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PD-1, PD-L1 mechanism and cancer treatment

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ABSTRACT

In response to a foreign organism or material, our bodies react with a variety of immunological agents. This response mechanism is called immunogenicity. While some of the proteins that regulate the immune response function to activate this response, some of them are responsible for inhibiting this response. The main "brake" proteins involved in this negative regulation are cytotoxic T-lymphocyte-associated protein-4, programmed cell death protein-1 (PD-1), T-cell immunoglobulin and mucin domain-containing protein-3, and lymphocyte-activation gene-3. The cell can differentiate endogenous and exogenous substances through these brakes and other signal pathways. In the presence of brake proteins, the tumor cells are not perceived as a threat by the immune system, so a corresponding response does not occur. To generate this response, drugs containing monoclonal antibodies are produced for use in cancer treatments. Monoclonal antibodies are designed to block braking processes while also eliciting an immunological response. In this review, the PD-1 and programmed cell death-ligand 1 pathway and cancer immunotherapy are mentioned.

Keywords: Cancer immunotherapy, immune checkpoint, monoclonal antibody, PD-1 inhibition, PD-L1 inhibition.

IMMUNE CHECKPOINTS

The proliferating tumor cells encounter various cell types involved in the immune system, and as a result of this encounter, the immune system creates a response. Receptors that activate and inhibit T cells regulate the balance between immune response and immune tolerance. T lymphocytes are divided into CD4+ (helper) and CD8+ (cytotoxic) T lymphocytes. For the immature CD8+ T (naive) cell to become active, a peptide presented by the T-cell receptor complex (TCR) and another by the major histocompatibility complex (MHC) must be combined, followed by secondary stimulation.[1] Self and foreign antigens in the body are presented to T cells by antigen-presenting cells (APCs). The antigen presented to T cells by the MHC is recognized by the TCR and the first signal is formed. Afterward, a secondary signal is required for T cells to complete the activation process. The most important secondary stimulus of CD8+ T cell precursors is the interaction of CD28 on the T lymphocyte surface with CD80/CD86 (B7 family molecules) on the APC.[1,2] Thus, the T cell passes from the naive form to the active form. CD80/CD86 is also the target site for cytotoxic T-lymphocyte-associated protein-4 (CTLA-4). If CTLA-4 binds with CD80/CD86, it cannot complete T cell activation, and subsequently, immune response is inhibited. In order to be active for the other immune checkpoint, programmed cell death protein-1 (PD-1) must combine with its ligands programmed cell death-ligand 1 (PD-L1) and programmed cell death-ligand-2 (PD-L2).[1,3-5] While PD-1 binding occurs only in tumor cells, CTLA-4 binding also occurs between T cells and other normal cells in the body. Unlike CTLA-4, the direct interaction of PD-1 with cancer cells is associated with the potency of PD-1 inhibitors to be more potent

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and less toxic. In addition, the different immune control points act through the inhibition of two or more control points in combination as a new strategy in the treatment of various diseases, especially cancer.[6] Allison[7] found that the CTLA-4 molecule acts as a brake on T cells in the 1990s and designed an antibody to eliminate this brake. In this study, tumor transplanted mice were treated with monoclonal antibodies against CTLA-4. This treatment designed for the immune system has been found to be curative in mice with tumors. Consequently, a number of studies were conducted, including studies on prostate cancer, breast cancer, skin cancer (malignant melanoma) samples, and animal tumor models.[8-10] Treatment with ipilimumab was successful in a clinical study conducted on malignant melanoma patients in 2010. Thus, ipilimumab, an anti-CTLA-4, immunoglobulin (Ig) G1 monoclonal antibody, was approved by the Food and Drug Administration (FDA) and European Medicines Agency (EMA) in 2011.[11] Ishida et al.[12] defined the PD-1 molecule and its functions in 1992 and showed that PD-1 also acts as a brake like CTLA-4 but works through different mechanisms. It has been shown in clinical studies that drugs providing PD-1 inhibition have positive results in the treatment of cancer, thus providing long-term treatment options in cancer patients with metastases, who previously could not be treated. Therefore, nivolumab, an antibody-based treatment used today, has been developed.[13] In 2018, Allison and Honjo[14] were awarded the Nobel Prize in Physiology or Medicine due to their work in the field of "discoveries in cancer treatment through suppression of the negative regulation of immunity."[6]

DEFINING CTLA-4 AS A NEGATIVE REGULATOR

Cytotoxic T-lymphocyte-associated protein-4 is a molecule that binds to CD80/CD86 with a higher affinity than CD28.[15] It is known that CD28-associated CTLA-4 protein is found inside non-activated T cells but is rapidly transferred to the membrane after activation.[16]

