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The Mechanisms of CD8+ T Cells Exhaustion in the Tumor Microenvironment and Immune Therapy

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Abstract

In the tumor immune microenvironment, CD8+ T cells differentiate towards functional failure. The exhaustion of CD8+ T cells (Tex) showed varying degrees of effect dysfunction, loss of proliferation ability, and sustained high expression of a variety of inhibitory receptors, with metabolic and epigenetic changes. Tex cells are heterogeneous, including several subsets with different characteristics at different stages of differentiation. Immune checkpoint inhibitors (ICIs) can restore the effect or function of Tex cells, indicating that this T cell subset plays a key role in tumor immunotherapy. The understanding of the mechanism of CD8+ T cell exhaustion will be helpful to the implementation of tumor immunotherapy. This article reviews the production, differentiation and functional characteristics of Tex cells and their relationship with tumor immunotherapy.

Keywords

CD8+ T Cell Exhaustion, Exhausted CD8+ T Cells, Immunotherapy, Tumor

1. Introduction

In tumors and chronic infectious diseases, CD8+ T cells show sustained high expression of multiple inhibitory receptors, changes in epigenetic and transcriptional profiles, and metabolic dysregulation and functional impairment, a state known as CD8+ T cell exhaustion. In acute infections, initial CD8+ T (naive CD8+ T) cells differentiate and proliferate into effector CD8+ T (Tef) cells, which kill target cells to control the infection. After antigen clearance, most Tef cells

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die, while a portion differentiate into memory T (Tmem) cells and survive long term [1] [2]. When these Tmem cells are re-stimulated with the same antigen, they proliferate and differentiate into Tef cells, exerting their function to kill target cells and control the infection. The disappearance of antigen stimulation is a necessary condition for Tef cells to transform into Tmem cells [3]. In tumors and chronic infections, antigen stimulation persists, and Tef cells cannot transform into Tmem cells and gradually lose their effector function and enter a state of exhaustion. $CD8^+$ T cell exhaustion often occurs in tumors, chronic infections, and autoimmune diseases. Tex cells are composed of different subgroups of cells, and early Tex cells have stem cell characteristics, capable of self-renewal and differentiation, producing terminal Tex cells. Terminal Tex cells have severely impaired effector function and proliferative ability.

$CD8^+$ T cell exhaustion plays a key role in immune dysfunction in malignant tumors. In various malignancies such as melanoma, non-small cell lung cancer, and ovarian cancer, tumor-specific $CD8^+$ T cells express multiple inhibitory receptors and exhibit functional exhaustion, showing characteristics of T cell exhaustion. In the tumor microenvironment, Tex cells lose the ability to effectively kill tumor cells, and Tex cells and tumor cells are in a state of mutual antagonism [4]. ICIs therapy regulates the activity of $CD8^+$ T cells through a series of pathways such as targeting co-inhibitory or co-stimulatory signals, to enhance the immune cells' anti-tumor effects. ICIs therapy has become a main method of tumor immunotherapy in recent years. Studies have shown that blocking the programmed cell death 1 (PD-1)/programmed cell death ligand 1 (PD-L1) pathway in tumors can reactivate Tex cells, enhance their proliferation ability and effector function, and thus kill tumor cells. Monoclonal antibodies targeting PD-1/PD-L1 and cytotoxic T lymphocyte antigen 4 (CTLA-4) signal pathways have shown good therapeutic effects in the clinical treatment of melanoma, non-small cell lung cancer and other tumors [5] [6]. Reactivating Tex cells with ICIs may be the main mechanism of clinical immunotherapy. Tex cells are the main cell type that produces anti-tumor immune responses in ICI therapy [7]. The regression and control of tumors caused by ICI therapy are usually associated with an increase in the number of T cells, an increase in the number of tumor-infiltrating lymphocytes, and the restoration of T cell function. However, the mechanism of action of these treatments and their long-term efficacy still need further research, which will also help to further understand the molecular mechanisms of $CD8^+$ T cell exhaustion and its reactivation. This article reviews the generation, differentiation, functional characteristics, and significance of Tex cells in tumors.

