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T Regulatory Cells in Self-Tolerance

Khalilur Rahman
Department of Pharmacy, the University of Sydney, Australia

***Corresponding author**
Khalilur Rahman

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Abstract: The function of the immune system is to protect our system from pathogens entering our body and react immunologically to cells from a genetically different person. At the same time, the immune cells maintain tolerance to our own body cells and do not reject them. This is called as self-tolerance. However, in case of autoimmune diseases and cancer, the immune system identifies our cells as foreign and generates immune reactions resulting in severe damage to the affected organ. However, on the other hand, the cancer cells are eliminated or rejected by our immune system in the early stages but support their growth at later stage. These immune reactions controlling the self-tolerance are mediated by T regulatory cells which maintain immune homeostasis under healthy condition by keeping the effector CD4+ and CD8+ T cells under regulated state. Upon loss of immune balance T regulatory cells cannot suppress the effector T cells' activity resulting in autoimmune or autoinflammatory reactions. This review will discuss the various subtypes of T regulatory cells and their role in autoimmune diseases and cancer.
Keywords: T regulatory cells, vitamins, autoimmunity, self-tolerance, cancer.

INTRODUCTION

Immunological tolerance is the key characteristic of the immune system. Tolerance can be central or peripheral and is important to discriminate the self and non-self-antigens [1]. The main mechanism which maintains the central tolerance is the clonal deletion or inactivation of self-reactive T cells.

Whereas, peripheral tolerance arises to limit the immune responses when the peripheral CD4+ or CD8+ cells get excited on encountering the foreign or self-antigens [2]. Being a specific subpopulation of CD4+ T cells, T regulatory cells control the activity of other self-reactive T cells in periphery. Despite identifying the potential tolerance mechanisms, more details about cell lineage that lead to immune suppression, differentiation factors, antigen specificity and subsets of T regulatory (T reg) cells are not clear. It is difficult to group all the T reg cells into category as they vary based upon origin (Thymus vs peripheral), mechanism of action, experimental/disease model and even their proliferation is very low *in vitro*.

The understanding of how the immune system does not react to self-antigens but can discriminate the foreign antigens or bacterial antigens is important. Tolerance to self-antigens occurs in the early stages of T cell development by multiple mechanisms including physical elimination, functional inactivation or anergy of auto responsive T cells by clonal deletion in the thymus [3, 4]. Initially anergy and suppression are defined as independent mechanisms. But they are not completely distinct. Anergy is the state of T cells in which they do not respond to antigenic stimulation, not proliferative and fail to produce IL-2. On the other hand, suppression is an active process where other cells including T regs inhibit their activity to reach threshold. Together, various mechanisms determine the peripheral immune tolerance [5].

Nutrition is key factor to boost and support the body immune system. Epidemiological studies have indicated that few infections and other immune diseases such as pneumonia, bacterial and viral diarrhea and tuberculosis take advantage of malnutrition to grow [6-8]. For other cases, the effect on nutrition is either low or moderate. The malnutrition impairs cell-mediated immunity, phagocyte function, and even cytokine and antibody secretion [9, 10]. Vitamins such as Vitamin E, and C are known as antioxidants and anticancer agents in the early days [11-14]. Later, the other functions of the vitamins in immune system were also identified. Vitamin C and E treated dendritic cells are resistant to change from inflammatory environment. Further, allogenic T cells in the environment were anergized to act as regulatory T cells independent of IL-10 [15]. Vitamin A metabolite, all-trans retinoic acid (RA), also concerts the naïve FoxP3 Cd4+ T cells into matured FoxP3 regulatory T cells in mouse and animal models. RA also induces the gene transcription in T reg cells to favor their gut homing capability [15, 16].

Most of the T regs are produced in the thymus as functionally distinct T cell population and few of the T regs can be generated *de novo* from peripheral naïve CD4+ T cells once they are exposed to antigens [17]. If the anergic T cells (CD4+ and CD8+) are activated with high doses of anti-CD3 antibodies, they can proliferate in regards to the antigenic stimulation and the subset of T regs in them will be activated. It indicated that, T regs can also be generated from T cells that are in anergy state [18]. Molecular markers including CD25 and transcription regulator, Foxp3 are used to identify the T regs population among other T cells. Further it is proven that Foxp3+CD25+CD4+ T regs negatively control immune responses and can be targeted to treat autoimmune and inflammation diseases and improve host defense immune responses [19].

