Review

Advances in the Study on the Relationship between Regulatory T cells and Human Papilloma Viral Infection

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Abstract

Regulatory T cells (Treg cells) are a group of negative regulatory cells that include non-specific immune regulation CD4⁺ T cells. Treg cells inhibit the function of other immune cells. CD4⁺ CD25⁺ FOXP3⁺ is a Treg cell that is co-expressed by CD25 and FOXP3. The expression of Treg cells is up-regulated in the focal microenvironment and peripheral blood of patients infected with human papilloma virus (HPV). Further studies on Treg cells indicate that their potential clinical applications in the treatment of HPV infection.

In the 1970s, some scholars first proposed the concept of inhibitory T cells [1-2]. They speculated that inhibitory T cells might play an important role in the development and progression of tumors [3]. Subsequently, spleen-derived cells in tumor-bearing mice were found to inhibit anti-tumor immune function [4-6]. This behavior confirmed previous hypotheses about the existence and function of inhibitory T cells. In 1995, Sakaguchi [7] *et al.* proposed the concept of regulatory T cells (Treg cells), a group of negative regulatory cells that inhibits the function of other immune cells. T cells participate in the development and progression of various diseases, such as allergic reactions, infections, graft-versus-host disease, autoimmune disease, and tumors. Thus, CD4⁺ CD25⁺ FOXP3⁺ Treg cells became a research hotspot. Human papilloma virus (HPV), a double-stranded DNA virus, is a member of the A subgroup of papovaviridae and is an epithelial virus. The persistent infection of the cervix with high-risk HPV strains can lead to cervical intraepithelial neoplasia or cervical cancer, whereas infection with low-risk HPV strains can induce condyloma acuminatum [8]. The expression levels of CD4⁺ CD25⁺ FOXP3⁺ Treg cells increase in the focal microenvironment and peripheral blood of patients with HPV infection [9-10]. This paper provides a review of the relationship between the CD4⁺ CD25⁺ FOXP3⁺ Treg cells, and HPV infection to provide new ideas for HPV

treatment.

CD4⁺ CD25⁺ FOXP3⁺ Treg cells

Origin and Differentiation of CD4⁺ CD25⁺ FOXP3⁺ Treg cells Function of T Cells

T lymphocytes, or T cells, are the main effector cells of cellular immunity and are derived from the thymus. T cells are highly heterogeneous and can be classified into several subsets in accordance with different classification methods. The balance between T cells and their subsets is a prerequisite for the effectiveness of immune defense, stability, and surveillance. Collectively, all T cell subsets function in immunity [12].

Based on their activation stages, T cells can be classified into: 1. Initial T cells, which are mature T cells that have never been stimulated by antigens. These cells are in the G0 phase of the cell cycle and survive only for a short duration. Initial T cells, which primarily recognize antigens, are activated by the stimulation of pMHC (antigen peptide MHC molecule complex), expressed in the peripheral lymphoid organ, and differentiate into effector and memory T cells. 2. Effector T cells are the main T cells with immune function. These cells have a short survival time. Effector T cells migrate to peripheral inflammatory sites or some organ tissues and

are no longer circulated to lymph nodes. 3. Memory T cells, which may be differentiated by effector T cells or directly differentiated from initial T cells with antigenic stimulation. These cells have long survival durations that may last for years. Memory T cells can be activated quickly with the same antigenic stimulation, differentiate into effector cells, and mediate immune response again [13].

T and Treg Cells

T cells can be divided into helper, cytotoxic, and regulatory T cells on the basis of their functional characteristics. $CD_4^+ CD_{25}^+ FOXP_3^+$ Treg cells are the most common Treg cells. Treg cells function in immune disability and immunosuppression, and their interactions with T cells are reflected in: 1. Activated Treg cells inhibit the activation and proliferation of normal T cells. 2. Treg cells exert immunosuppressive effects to inhibit the expression of IL-12 by effector T cells and other cytokines. 3. Treg cells mediate the cracking of T cells or antigen-presenting cells (APCs) in the granzyme-B- or perforin- dependent manners of the target cells, thereby inhibiting immune response. 4. Treg cells negatively regulate APC by attenuating co-stimulatory signals and inhibiting antigen presentation [14].