In a study on CTLA-4 gene inactivation in mice, it was confirmed that CTLA-4 is a negative regulator, since these mice develop very severe autoimmune disease.[17] In 1999, an anti-CTLA-4 IgG1 monoclonal antibody named MDX-010 was developed and later named ipilimumab.[18,19]

Defining LAG-3 as a negative regulator

Lymphocyte-activation gene-3 (LAG-3 or CD223) is a cell surface molecule expressed on the surface of activated T cells, natural killer (NK) cells, B cells, and plasmacytoid dendritic cells.[20-24] The interaction between LAG-3 and its major ligand, MHC class II molecule, plays an important role in regulating dendritic cell function.[24,25] In addition, LAG-3 has been shown to have various roles on CD8+ T cell exhaustion.[26] Inhibition of the interaction between LAG-3 and MHC class II with the LAG-3 Ig fusion protein continues to be applied as a therapy in many cancer patients.

Defining TIM-3 as negative regulator

T-cell immunoglobulin and mucin domain-containing protein (TIM)-3 is a member of the TIM gene family, which includes TIM-1, TIM-3, and TIM-4 in humans, and it is expressed particularly on the surface of cells belonging to the myeloid family of T helper (Th) 1, Th17, and CD8+ T cells and in mice.[27-29] T-cell immunoglobulin and mucin domain-containing protein-3 and its ligands have been found to suppress Th1 and Th17 responses.[30] It has four different ligands shown to interact with the immunoglobulin variable (IgV) domain.[31] These are galectin-9 (Gal-9), high mobility group protein B1 (HMGB1), carcinoembryonic antigen cell adhesion molecule-1 (Ceacam-1), and phosphatidylserine (PtdSer).[32] Antibodies inhibiting TIM-3 have been shown to exacerbate experimental autoimmune encephalomyelitis (EAE) in a mouse model for human multiple sclerosis (MS).[29] This shows that TIM-3 has a negative regulatory role.

Discovery of the PD-1 receptor

Programmed cell death protein-1 is a 55 kDa transmembrane protein containing an immunoreceptor tyrosine-based inhibition motif (ITIM), first described by Ishida et al.[12] in 1992.[33] Ishida et al.[12] identified two ligands of PD-1 to characterize the function of PD-1. In a later study, they analyzed signal transduction and examined the pathophysiology of autoimmune diseases in PD-1 knockout mice.[33]

The most prominent feature of autoimmune diseases observed in PD-1 knockout mice is the variation in symptoms between strains. Mice in the C57BL strain developed lupus-like arthritis and glomerulonephritis.[2,33] In contrast, mice in the BALB strain produced autoantibodies against cardiac troponin I and developed dilated cardiomyopathy.[2,33-35] In addition, mice in the non-obese diabetic (NOD) strain developed type 1 diabetes as a result of extensive destruction of the islets of Langerhans.[36] From these observations, it was revealed that PD-1 provides negative costimulation against lymphocytes critical for establishing or maintaining self-tolerance.

The occurrence of autoimmune diseases in PD-1 knockout mice suggests that blocking the PD-1/PD-L1 pathway will facilitate the recognition of cancer cells as foreign. One study showed that tumor cells in mice express PD-L1 to escape the immune system by inhibiting T cell activity.[36] Similarly, it has been observed that cancer patients who express high levels of PD-L1 in cancer cells have a worse prognosis.[37]

PD-1 LIGANDS

Programmed cell death-ligand 1 and PD-L2 are type 1 transmembrane proteins with regions similar to IgV and immunoglobulin constant (IgC) in their extracellular regions.[2] Programmed cell death-ligand 1 and PD-L2 were discovered in 2000 and 2001 as ligands of PD-1.[4,5] Programmed cell death-ligand 1 is expressed in both lymphoid and non-lymphoid tissues. In particular, it shows up-regulation in the activation of APCs such as dendritic cells, macrophages, and B cells.[2,38,39] Programmed cell death protein-1 is also expressed in activated T cells. It is upregulated by interferon (IFN)-γ and other inflammatory cytokines secreted by activated T cells.[36,40,41] In addition, PD-L1 is expressed in a variety of tumor cells and virus-infected cells. Programmed cell death-ligand 1 expression in target cells enables PD-1 to directly inhibit the T cell response against the target cell.[42] Unlike PD-L1, PD-L2 is expressed only in APCs such as dendritic cells and macrophages.[39]