Recent advancements in the T regs explained the role of these in controlling the severe autoimmune diseases such as arthritis. Any abnormality in the number, functionality or mutations in these T regs will affect the autoimmune and inflammatory diseases in humans [20]. T regs also play important role in tumor growth and tolerance. Immune microenvironment of tumor is controlled mostly by tumor associated macrophages, mesenchymal stem cells, T regulatory cells and functionally modified Antigen presenting cells (APCs) [21, 22]. Human tumors express wide range of tumor associated antigens (TAA). T reg cells suppress the effector activity of CD4+ or CD8+ T cells and maintain obstacle to generate TAA and TCR activity. Hence successful immunotherapy and active vaccination against cancer are becoming challenging [23].

I summarize and review subcategories of T regulatory cells in this paper, demonstrate their role in cancer and mechanism of T reg cells' immune suppression

CD25+ T regulatory cells in autoimmunity/ self-tolerance

Approximately 10% of peripheral CD4+ cells in normal adult mice express the IL-2 receptor a-chain (CD25) molecules. In early studies it was found that CD25 molecule present on few CD4+ T cells or CD4+CD8– mature thymocytes can differentiate the immune-regulative T reg cells from other T cells [5]. To define the role of CD25+ T cells in immune regulation, research was conducted by depleting or populating CD25+ T cells in the system and resulting immune conditions were observed [24]. Even though the molecular mechanisms and etiology that lead to autoimmune diseases are not clear until now, it is shown that simple altered manipulation of effector T cells in the absence of T regs can result in autoimmunity in mice. In a recent study, Thymectomy was performed on neonatal mice (NTx) on Day 3 after birth to prove that CD25+ T cells appear in the periphery after Day 3 and populate to normal levels by 2 weeks [20]. NTx on Day 3 eliminated most of the CD25+ T regs from the periphery resulting in autoimmune development. However, inoculation of CD25+ splenic T cells from syngeneic adult mice immediately after NTx prevents this autoimmunity occurrence. At the same time, inoculation of CD25- splenic T cells did not prevent the autoimmune reactions in these mice. This implies that self-reactive T cells that were generated before Day 3 or NTx in mice can be activated by elimination of CD25+ T cells and can cause auto immunity. These auto reactive T cells exist in periphery by ignorance. The ignorance is broken and is reactivated when exposed to auto antigens in immunogenic form [20]. CD25+ T cells keep the self-reactive cells in suppressed state throughout the life to prevent autoimmunity. CD4+ CD25+ T cells not only inhibit the organs specific autoimmunity but also can suppress the disease caused by cloned autoantigens. Further, these CD25+CD4+ T cells maintain anergy and do not proliferate *in vitro* to TCR stimulation but suppress the proliferation of other CD4+ or CD8+ T cells. Their anergy state is broken by high doses of IL-2 (ligand for CD25) and their suppressive activity is inhibited and CD4+ or CD8+ T cells co-cultured with CD25+ T cells will regain their auto reactivity to self-antigens and generate autoimmune disease in nude mice. As CD25+CD4+ T cells are generated by thymocytes early in the neonatal mice or generated from CD25-CD4+ T cells during various antigen exposures in periphery. Both the subsets express similar surface markers but display distinct immune regulatory capabilities. This may be due to acquiring anergy in thymus by selection process [1].

CD25+ CD4+ T cells maintain self-tolerance by down-regulating immune response to self and non-self Ags in an Ag-nonspecific manner. When CD25 depleted CD4+ cell suspension from BALB/c nu/+ mice lymph nodes and spleens were inoculated into BALB/c nude mice, they showed significant immune responses to the allogenic skin grafts, and reconstitution of CD25+ CD4+ T cells normalized the responses. Hence, elimination of CD4+CD25+ cells relieved general immune suppression [24].

Types of T regulatory cells

T reg cells are functionally and phenotypically diverse. Human T reg cells were first characterized as CD4+ and CD25+ and they were proposed to constitutively express CD25 cell surface molecule. Subsequently, *Foxp3* was described as a master regulator of immune system by controlling the immune related gene expression in T cells and used as a specific marker for human T reg cells. However, CD25 and FOXP3 can be induced in naive CD4+ T cells through cell activation. Hence it became challenging to define T reg cells [17].

