The Research Progress of Cancer Immunotherapy

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Abstract. The binding of cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) and programmed death 1 (PD-1) immune checkpoints on T cell and B7 family will serves as an inhibitory signal of T cells' activation, which reduces the immune response toward cancer cells. The blockade of the interaction between the ligands and receptors can prevent the deactivation of T cells. CTLA-4 and PD-1/PD-L1 pathways showed promising efficacy and were suggested as first-line medications in melanoma, among another guideline, like cytokines, and cancer vaccines, which still undergo a stage of development. Though all treatments experience resistance, toxicities, and challenges, ways of improvement continue to be developed.

Keywords: Canner, Immunotherapy, Vaccine.

1. Introduction

Signaling in the immune system enables the complex system to accomplish the goal of identifying self and non-self. For Identifying the non-self, MHC on the present antigen is aimed by T cell receptor. T cell proliferation in lymph nodes, composited with lymphocytes in order to help people to fight infections, is mainly regulated by CTLA-4, while PD-1 plays role in peripheral tissue (not center concern tissues) especially after T cell activation. Blocking CTLA-4 and PD-1 with inhibitors can suppress the de-activation of T cells, which provides implications for cancer immunotherapy. This report review overviews introduce the inhibitor of immune checkpoints and the mechanism of CTLA-4 and PD-1 inhibitors, as well as discusses the applications of immune checkpoints to cancers and other cancer immunotherapies such as vaccines and cytokines.

2. T cell and receptors

T cell and B cell are essential to adaptive immune system in two types of responses (antibody responses and cell-mediated immune responses), creating a solid firewall to overcome the infection. After T cells have been well-developed in the thymus, they will enter and circulate in the bloodstream. When it reaches to peripheral lymphoid organ, it will migrate through the lymphoid tissue until it reaches the antigen. Helper T cell (CD4+), cytotoxic T cell (CD8+), and regulatory T cell are fight against the antigens invading the body. Co-receptor, CD8+ connect to MHC class I molecules and T cell receptors. When the T cell recognizes the infected or diseased cells, it releases cytotoxic molecules killed the target cells. CD4+ assists with helper T cells to receive signals and interacts with MHC class 2 molecules. When it recognizes the antigen, the helper T cells produce cytokines, leading to further signaling processes. Depending on the specialized antigen, the subtype of helper T cells (Th1, Th2, Th 17) will produce different types of cytokines. Regulatory T cells, like Treg cells, turn off the immune responses to prevent overexpressing and maintain immune homeostasis.

3. Immune checkpoint inhibitor (ICI)

Immune checkpoint inhibitor (ICI) is a type of drug that can block the checkpoints on immune cells, which prevent the immune system's overreaction. In the blocking situation, T cells can act actively and kill target cells. In multicellular organisms, cells communicate by binding ligands - such as proteins, peptides, and fatty acids - to receptors on target cells. These signaling processes offer instructions to cell functions, including proliferation and differentiation [1]. Signaling in the immune

system enables the complex system to accomplish the goal of identifying self and non-self. Identifying the non-self T cell receptor binding to the MHC on the presented antigen involves other factors that influence the effectiveness of the T cell, which is the main center of the complex system [2].

3.1. Mechanism

3.1.1. PD-L1/anti-PD-L1

As a costimulatory receptor of B7/CD28, programmed death ligand 1(PD-L1) and PD-L2 regulate the immune response in adaptive immune system. PD-L2 binds to cell which presents antigen, while PD-L1 mainly focuses on detecting and reducing the immune responses towards self-antigen.2 PD-1 binding reduces the proliferation of T-cell, interferon-y, IL-2 and tumor necrosis factor-a, it also decreases the successful survival rate [3].

After the antigen on the MHC of cancer cells was recognized, the T cell will be activated and produce IFN-y and cytokines. Interferon-y plays a vital role in immune response for anti-tumor and anti-viral. IFN-y receptors are composed of two chains: IFNyR1 and IFNyR2, which will be oligomerized and trans-phosphorylated in the IFN-y canonical signaling pathway. Then, two ligands-binding receptors will activate the Janus-activated kinases (JAK 1 and JAK2), which further induce the signal transducer and transcriptional activator (STAT). The STAT then induces the interferon-response genes. As the product of the regulation of genes, interferon regulatory factor 1 (IRF-1) binds to the PD-L1 promotor. The binding enables PD-L1 to express on the surface. Then, PD-L1 on the surface stops T cells from attacking cancer cells. The PD-L1 inhibitor prevents binding between PD-1 and PD-L1, recovering the proliferate T cells.