PD-1 INHIBITION MECHANISM

Programmed cell death protein-1 is mainly expressed on activated CD4+ and CD8+ T cells and B cells.[2,12,43] Inhibition occurs by binding with the PD-1 ligand PD-L1. The expression of PD-1 induced by activation regulates the late-phase immune response (memory response, chronic infection, etc.) in peripheral tissues while regulating early stimulation in lymphoid organs. The extracellular domain of PD-1 consists of a single IgV region-like fragment, 20-amino acid stem, transmembrane fragment, and cytoplasmic tail, and the cytoplasmic tail consists of an ITIM and an immunoreceptor tyrosine-based switch motif (ITSM).[2] Phosphorylated tyrosines on ITIM and ITSM bind to Src homology protein (SHP), a phosphatase. The main purpose of SHPs is to suppress T cell receptor signals by blocking intermediate molecules during T cell activation.[1] While ITIM binds to SHP-2 only, ITSM binds to both SHP-1 and SHP-2. PD-1/PD-L1 pathway inhibition loses its function in the presence of ITSM mutation.[30,31,44,45] When PD-1 binds to its ligands, PD-L1 and PD-L2, it suppresses T cell activation by recombining with SHP-2, which dephosphorylates and inactivates ZAP-70, an important integrator of TCR-mediated signaling.[45-47] As a result, PD-1 prevents responses such as T cell proliferation, IFN production, and cytotoxic activity. The main target of the PD-1 pathway is the PI3K/Akt/mTOR pathway, which is involved in cell survival, development, and proliferation.[1]

Under normal conditions, protein tyrosine phosphatase and tensin homolog (PTEN) is inactivated when phosphorylated, while dephosphorylated it is activated and inhibits the PI3K/Akt/mTOR pathway.[1] Another important pathway targeted by PD-1 is the Ras/MEK/ERK pathway. It inhibits this pathway, which affects cell proliferation, cell division, and differentiation, and enables the release of interleukin (IL)-2, an inflammatory cytokine.[48]

Two transcription factor binding sites in the promoter region of the programmed cell death 1 (PDCD1) gene are important in regulating PD-1 expression. T-cell receptor complex-mediated calcium influx in undeveloped T cells initiates transcription of PDCD1 by activation of NFATc1, which binds to the 5' promoter region of the PDCD1 gene.[49] On the other hand, IFN-α and interferon regulatory factor 9 (IRF9) in chronically activated T cells cause long-term transcription of PDCD1 by binding to the PDCD1 promoter.[50]

In addition, the PDCD1 promoter region becomes demethylated during chronic infection, causing high PD-1 expression in fatigued CD8+ T cells.[51] Fatigued CD8s+ T cells express high amounts of Eomesodermin (EOMES), a protein regulated by the FoxO1 transcription factor. The FoxO1 also binds to the PDCD1 promoter and enhances PD-1 expression.[52,53]

TREATMENT METHODS USED IN CANCER IMMUNOTHERAPY

Two different approaches have been developed to activate the immune system against cancer. The first is antigen-specific immunotherapy or therapeutic cancer vaccines, and the second is non-antigen-specific immune system modulation or immune checkpoint inhibition.[54] Immune checkpoint inhibitors are currently used in the treatment of many solid tumors such as malignant melanoma, renal cell cancer, and urothelial cancer.

Among other immunotherapeutic methods, monoclonal antibodies have the most clinical studies and are the most validated ones.[55,56] The working principle of monoclonal antibodies is that they activate the immunity by connecting with receptors on the cell surface. This activity varies: While the anti-CD20 (B lymphocyte antigen) monoclonal antibody induces apoptosis, the epidermal growth factor receptor (EGFR) binding antibody blocks the receptors by preventing the binding of naturally occurring ligands.[56]

In the adaptive immunotherapy method, immunologically active cells are given to patients, and T cells are used in this treatment method. These cells are tumor-infiltrating lymphocytes, T cells engineered for cancer-specific TCR expression, and T cells engineered for chimeric antigen receptor expression.[56]

In the formation of antibodies against the antigen in the tumor vaccines, it is aimed to activate CD4+ helper and CD8+ cytotoxic T cells.[54,57] An ideal tumor antigen should be expressed in an excessive amount and only on tumor cells, and the expression of the antigen should continue during the progression of the disease. Vaccines are developed on antigenic targets such as a recombinant protein, peptide, ganglioside, or tumor cell and in combination with an adjuvant that enhances the immune response.[54]

IMMUNOMODULATORY THERAPIES IN NON-SMALL CELL LUNG CANCER

Non-small-cell lung carcinoma (NSCLC) is one of the leading causes of cancer-related death in the world. Five-year survival rates of NSCLC cases are less than 20% despite chemotherapy and targeted treatments.[54,58] Nivolumab and pembrolizumab, which are PD-1 antibodies, and atezolizumab, durvalumab, and avelumab, which are among the PD-L1 antibodies, are agents that have been shown to be effective in different stages of NSCLC. Currently, three immunotherapy agents, approved by the FDA for use in patients with advanced-stage NSCLC, are nivolumab, pembrolizumab, and atezolizumab. All three agents have shown an overall survival (OS) advantage over docetaxel, a chemotherapy drug.[54,59-62]