$CD_4^+ CD_{25}^+$ Treg Differentiation

$CD_4^+ CD_{25}^+$ Treg differentiate in two ways: 1) During the maturation of immature T lymphocytes in thymocyte cells, CD_4^+ cells can be transformed into $CD_4^+ CD_{25}^+$ Treg cells via FOXP₃ expression. 2) When some naive $CD_4^+ CD_{25}^-$ T cells are stimulated by antigens, TGF- β induces FOXP₃ expression to form $CD_4^+ CD_{25}^+$ Treg cells. $CD_4^+ CD_{25}^+$ Treg cells that differentiate via this process are called induced regulatory T cells.

Based on the different degrees of CD_{25} expression and flow cytometry, CD_{4+} T cells can be divided into three groups of $CD_4^+ CD_{25}^-$, $CD_4^+ CD_{25}^{mid}$, and $CD_4^+ CD_{25}^{high}$ by flow cytometry. Nearly all $CD_4^+ CD_{25}^{high}$ express FOXP₃, whereas only some $CD_4^+ CD_{25}^{mid}$ cells express FOXP₃. Thus, the Treg cell phenotype is thought to be $CD_4^+ CD_{25}^+ FOXP_3^+$.

Main Regulatory Genes of $FOXP_3^+ CD_4^+ CD_{25}^+$ Treg Cells

FOXP₃, a member of the forkhead/winged helix transcription factor family, is a central transcription factor that regulates the formation and maintains the function of CD_4^+ T cells [16–17]. FOXP₃ also regulates Treg cells and is necessary for the differentiation and development of $CD_4^+ CD_{25}^+$ Treg cells in

the thymus and their expression levels in peripheral blood.

FOXP₃, a highly-conserved gene that belongs to the intracellular marker of Treg cells, is specifically expressed in $CD_4^+ CD_{25}^+$ Treg cells. The gene is not only a marker of the activation of $CD_4^+ CD_{25}^+$ Treg Cells, but also plays a role in the development and inhibitory function of Treg cells. The FOXP₃ gene has multiple CpG sites, and the corresponding methylation level is directly related to FOXP₃ expression. A number of transcription factor binding sites that are important for FOXP₃ expression are located in these methylated regions. FOXP₃ expression stabilizes only when the methylation site is de-methylated. Therefore, FOXP₃ methylation inhibits the differentiation of Treg cells [18–21].

Functional Characteristics of $CD_4^+ CD_{25}^+$ Treg Cells

$CD_4^+ CD_{25}^+$ Treg Cells are subsets of CD_4^+ T cells and play important roles in immune regulation. T cells regulate the immune function of the organism by controlling cell viabilities and B cell proliferation, as well as by inhibiting antibody production [22–24]. Treg cells inhibit immune function through various mechanisms, including [25] the destruction of cell metabolism, the regulation of antigen-presenting cell function, the inhibition of cytokine dependence, and the dissolution of cells. Treg cells can also inhibit immune responses to tumors, leading to tumor immune tolerance [26]. Treg cells in the autoimmune state and T cell cytokines are involved in the differentiation of benign and malignant tumors *in vivo*, as well as in the immune response rate of the tumor [27].

The Treg cells of FOXP₃⁺ enhance autoimmune tolerance and balance immune functions [28]. $CD_4^+ CD_{25}^+ FOXP_3^+$ Treg cells participate in the immune escape of various diseases, such as infectious diseases, allergic reactions, graft-versus-host disease, autoimmune diseases, asthma, and tumors [29]. Thus, $CD_4^+ CD_{25}^+ FOXP_3^+$ Treg cells are also cytokines that regulate immune suppression in cervical cancer and condyloma acuminatum.