3.1.2. CTLA-4/anti-CTLA-4

T-cell activation needs more than one stimulatory factor. T cells activation initiates when TCR, the surface receptor on T cell which can recognize the antigen-major histocompatibility complex (MHC), is activated by the stimulus with the co-stimulation of the interaction between B7-CD28, which serves as the determinable process. Tumor-related antigens are presented as peptide-MHC and bind to the TCR on antigen-specific T cells to trigger the killing. The costimulatory factor is needed for full T cell activation. Otherwise, it will cause the energy or inactivation of the immune response. B7-1(CD80) or B7-2(CD86) on the APC binding to CD28 on the T cell serves as a co-stimulatory signal. The combination of stimulatory and costimulatory factors enhanced the level of Interleukin-2 (IL-2), which determines the T cell's differentiation, homeostasis, and metabolic progress. The increased numbers of IL-2 lead to the T cell activation and promotes the expression of Th17 and T follicular helper cell (Tfh) and enhances cell's energy metabolism [4].

Cytotoxic T lymphocyte antigen-4 (CTLA-4 or CD152) is the homolog of CD 28 and shares the same genetic motif MYPPY with CD28. CTLA-4 origins from the intracellular vesicle and is expressed on the surface, induced by the upregulation of Calcium level in the cell [5]. The soluble CTLA-4 fused to an immunoglobulin tail (CTLA-4-lg) and then bind to B7 family protein, which indicates that CTLA-4-lg is a potent inhibitor depending on the T and B lymphocytes [6]. The intensity of TCR signaling determines the expression of CTLA-4 on the cell's surface. Also, CTLA-4 will compete with CD28 to form the CTLA-4 and B7 binding, reducing the number of IL-2 and T cell [7]. Because CTLA-4 can bind to B7-1 and B7-2 more easily than that of CD28, interaction between CD28 and B7-1 and B7-2 has been interrupted by preferential bindings between CTLA and B7 group which does not induce the stimulatory factors [3]. In some studies, CTLA-4 not only plunder the signal required for T activation but also provides some inhibitory signals by inducing the indoleamine 2,3-dioxygenase (IDO) in APC which causes effector T cells to face restriction and cancel out the TCR-MHC binding's effectiveness. The T cell will integrate the stimulatory and inhibitory signals to give the response toward activation. The presence of CTLA-4 diversifies the responders to the stimulus, while the absence of CTLA-4 enables the responders to concentrate in the high TCR affinity domain. Threshold and attenuation models provide different prediction on how

CTLA-4 affect T cell's responses. In the threshold model, presented CTLA-4 reduces the T cell responses to the stimulus, and the absence of CTLA-4 enables T cell to activated by only less stimulus. The end to set the threshold of CTLA-4 might be setting the minimum quantity of TCR signal and CD28-B7 interaction to activate the T cell. The upregulation of CTLA-4 will suppress the CD28 and B7 bindings and generates an inhibitor to react with the product produced by CD28. In the attenuation model, CTLA-4 is not required or presented in the T cell activation [8]. However, after the T cell activation, CTLA-4 limits the divided T cells, which means CTLA-4 does not serve co-stimulatory signals but rather an inhibitory signal by blocking the signaling using the product triggered by the presence of CTLA-4. Cells remain in the lower division stage, while cells experience more division under the deficiency of CTLA-4.

The mechanism of anti-CTLA-4 is not clearly explained. The binding of anti-CTLA-4-antibody and CTLA-4 can re-active the T cell, enhancing the effective immune responses [9].

3.2. Application

Anti-CTLA-4 and anti-PD-1 are changing the market for many cancer treatments, such as melanoma and Non-Small-Cell Lung Cancer.

Immune inhibitor shows advantages in applicability and effectiveness. ICI appears safe to HIV patients, and transplanting organs can respond to ICI but may suffer from transplant loss. Among the treatments for melanoma, Ipilimumab is a designed antibody of the IgG1 isotype, which binds with CTLA-4 and modulates antitumor immunity with TCR expression. Ipilimumab can increase the percentage of patients able to achieve tumor regression and control of tumors from 10% to almost 50% [10]. The combination of ipilimumab and other chemicals is tested. Patients treated with the combination of ipilimumab and dacarbazine had a longer overall survival time than those who received a placebo and dacarbazine [11]. The nivolumab-plus-ipilimumab group got overall higher survival than the alone nivolumab group under a minimum 60 months trial [12]. The combination of ipilimumab and nivolumab is better than chemotherapy in treating non-small cell lung cancer (NSCLC).22 Another CTLA-4 inhibitor, tremelimumab, is a designed monoclonal antibody of IgG2 isotype. Unlike ipilimumab, tremelimumab does not show significant results in clinical trials about melanoma treatment and takes a chance on other cancers. Also, PD-1 inhibitors serve as accompanying therapy in the high-risk stage of melanoma.

