Journal of Experimental and Basic Medical Sciences 2023;4(3):187-191

Review

Regulatory T Cells and Their Implications in Cancer Immunotherapy

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ABSTRACT

The regulatory T (Treg) cells, an essential cell type in the immune system, control various immune responses, including autoimmune diseases, inflammatory conditions, and cancer. These cells, preventing the development of abnormal immune responses against self-antigens, have complex roles in cancer. Treg cells are abundant in the tumor microenvironment, supporting progression by suppressing anti-tumor immune responses. Ongoing research explores several cancer immunotherapy strategies, including methods aiming to deplete Treg cells. However, the depletion of Treg cells may lead to autoimmune-related adverse events, and investigations into this phenomenon are ongoing. This review examines several strategies developed for cancer immunotherapy.

Keywords: Cancer, immune response, immunotherapy, regulatory T cells, Treg cells

The common goal of all living organisms is to sustain life. The human body serves as a host for many organisms to perpetuate their generations.[1] Consequently, the human body is constantly exposed to invasion by external factors. A defense system is necessary to counteract these threats.

The immune system is a complex system that detects various foreign pathogens and develops different responses to defend the organism against these pathogens. Additionally, the immune system has developed various effector mechanisms to prevent autoimmunity and assist in tissue repair. It can activate many protective mechanisms against inflammatory diseases, tissue dysfunction and destruction, inflammation, and cancer. At the center of these mechanisms performed to protect the host are $CD4^+$ regulatory T (Treg) cells.[2]

Treg cells express high levels of CD25 together with the high-affinity interleukin (IL)-2 receptor alpha (α) chain and represent a subset of $CD4^+$ T cells.[3] They play a crucial role in maintaining immunological self-tolerance and immune homeostasis.[4]

The regulatory functions of Treg cells also impact various other immune cells, including dendritic cells (DCs), macrophages, gamma/delta (γδ) T cells, neutrophils, and natural killer (NK) cells. Therefore, preserving a balanced and functional Treg population is crucial for preventing both autoimmune and chronic inflammatory diseases. Treg cells suppress abnormal immune responses against self-antigens and contribute to preventing autoimmune diseases.[5] However, their limiting roles in immune responses against self-antigens can also lead to adverse outcomes.

Treg cells are characterized by the expression of the transcription factor forkhead box protein 3 (FoxP3), also known as Scurfin.[6] According to research, FoxP3 is the best marker for $CD4^+$ Treg cells in both mice and humans, although there are some differences between FoxP3 in humans and mice. Human FoxP3 has two isoforms, one encoding the full-length FoxP3 and the other encoding a short version of the protein lacking exon 2. However, the short form described in human FoxP3 is not present in murine $CD4^+$ Treg cells. Another difference between human and mouse FoxP3 is that in human $CD4^+$ T cells, FoxP3 expression

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Cite this article as: Gemici SS, Erbaş O. Regulatory T Cells and Their Implications in Cancer Immunotherapy. JEB Med Sci 2023;4(3):187-191.

doi: 10.5606/jebms.2023.1064

Received : October 26, 2023
Accepted : November 21, 2023
Published online : December 25, 2023

is dependent on T-cell receptor-mediated activation.[7] The FoxP3 regulates the differentiation and functions of Treg cells. In humans, any mutation in the FoxP3 gene results in an X-linked disorder characterized by severe allergies and excessive inflammation. Therefore, the FoxP3 transcription factor is considered crucial for the development of Treg cells. It is known that Treg cells maintain peripheral tolerance.[8]

There are several subgroups of Treg cells that suppress lymphocyte activity specific to tumor-associated antigens and tumor eradication.[7,9] Some of these subgroups include naturally occurring $CD4^+$ $CD25^+$ Treg cells, adaptively induced $CD4^+$ Treg cells, $CD8^+$ Treg cells, and natural killer T cells (NKT) cells.[7] However, Treg cells are generally studied in two main subgroups: natural Treg cells (nTreg) and adaptive or inducible Treg (iTreg) cells. The exact differences between these two types of Treg cells are not fully understood. It is observed that nTreg cells play an important role in recognizing self-antigens, while iTreg cells are involved in creating tolerance to non-self antigens, such as antigens from certain bacterial species, which are harmless.[10] Additionally, nTreg cells inhibit the function and maturation of NK cells, NKT, and DCs. On the other hand, the proliferation of B cells, immunoglobulin production, and class switching of the produced Igs can be suppressed by nTreg cells, partially mediated by the secretion of transforming growth factor-beta (TGF-β).[11]