Relationship between Regulatory T Cells and HPV Infection

At present, more than 100 types of HPV have been identified, 40 of which can damage the female genital tract, and at least 14 of which are associated with invasive cervical cancer. HPV infection can be detected in more than 75% of sexually

active women. The vast majority of infected women have no symptoms, whereas few have pathological or clinical impairment. HPV infection mainly includes condyloma acuminatum and cervical cancer.

Condyloma Acuminatum and Treg Cells

Condyloma acuminatum manifests as hyperplastic skin lesions proximal to the genitals and anus. Condyloma acuminatum is a common sexually transmitted disease that is easily transmitted, with rapid growth and recurrent episodes. HPV infection is the main cause of its pathogenesis and recurrence, with low-risk HPV subtypes including HPV-6, 11, 16, 18, 31, and 33, as the main cause. HPV typing is crucial in the clinical treatment and prognosis of condyloma acuminatum [30–31].

HPV exposure does not necessarily result in HPV infection. HPV infection is closely related to the immune function of the organism [9]. In a population with normal immune function, HPV infection will be cleared from 70% to 90% of sexually active adult women or young women after 12 to 30 months. By contrast, among women with immunosuppression or HIV infection, the risks of HPV infection and tumorigenesis increase; in addition, the duration of infection is prolonged [32–33]. The decline of the whole immune level, especially the cellular immunity level, is closely related to the development of condyloma acuminatum.

Immune function is closely related to the occurrence, development, and recurrence of condyloma acuminatum, as well as the elimination of HPV. Xu Yan *et al.* [34] detected $CD_4^+ CD_{25}^+ FOXP_3^+$ in the peripheral blood of patients with condyloma acuminatum and found that the numbers of $CD_4^+ CD_{25}^+$, $CD_4^+ FOXP_3^+$, and $CD_4^+ CD_{25}^+ FOXP_3^+$ cells are significantly higher than those in healthy individuals. The use of flow cytometry to detect $CD_4^+ CD_{25}^+ FOXP_3^+$ Treg cells in the peripheral blood of patients with condyloma acuminatum revealed that [35] the increase degree was closely related to the duration, initial onset, and recurrence of infection. HPV infection may cause the activation and proliferation of T cells, increase the $FOXP_3^+$ expression levels of transcription factors, and inhibit the antiviral immune response of the organism through various mechanisms. The presence of $CD_4^+ CD_{25}^+ FOXP_3^+$ and Treg cells in the microenvironment and peripheral blood of patients with condyloma acuminatum results in the immune escape of HPV and promotes the incidence of condyloma

acuminatum.

Cervical Cancer and Treg Cells

Cervical cancer is the most common cancer of the female reproductive system. Cervical cancer can be prevented given its clear etiology and perfected screening method [36]. Infection with high-risk HPV subtypes is the main cause of cervical cancer. These subtypes include HPV-16, 18, 31, 33, 35, 52, and 58. HPV-16 is the main subtype present in patients with cervical cancer and is present in more than 50% of all HPV cases [37]. When cervical cancer patients are infected with HPV, the E6 protein is highly expressed in cancer cells. The high expression of E6 inhibits p53, a tumor suppressor gene, thereby participating in and promoting the occurrence of cervical cancer [38].

In the United States, a study that screened for the high-risk factors (high-risk HPV-DNA) of cervical cancer found that HPV types in women over the age of 25 are mainly HPV-31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68; in addition, infection with HPV-16 and 18 are independent risk factors for cervical cancer. These results indicated that the pathogenesis of cervical cancer is closely related to HPV infection [39]. Austin *et al.* [40] studied and found that routine cytology and HPV joint testing provide strong and effective evidence for cervical cancer screening and cancer prevention.

