissues, but when they work too well, they can reduce the effectiveness of the body’s defense against diseases like cancer.

5. Clinical Studies

It has been shown that PD-1/PD-L1 inhibitors can induce HBV-specific T-cell activation and thus exert antiviral effects. Based on clinical observation, academician Fan Jia's team from Institute of Liver Cancer Research and Department of Liver Surgery, Zhongshan Hospital, Fudan University, who has long been deeply engaged in the research on the clinical application of interferon against hepatocellular carcinoma, has found that, for advanced hepatocellular carcinoma, the combination of interferon alpha (IFN alpha) and anti-PD-1 antibody can significantly reduce the size of the tumor, and has obvious effect on the inhibition of metastasis of hepatocellular carcinoma.

ICB therapy has also achieved unprecedented results in the clinical treatment of non-small cell lung cancer (NSCLC). Recently, a research team led by Prof. Zemin Zhang from Peking University and two scientists, Weidong Han and Yi Hu from the General Hospital of the Chinese People's Liberation Army (PLA), explored the cellular basis and molecular mechanism of immunotherapy using single-cell data from NSCLC patients before and after PD-1 inhibitor treatment, and found that immune checkpoint blockade enhances their anti-tumor activity. However, data show that the number of NSCLS patients who have a significant response to immunotherapy is still only a few [27].

In colon cancer, although the mechanism is not yet clear, it was found that patients with genomic microsatellite instability (MSI) responded better to treatment with immune checkpoint inhibitors than patients with microsatellite stability (MSS). And among the responding patients, the long-term survival of the patients was excellent by generating tumor-specific memory T-cell responses. This

suggests that patient response to immune checkpoint inhibitor therapy correlates with pretreatment tumor-infiltrating T cells.

6. Conclusion

In this article, we discuss the term and the molecular mechanisms of CD8+ Tex in TME together with the existing ICB therapies targeting this change, which attempt to restore the function of TEX. We focus on the mechanisms of operation of four major upregulated ICPs, and discuss ICB therapies that block their operation for each distinct ICP. However, TEX that are in the later stages are shown less likely to respond to ICB therapies due to the stability of their epigenetic state. In contrast, recent clinical data have demonstrated the limitations of ICB as a treatment with significant efficacy while at the same time being limited in patient selection, suggesting that future research should focus on its generalization on patient selection, aiming for a better overall treatment outcome.

Authors Contribution

All the authors contributed equally and their names were listed in alphabetical order.

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