Nivolumab is an IgG4 type anti-PD1 monoclonal antibody of human origin. Nivolumab's efficacy was investigated in phase III studies after 17.6% response rates, and 42% OS rates for one year were reported in phase I studies with nivolumab in intensively treated non-squamous NSCLC cases. As a result, nivolumab was shown to be more effective than docetaxel.[54,63]

Pembrolizumab is a human IgG4 type monoclonal antibody developed against PD-1. In a study; in a group that had previously received platinum-based chemotherapy and tumor PD-L1 expression greater than 50%, regard to their OS results, it was approved by the FDA.[54,64] In another subsequent study, an advantage over docetaxel on OS was demonstrated in NSCLC cases who had previously received platinum-based chemotherapy and had PD-L1 expression of more than 1%.[65] The survival advantage is particularly pronounced in the group with PD-L1 expression greater than 50%, and the risk of death was reduced by approximately 50% with pembrolizumab.[54]

Atezolizumab is an IgG1 type anti-PD-L1 monoclonal antibody of human origin. The approval of atezolizumab for use in NSCLC cases is based on the results of an international phase III study.[62] The use of atezolizumab compared with

docetaxel has been shown to provide a significant survival advantage in previously treated NSCLC patients.

METASTATIC RENAL CELL CARCINOMA TREATMENT WITH THE NIVOLUMAB AND IPILIMUMAB COMBINATION

Renal cell carcinoma (RCC) accounts for 2.4% of cancer cases worldwide, with 338,000 new diagnoses each year.[65,66] At the time of diagnosis, 25 to 30% of patients show metastatic disease associated with high mortality.[65,67,68] The combined use of immune checkpoint inhibitors results in an increased anti-tumor activity in tumor types such as melanoma compared to monotherapy.[69]

In the reference study, different doses of anti-PD-1 monoclonal antibody nivolumab and anti-CTLA-4 monoclonal antibody ipilimumab were used. A study was designed to find out both combination efficacy and which pathway inhibition is more effective. Nivolumab and ipilimumab doses were equal in the first group of the study, the dose of nivolumab was higher in the second group, and the dose of ipilimumab was higher in the third group.[65] Less adverse events (AEs) were encountered in the arm where nivolumab was used more than ipilimumab, and fewer patients required immune-modulating medication to cope with the side effects of AEs (mainly in the skin, endocrine and gastrointestinal system).[65]

PROGRESSIVE MULTIFOCAL LEUKOENCEPHALOPATHY TREATMENT WITH PEMBROLIZUMAB

Progressive multifocal leukoencephalopathy (PML) is a brain infection caused by the human polyomavirus-2 (JC virus) and is fatal unless the immune system is repaired.[70] The virus is estimated to be latent in the kidneys of more than 50% of healthy adults. It can transform into a neurotropic virus by undergoing genetic rearrangements in non-coding regions, thus infecting glia cells and causing PML.[70,71]

Programmed cell death protein-1 expression is upregulated in CD4+ and CD8+ T cells of PML patients.[70,72] An autopsy revealed increased PD-1 and PD-L1 expression in PML lesions. Pembrolizumab negatively regulates PD-1 expression in peripheral blood and cerebrospinal fluid. Programmed cell death protein-1 inhibition reactivates antitumor immune activity against various types of cancer.[70,73]

Findings suggest that pembrolizumab decreases JC viral load and increases CD4+ and CD8+ activity against the JC virus in some PML patients. In addition, magnetic resonance imaging showed that the size of PML lesions decreased.[70]

In conclusion, while some of the proteins activate the immune response, others are responsible for inhibiting this response. The main brake proteins involved in this negative regulation are CTLA-4, PD-1, TIM-3, and LAG-3. The cell can differentiate endogenous and exogenous substances through these brake proteins. Monoclonal antibodies are used to inhibit negative regulation in the treatment of various diseases. The main monoclonal antibodies that inhibit the PD-1/PD-L1 pathway are nivolumab, pembrolizumab, atezolizumab, durvalumab, and avelumab. Recently, it has been proven that cancer immunotherapy based on PD-1 and PD-L1 is effective in generating an antitumor immune response with less toxicity in many tumor types. As a result of studies on melanoma and other diseases, it has been found that treatment with a combination of monoclonal antibodies is more successful than monotherapy.

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