2. Generation of Exhausted $CD8^+$ T Cells

In tumors and various chronic infectious diseases, long-term high antigen load is crucial for the generation of Tex cells. Dysfunction of antigen-specific $CD8^+$ T cells was first reported in chronic lymphocytic choriomeningitis virus (LCMV)

infection in mice. Three different viral strains with varying antigen loads were used in the model of LCMV infection in mice. The Armstrong strain, with lower antigen load, caused an acute infection that was cleared and resulted in effective responses by antigen-specific $CD8^+$ T cells. The LCMV-t1b strain, with moderate antigen load, maintained antigen-specificity at 60% after the first month of infection, leading to ineffective responses by $CD8^+$ T cells in the lesion, but the effect of Tex cells could be restored by reducing antigen load until the 90^{th} day. The Clone 13 strain, with higher antigen load, resulted in 80% of antigen-specificity persisting after infection, causing long-term ineffective responses by $CD8^+$ T cells that could not be restored [8]. Similarly, in chronic infections caused by human immunodeficiency virus, hepatitis B virus, hepatitis C virus, etc., severe exhaustion of $CD8^+$ T cell function occurs with high antigen load [9] [10] [11]. A mouse liver cancer model showed that tumor-specific $CD8^+$ T cells experience functional impairment in the early stages of tumor formation. When tumor-specific $CD8^+$ T cells experience functional impairment in the early stages, removal of antigen stimulation can restore their effector function and differentiate into Tmem cells, but their effector function cannot be restored under continuous antigen stimulation [3].

In chronic inflammation and cancer, various cytokines and other immune regulatory molecules affect the function of $CD8^+$ T cells through complex regulatory patterns. Interleukin-10 (IL-10) and transforming growth factor-β (TGF-β) inhibit immune cell function, and increasing the expression of IL-10 and TGF-β can enhance the immune system's control of chronic viral infections [12] [13]; interleukin-2 (IL-2) and interleukin-21 (IL-21) can enhance the effector function of $CD8^+$ T cells and counteract $CD8^+$ T cell exhaustion [14] [15]. $CD8^+$ T cell exhaustion in the early stages of tumors occurs in an environment lacking inflammation and is mainly driven by sustained antigen stimulation [16]. The low reactive state of $CD8^+$ T cells allows tumor cells to continue to grow. As the tumor develops, $CD8^+$ T cells are continuously stimulated by tumor antigens and enter a state of late-stage functional impairment.

3. Differentiation of Exhausted $CD8^+$ T Cells

The differentiation and phenotype of T cells are strictly regulated by various transcription factors, cytokines, chemokines, integrins, and metabolic signals. After antigen recognition, initial $CD8^+$ T cells proliferate and differentiate into memory precursor T cells, driven by transcription factors such as basic leucine zipper ATF-like transcription factor (BATF), interferon regulatory factor-4 (IRF-4), and nuclear factor of activated T cells (NFAT). Memory precursor T cells have low expression of killer cell lectin-like receptor subfamily G member 1 (KLRG-1) and high expression of CD127. During acute infection, memory precursor T cells proliferate and differentiate into Tef cells, driven by cytokines such as T-box expressed in T cells (T-bet) and B lymphocyte-induced maturation protein 1 (Blimp-1). Tef cells have high expression of KLRG-1 and low expres-

sion of CD127 and clear antigens by secreting perforin, granzymes, and granulysin to kill target cells. After antigen clearance, most Tef cells die, and a portion of Tef cells differentiate into Tmem cells. Tmem cells have low expression of KLRG-1 and high expression of CD127 and can survive long-term in the body, maintaining a certain number by spontaneous proliferation using interleukin-7 (IL-7) and interleukin-15 (IL-15). Upon receiving the same antigen stimulation again, Tmem cells rapidly activate and differentiate into Tef cells. In tumors and chronic infections, Tef cells cannot completely clear antigens, and memory precursor T cells differentiate into Tex cells. Tex cells are a distinct cell population from Tef and Tmem cells.

Tex cells are composed of subpopulations of cells at different differentiation stages, which are formed by specific differentiation of $CD8^+$ T cells [17] [18]. These subpopulations of cells have different biological characteristics in terms of proliferation capacity, effector function, and transcription factor expression. Tex cells can be divided into four subgroups at different developmental stages based on the expression of Ly108 and CD69 molecules [19]. Among them, Ly108+CD69+ Tex cells and Ly108+CD69− Tex cells are two subgroups in the progenitor cell state that can transform into each other and have strong proliferation potential. Ly108+CD69+ Tex cells are in a quiescent state and cannot enter the bloodstream, while Ly108+CD69− Tex cells can enter the bloodstream and differentiate into Ly108-CD69− Tex cells. Ly108-CD69− Tex cells have partial effector function and can differentiate into the terminal stage Ly108-CD69+ Tex cells. Ly108-CD69+ Tex cells lose effector function and proliferation capacity, and are eventually cleared.