However, there is still room for improvement since there are no biomarkers that are sensitive to checkpoint inhibitors and give feedback in leukemia treatment. At the same time, there are some perceived disadvantages of immune inhibitors, like autoimmune toxicities they induce. The toxicities can affect the human organ system and induce autoimmune diseases.

4. Cytokines

The receptor-expressed high-affinity binding to the surface of cytokine can cause an intracellular signaling cascade to change the response [13]. Cytokines transmit the intracellular signs that perform autocrine and paracrine actions. Cytokines have been categorized into type one cytokines and type two cytokines. The Janus Kinase-signal transducer and activator of transcription (JAK-STAT) pathway is one of the signaling channels used by cytokines, and it is a quick mechanism to transfer external stimulation from the cytosol to the nucleus [14]. Ligands and receptors are involved in JAK-STAT signaling. The JAK family is made up of the four proteins JAK-1, JAK-2, JAK-3, and JAK-4. STAT1, STAT2, STAT3, STAT4, STAT5A, STAT5B, and STAT6 are the seven STATs that have been identified in humans. When cytokines engage with the receptor, dimerization occurs, which recruits the JAK. The JAK is further activated by cytokine stimulation, which also creates the docking site via tyrosine-phosphorylating STATs. The STAT will dispatch from the receptor and form the homodimers and heterodimers. Then, STAT will translocate the nucleus in order to trigger the activation or suppression of transcriptional genes in the nucleus. These have a tyrosine residue, a

DNA-binding domain, a Trans-Activation Domain (TAD), and the N-terminal FERM domain, which is crucial for protein-protein interactions [15].

T cells secrets the interferons and interleukins to adjust the immune response toward the presented antigen. These cytokines produced by T cell work in different ways: enhance the stimulatory factor, suppress the growth of cancer cells, and consolidate the immune system. Single and combination of pro-inflammatory cytokines are candidates for cancer immunotherapy, and they can enhance the activity of antigen-priming, which is the first contact between antigen and antigen-specific helper T cells.

Interferons (INF) have anti-viral properties by inhibiting the transcription of the viral nucleic gene and can regulate cell growth. This cytokine can be exploited by its apoptotic enhanced and proliferative suppressed activities in the tumor cell. However, for its application in clinical, cytokines treatment is reduced use because of its safety and effectiveness [13].

Interleukins (ILs) are categorized into interleukin-1 alpha and -1beta, responsible for immune responses, such as lymphocyte activation and growth of T helper cells. IL-2 can promote the separation of natural killer (NK) cells and T lymphocytes. This implies that using IL-2 creates intense lymphocyte cultures and enhances other cells' lasting periods.24 Interleukin-3 stimulates the growth and differentiation of stem cells which have a variety of potentials in development.

IL-4 regulates the Th-2 immune responses and is founded in immunological cells such as mast cells, NK, and innate-like skin keratinocytes. Like IL-4, IL-13 can increase the level of MHC cells and co-stimulatory signal in T cell activation, but IL-13 does not regulate the Th-2 responses.

5. Cancer Vaccine

Cancer vaccine aims to enhance the effectiveness and time of duration of immune responses [16]. Cancer vaccines are categorized into predefined vaccines in which the antigen needs to be determined based on tumor biopsy and analysis of common shapes depending on previous cases and anonymous antigen vaccines. In the anonymous antigen category, cases are divided based on the question: are antigens loaded onto APCs outside the body? Moreover, with the predefined antigen, the personalized vaccine, based on the patient’s mutation, only occurred in a specific person, and the standard vaccine, in which the patient shared a common mutation in many other patients [17]. Optimal cancer vaccines can send antigens to different classes of DCs and induce the stimulatory ligands (CD4 and CD8) in T cell activation. The antigen in the vaccine can be selected from mutant sequences, a typical cancer antigen, and an antigen that can cause infection. T cells can recognize these antigens quickly because of the tolerance and memory mechanism established the first time the cancer cell was first presented [18].

6. Conclusion

Cancer mentioned above immunotherapies are all designed to control tumor growth by enhancing the immune strength and duration of one's anti-tumor. Immune checkpoint inhibitor ultimately promotes anti-tumor activity by matching ligands, thereby blocking the binding of ligands to the DC family. The JAK-STAT pathway is the primary channel through which cytokines communicate with the nucleus from the cytoplasm. Interferons and Interleukins secreted by t cells promote the production of the stimulatory factor for t cell activation and stabilize the immune system. Cancer vaccine works by exposing defined or personalized antigens to the DC family and causing further t cell activation. Although the objectives are the same, different treatments face different difficulties. ICI is currently the most promising in terms of its effectiveness, practicability, and collateral benefits for cancer treatment.

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