REGULATORY T CELLS AND CANCER

The effects of Treg cells on cancer are complex. However, numerous studies have reported that the infiltration of Treg cells into various tumor tissues supports tumor progression by limiting anti-tumor immunity and promoting tumor immune escape.[12] Treg cells normally constitute approximately 4% of $CD4^+$ T cells. However, they can make up 20-30% of the total $CD4^+$ population around the tumor microenvironment.[9]

According to examinations conducted in most cancer cases, the identified Treg cells belong to the $CD4^+$ $CD25^+$ Treg cell population. These cells are highly proficient in suppressing immune responses in *in vitro* functional experiments.[7] The infiltration of a large number of Treg cells into tumor tissues is generally associated with a poor prognosis in many cancer types, including melanoma, non-small cell lung, gastric, hepatocellular, pancreatic, renal cell, breast, and cervical cancers.[5] At the same time, Treg cells in the tumor microenvironment encompass heterogeneous cell subsets expressing different immunosuppressive molecules that support tumor progression. In other words, Treg cells induce an immunosuppressive microenvironment, which is a significant barrier to successful tumor immunotherapy.[13,14] However, this situation varies in some cancer types.[15]

In certain cancers such as colorectal carcinoma, Treg cells suppress bacteria-induced inflammation, promoting carcinogenesis and creating a beneficial condition for the host. Here, a high level of Treg cells is associated with a positive prognosis. It is believed that this association arises from the known ability of Treg cells to suppress general inflammation, triggering cell proliferation and metastasis.[9] Therefore, the direct correlation between a high percentage of Treg cells and patient survival prognosis requires further investigation. The accumulation of Treg cells in the tumor microenvironment and the role of antigen specificity in this process are crucial aspects to consider.[7] The dual role of Treg cells in cancer and their role in cancer development significantly depends on both the type and location of the tumor.

Increase of Treg Cells in the Tumor Microenvironment

The infiltration of Treg cells into the tumor microenvironment occurs through chemokines. For example, in breast cancer, this process involves the binding of the chemokine receptor CCR4 expressed by Treg cells to the ligand CCL22 secreted by many tumor cell types.[16,17]

Transforming growth factor-beta, a cytokine generally produced by tumor cells induces the differentiation and activation of Treg cells. Additionally, IL-10 also plays a role in the induction and differentiation of Treg cells. The TGF-β has a significant role in the induction and maintenance of FoxP3 expression, suppressive function, and homeostasis in peripheral $CD4^+$ $CD25^+$ Treg cells.[17,18]

Tumor cells not only produce IL-10 or TGF-β but also induce the secretion of TGF-β/IL-10 by immature myeloid DCs or immature myeloid suppressor cells. This leads to the formation of $CD4^+$ $CD25^+$ Treg cells.[18]

At the same time, it is thought that some naturally occurring $CD4^+$ $CD25^+$ Treg cells directly produced by the thymus may have cross-reactivity with antigens expressed by cancer cells. As a result, these types of Treg cells accumulate in tumor areas.[17] These cells are significantly different from conventional T cells that penetrate the tumor. However, there is still no

definitive information on whether these antigens are recognized only by these Treg cells or if recognition is shared by helper $CD4^+$ T cells.[5] Additionally, another reason for the high percentage of Treg cells in the tumor microenvironment is speculated to be the transformation of peripheral $CD4^+$ $CD25^-$ naive T cells into Treg cells.[17]

TARGETING TREG CELLS IN CANCER IMMUNOTHERAPY

Evading the immune system is a distinctive feature of cancer cells. Strategies are being developed to prevent this phenomenon and to eliminate tumor cells. Treg cells play a crucial role in shaping the tumor microenvironment to prevent optimal function of effector cells, prompting increased research on the manipulation of Treg cell reduction or suppressive functions.[9] Studies indicate that systematic depletion of Treg cells enhances the anti-tumor response.[13] However, systemic depletion of Treg cells can lead to autoimmunity. Consequently, immune system cells recognize their own self-antigens as foreign, activating an immune response against these antigens. This process results in the elimination of self-antigens, leading to the development of autoimmune diseases.

With the implementation of specifically targeting Treg cells by administering CD25 monoclonal antibody (mAb), the immunological unresponsiveness against tumor cells has been eliminated.[18] Simultaneously, it induced the spontaneous development of tumor-specific $CD8^+$ effector T cells and NK cells. However, the depletion of Treg cells has led to cross-reactive tumor immunity against tumors of various origins, and independent of this depletion, the application of CD25 mAb has not resulted in any tumor regression. Following the administration of this process, the number of Treg cells tends to increase over time, and the capacity to generate an anti-tumor response gradually diminishes.