4. Characteristics of Exhausted $CD8^+$ T Cells

Tex cells are composed of different subpopulations of cells formed by specific differentiation directions. Compared with Tef and Tmem cells, Tex cells mainly have the following six characteristics: 1) gradually losing effector function and proliferation capacity; 2) sustained high expression of multiple inhibitory receptors; 3) changes in responsiveness to cytokines; 4) metabolic changes; 5) changes in expression and utilization of transcription factors; 6) changes in epigenetic programs [20].

Tex cells persist in tumors and chronic infectious diseases but their effector function is impaired. Under prolonged antigen stimulation, Tex cells first lose their ability to produce IL-2, followed by the loss of tumor necrosis factor (TNF) production. Finally, terminally exhausted Tex cells exhibit loss of interferon-γ (IFN-γ) production [21]. The severity of $CD8^+$ T cell exhaustion is related to antigen load, $CD4^+$ T cell help, and duration of antigen stimulation, and therefore $CD8^+$ T cells can exhibit varying degrees of functional impairment. Prolonged high expression of various inhibitory receptors is one of the important features of Tex cells. At the end of acute infection, inhibitory receptors are briefly expressed on Tef cells, playing a role in controlling the immune response by inhi-

biting T cell function. During the development of $CD8^+$ T cell exhaustion, inhibitory receptors such as PD-1, T-cell immunoglobulin and ITIM domain (TIGIT), lymphocyte-activation gene 3 (Lag-3), and T-cell immunoglobulin and mucin-domain containing-3 (Tim-3) continue to be expressed. These inhibitory receptors on the cell surface, when activated by binding to their ligands, suppress the effector function of $CD8^+$ T cells. Tex cells utilize cytokines such as IL-7 and IL-15 differently from Tmem cells. Tmem cells express interleukin-7 receptor α (IL-7Rα) and interleukin-2/15 receptor β (IL-2/15Rβ), and use IL-7 and IL-15 for self-renewal and long-term survival. Tex cells do not express IL-7Rα and have impaired IL-15Rβ pathway function. Early Tex cells undergo proliferation and produce a large number of terminally exhausted Tex cells under antigen stimulation signal. Terminally exhausted Tex cells lose their proliferative ability and are eventually cleared. The metabolic dysfunction of Tex cells is manifested as respiratory inhibition, reduced glucose uptake, and mitochondrial energy dysfunction [22]. Tex cells in tumors and chronic infectious diseases exhibit similar epigenetic characteristics. The Programmed cell death protein 1 (Pdcd-1) gene encoding PD-1 is demethylated in Tex cells, leading to sustained transcription and expression of PD-1.

5. Immunotherapy of $CD8^+$ T Cell Exhaustion and Tumor

$CD8^+$ T cells are the main effector cell population involved in tumor immunity, capable of killing tumor cells and controlling tumor progression. Tumor-specific antigens (TSA) and tumor-associated antigens (TAA) produced by tumor cells can activate $CD8^+$ T cell-mediated anti-tumor immune responses. Effective anti-tumor immune responses can clear tumor cells in the early stages of tumor development. However, in advanced tumors, the effector function of $CD8^+$ T cells is low and unable to effectively kill tumor cells, leading to a coexistence of tumor growth and $CD8^+$ T cells. The activation of inhibitory receptors is considered one of the main mechanisms of effector dysfunction in Tex cells. When PD-1 expressed by $CD8^+$ T cells binds to PD-L1/PD-L2 expressed by tumor cells, the tyrosine residues on the cytoplasmic domain of PD-1 are phosphorylated, leading to the recruitment of tyrosine phosphatase-2 (SHP-2) and the weakening of the phosphatidylinositol-3-kinase-protein kinase B (PI3K-Akt) and mitogen-activated protein kinase-extracellular signal-regulated kinase (MEK-ERK) signaling pathways [23]. The activation of the PD-1 signaling pathway antagonizes the co-stimulatory effect of CD80-CD28 and weakens T-cell receptor (TCR) signaling transduction. In addition, PD-1 is involved in the metabolic transition of $CD8^+$ T cells from glucose metabolism to fatty acid oxidation, leading to the accumulation of reactive oxygen species and mitochondrial damage [24]. Ultimately, the cytokine production and proliferative capacity of $CD8^+$ T cells decrease, inhibiting $CD8^+$ T cell-mediated immune responses. For example, activation of the WNT–β-catenin pathway in cancer cells results in inadequate dendritic cell (DC)-mediated priming of antigen-specific $CD8^+$ T cells in tu-