The pathogenesis of cervical cancer is complex and involves immune escape [10]. Immune escape causes HPV to remain latent and undetected in the body for a long period. The up-regulation of Treg cells results in immunosuppression and the secretion of immunosuppressive factors [10]. This expression pattern is closely related to the FOXP3 gene and affects the development and function of Treg cells. FOXP3 is also expressed in cervical cancer cells. Its expression level is related to the stage of cervical cancer and the size of the tumor: the more advanced the stage of cervical cancer and the greater the tumor volume, the higher the expression of Treg cells [11].

Cellular Immune Mechanism of Condyloma Acuminatum and Cervical Cancer

Treg cells regulate immune function, thus playing a crucial role in the pathogenesis of condyloma acuminatum and cervical cancer. Treg cells, on the one hand, protect tissues

and cells from immune injury by inhibiting the production of antibodies or the cytotoxicity of cytotoxic T lymphocytes. On the other hand, Treg cells lead to the persistence of HPV infection and chronic disease.

Based on the clinical immunological characteristics of condyloma acuminatum, as well as the biological characteristics and functions of Treg cells, Treg cells may be involved in and become one of the most important causes that affect the low cellular immune function of patients with condyloma acuminatum. In the pathogenesis of condyloma acuminatum, the imbalance between T lymphocytes and its subpopulation causes the patient to suffer various viral infections, including HPV. In patients with HPV infection, the numbers of total and auxiliary T cells in peripheral blood decrease; inhibitory T cells increase; and the ratios of auxiliary T cells and inhibitory T cells are significantly lower than in healthy individuals. These results suggest that cellular immune function is inhibited [41].

In patients with cervical cancer, the immune functions of T lymphocytes, particularly cytokine secretion, are inhibited [42]. $CD_4^+ CD_{25}^{high} FOXP_3^+$ T reg cells are highly expressed in the peripheral blood of patients with persistent HPV infection and cervical carcinoma in situ. For the in vitro consumption of CD_{25}^+ T cells in HPV-16 patients with cervical cancer, the secretion of HPV-16, E6, and E7 antigen peptides is stimulated by IFN- γ . These factors then combine with the tumor suppressor genes p53 and Rb, resulting in abnormal cell cycle control and promoting carcinogenesis. Moreover, the increased number of Treg cells in the peripheral blood of patients with cervical cancer may be an important reason for the suppression of HPV-specific immunity [35]. More than 90% of cervical cancer patients are co-infected with HPV. In the presence of HPV, the combined effects of many factors undermine cervical epithelial cells and cause the imbalance of immune response. The expression levels of Th17 and TH1 cells in the peripheral blood and tumor tissues of patients with cervical cancer are negatively correlated: as the expression of Th17 cells increases, the expression of TH1 cells decreases. Th17 and Th1 cells are imbalanced in the tumor tissues of cervical cancer patients, thus inhibiting the anti-tumor effects of the organism [43].

Conclusion

$CD_4^+ CD_{25}^+ FOXP_3^+$ Treg cells play an important role

in HPV infection. FOXP₃ expression is up-regulated in patients with condyloma acuminatum and cervical cancer. In these patients, the number of $CD_4^+ CD_{25}^+ FOXP_3^+$ Treg cells is higher than in healthy individuals. Moreover, the increase in the number of $CD_4^+ CD_{25}^+ FOXP_3^+$ Treg cells is negatively correlated with disease progression, prognosis, and survival. The results of continuous research have indicated that targeting Treg cells is a novel approach for the immunotherapy of HPV infection. To understand the expression and mechanism of Treg cells, further research should focus on the effective regulation of immune suppression, the suppression of the differentiation and amplification, and the inhibition of the immune function of Treg cells.

Declarations

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No.

Competing interests

The author declares that she has no competing interest.

Authors' contributions

YT Wang made the literature analysis and wrote, discussed and revised the manuscript of this review.

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