Various cell surface molecules, including chemokine receptors such as CCR4 specifically expressed by effector Treg cells, can be candidates for consumption by monoclonal antibodies that specifically target effector Treg cells.[13] An important application in immunotherapy involves the use of immune checkpoint inhibitors (ICI), which are antagonistic antibodies blocking crucial immune regulatory molecules (checkpoint molecules) such as cytotoxic T lymphocyte-associated antigen 4 (CTLA-4).[10] It is known that human CTLA-4 antibodies provide long-term protection against melanoma. These antibodies are believed to block negative signals from B7-CTLA-4 interactions to increase the activation of naive T cells, thereby leading to tumor rejection. Additionally, anti-CTLA-4 antibodies selectively induce the depletion of Treg cells in the tumor microenvironment. Therefore, anti-CTLA-4 antibodies induce tumor rejection. The first anti-CTLA-4 antibody for melanoma treatment, Ipilimumab, has shown significant and lasting effects. According to the checkpoint blockade hypothesis, anti-CTLA-4 antibodies block the B7-CTLA-4 signal in peripheral lymphoid organs, supporting the activation of naive T cells and resulting in tumor rejection. However, the accuracy of this hypothesis has not been proven.[19] The B7 ligands are common stimulatory molecules expressed on the cell surface, and therefore, it is recommended to conduct studies on whether Ipilimumab effectively blocks B7-CTLA-4 interactions under physiological conditions.

As a result of studies on this subject, it has been found that blocking the B7-CTLA-4 interaction is unnecessary for the immunotherapeutic effect of anti-CTLA-4 antibodies. It has been noted that the idea that anti-CTLA-4 antibodies induce tumor rejection by promoting T cell activation in lymphoid organs is not accurate. As studies continue, it has been observed that mouse anti-CTLA-4 antibodies induce tumor rejection through the selective depletion of Treg cells in the tumor microenvironment. The results obtained with mouse anti-CTLA-4 antibodies are similar to the results obtained with human anti-CTLA-4 antibodies.[16-19]

An important barrier to the effectiveness of ICI immunotherapy is tumor resistance due to the immunosuppressive nature of the tumor microenvironment, and Treg cells are among the most abundant suppressive cells in the tumor microenvironment. The primary reason for Treg cells representing a direct target of ICI immunotherapy is the abundant expression of checkpoint molecules such as CTLA4, PD1, and LAG3.[18] With the widespread use of ICI for cancer treatment, an increase in immune-related adverse events that pose a serious threat is likely. This challenge has prompted the consideration of ways to maintain immune homeostasis after ICI therapy. Therefore, understanding the role of Treg cells is crucial for triggering an effective anti-tumor response while maintaining immune homeostasis, and ongoing research aims to achieve this goal.[20-23]

Efforts in cancer immunotherapy are focused on eliminating the suppressive functions of Treg cells.

One approach to this issue involves targeting $CD25^+$ Treg cells with a specific antibody or ONTAK (an IL-2 toxin fusion protein). However, this approach may not efficiently eliminate Treg cells or may result in the removal of both Treg cells and activated effector cells. The reason for this is that the CD25 marker is not specific to Treg cells; it is also positive in all activated T cells.[7] Therefore, a more specific method is needed.

Targeting a subset of highly suppressive effector Treg cells based on specific molecules uniquely upregulated in response to tumor antigens in the tumor microenvironment is considered a viable option for cancer immunotherapy. Studies have tested the impact of anti-CCR4 antibodies on subsets of human Treg cells in melanoma patients.[3] As a result of this process, it was observed that effector Treg cells expressing CCR4 were selectively eliminated, while pure Treg cells were preserved. Research on this topic is ongoing.

In conclusion, the results obtained from studies suggest that Treg cells in the tumor microenvironment inhibit anti-tumor immunity, posing a significant obstacle to the development of effective treatment methods. Tumor cells not only recruit Treg cells to the tumor site but also induce the transformation of naïve or effector T cells into Treg cells through various cytokines mediated by the natural immune system. Particularly, a decrease in the ratio of $CD8^+$ T cells to Treg cells is associated with poor prognosis in various cancer types. To advance cancer immunotherapy, a thorough understanding of Treg cell biology in the context of tumor development and progression is essential. Identifying more specific cell surface markers is a crucial step. The mechanisms through which Treg cells contribute to the establishment of tumor-specific tolerance are becoming an increasingly important focus of research. Understanding Treg cells in depth is necessary to develop new strategies that intervene in the steps leading to Treg cell depletion or the functions they perform. One of the most critical issues in cancer immunotherapy is the autoimmune-related adverse events resulting from the process of targeting Treg cells. This poses a challenging step in cancer treatment.

Declaration of conflicting interests

The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

Funding

The authors received no financial support for the research and/or authorship of this article.

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