mour-draining lymph nodes and poor tumour infiltration [25].

Immune checkpoint therapy improves anti-tumor immune responses by targeting a series of pathways such as co-inhibition or co-stimulation signals to regulate T cell activity. The breakthrough combination of immune checkpoint therapy and chemotherapy has expanded the treatment options for tumors. Monoclonal antibodies targeting PD-1, PD-L1, and CTLA-4 have been used to treat various tumors, such as melanoma and non-small cell lung cancer. Tex cells are the main cell type that produces anti-tumor immune responses in ICI therapy [7]. The regression and control of tumors caused by ICI therapy are usually associated with an increase in the number of T cells, an increase in the number of tumor-infiltrating lymphocytes, and the restoration of T cell function. ICIs enable some tumor patients to achieve sustained therapeutic responses [26]. In these patients, Tex cells restore effector function, quickly clear tumor cells, and may develop into T cells that produce persistent responses to tumors. However, most patients achieve therapeutic responses in the initial stages of immune checkpoint therapy but subsequently develop resistance. Immune checkpoint therapy temporarily restores effector function in Tex cells in these patients, but $CD8^+$ T cells again develop into an exhausted state during continued treatment. Another group of patients does not respond to immune checkpoint therapy. The limitation of immune checkpoint therapy is that Tex cells fail to produce long-term effector function after activation. Blocking the PD-1/PD-L1 pathway can only cause slight epigenetic changes in Tex cells [15], indicating that restoring Tex cell function to achieve long-term control of tumors requires the use of regulatory mechanisms beyond immune checkpoint blockade.

ICI therapy has shown a powerful anti-tumor immune effect in various tumor models. Anti-PD-1 or PD-L1 monoclonal antibodies can block the signal transduction of the PD-1/PD-L1 pathway. Therefore, the expression of PD-L1 in tumor cells may be a prognostic factor for PD-1/PD-L1 targeted therapy. In fact, in PD-1/PD-L1 targeted ICIs therapy, tumors with high PD-L1 expression do have better therapeutic responses. However, some tumors expressing PD-L1 do not respond to treatment, while some tumors that do not express PD-L1 can show persistent therapeutic responses. The expression of PD-L1 in tumor cells is the most commonly used biomarker for ICI therapy, but its predictive role in treatment efficacy is limited.

Although ICIs have achieved great success in the treatment of tumors such as melanoma and non-small cell lung cancer, and have good application prospects in more types of tumors, ICIs cannot prevent $CD8^+$T cells from becoming exhausted. Most patients do not receive long-term survival benefits from ICI treatment. In addition, immune-related adverse events (irAEs) reduce the quality of life of some patients, increase medical costs, and may cause serious injury or death [27] [28]. Further research on effective biomarkers is necessary, which can help select patients who may benefit and exclude those who are at increased

risk of weak efficacy or irAEs. The molecular mechanisms of ICIs treatment inefficacy are still unclear. Therefore, it is important to understand the mechanism of ICIs on Tex cells in order to study more effective immune checkpoint therapy methods.

6. Conclusion and Outlook

This article reviews the generation, differentiation, functional characteristics, and significance of Tex cells in tumors. Tumor-associated antigens change the differentiation of $CD8^+$ T cells, leading to a state of functional exhaustion. Tex cells are a type of T cell composed of different subgroups. Understanding the mechanism of $CD8^+$ T cell exhaustion provides new ideas for the immunotherapy of tumors. In order to improve the effectiveness and precision of immune checkpoint therapy, further research is needed on the molecular mechanisms of $CD8^+$ T cell exhaustion and Tex cell reactivation.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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