Recently, CD25-CD4+ T cells with immune suppression function are identified. They don't express

CD25 and the immune regulatory mechanism is independent of IL-10/TGF-β like CD4+ CD25+ T reg cells. Mice transgenic for a TCR that is directed against the self-antigen MBP were crossed with mice deficient in recombinase activation genes; the F1 generation developed spontaneous autoimmune encephalomyelitis due to the lack of CD4+ T reg cells which were not contained in the CD25+ compartment [25]. When T reg cells were transferred into these mice and activated *in vivo* by ovalbumin, the T reg cells were not able to protect the mice from encephalomyelitis. This indicates the presence of other types of T reg cells and their control over auto immune diseases. In contrary to the CD25+ T reg cells, these special regulatory cells depend upon IL-2 and IFNγ production for their regulatory activity [25].

While large proportion of T reg cells belong to the CD4+ T cell sub population, small fraction of the CD8+ T cells also showed regulatory features in immune suppression. However, so far they were shown tolerance in intestinal immune suppression and systemic immune responses are not yet observed [26, 27]. When *in vivo* vaccination was performed in humans with autoreactive CD4+ T cells, CD8+ T reg cells were observed and CD4+ auto reactivity was suppressed by both antigen specific and non-specific mechanisms. They show great resistance against autoimmune diseases and maintain self-tolerance. In addition, Allo- and xeno-specific CD8+CD28– T reg cell lines that may induce transplantation tolerance have been described in the human. They could be isolated *in vitro* following multiple stimulations and can suppress alloreactive CD4+ T helper cells and impair the CD40 signaling pathway in Antigen presenting cells [28, 29].

While multiple subcategories of T reg cells exist, it is hard to identify and characterize them as they depend upon experiment/disease model. Different assays will show various populations of T reg cells. It is very important to identify the correlation between the subsets.

T regulatory cells in cancer

Studies reported high frequency of T reg cells in tumor micro environment or peripheral blood of patients with multiple forms of cancer [23]. As T reg cells express high CD25 molecule, it was targeted for cancer immuno therapy. CD25 specific antibody (PC61) or depletion of total CD4+ T cells were found to improve tumor immunity and suppress tumor growth [30]. Tumor specific CD8+ T cells were inoculated in mice bearing melanoma along with either CD4+CD25+ T reg cells or CD4+CD25– T cells. In this scenario, coadministration of CD4+CD25+ T cells inhibited the CD8+ T cells' immune effector function but the CD4+CD25– T cells did not have any effect on inoculated CD8+ T cells functionality [31]. Antibodies against the check point inhibitors CDLA4, PD1 or PDL1 molecule also showed significant benefit in cancer treatment [32, 33]. Mechanism of T reg cells in cancer is not yet clear, but following are few of the pathways.

Competitive consumption of IL-2: The IL-2 receptor (IL-2R) is a heterotrimeric complex made up of CD25 (α-chain), CD122 (β-chain) and CD132 (γ-chain). IL-2 promotes CD8+ or CD4+ T cell differentiation and proliferation. However, T reg cells IL-2R show 100-fold higher affinity towards the ligand, IL-2. Therefore, competition for IL-2 between T reg cells and conventional T cells was suggested as a suppressive mechanism. Established tumor microenvironment is populated richly with CD4+CD25+ T reg cells. So, the limited supply of IL-2 is utilized only by T reg cells leaving the other effector T lymphocytes not accessible to IL-2 [34].

Perforin and granzyme pathway: CD8+ T cells and NK cells use the perforin and granzyme pathways to kill tumor cells. However, activated human T reg cells express granzyme A and kill the T cells and APCs through perforin [35]. *CTLA4 induction of IDO:* Indoleamine 2,3-dioxygenase (IDO) is an enzyme that degrades the essential amino acid tryptophan. T reg cells constitutively express CTLA4 and have high IDO levels. It can reduce tryptophan in Antigen presenting cells, and suppress T-cell activation and promote tolerance [36].

CONCLUSION

Immune system protects us from pathogens entering our body from environment, food or any other means and maintains the tolerance against the self-antigens. T regulatory cells are important to maintain tolerance against autoimmune diseases. Extensive work in the field shed light on the fact that cytotoxic cells that can initiate autoimmune diseases exist in our body but kept in suppressed state by T regulatory cells. More than one subtype of T regulatory cells exists in the body arising from various pools of T cells or from thymus and their mechanism of actions are not same. It is evident that tumors take advantage of T regulatory cells to escape immune evasion from cytotoxic CD8+ T cells. Regulatory T cells inhibit TAA-specific priming of T cells which further are recruited into tumor with suppressed effector functions. It is quite important to target T regulatory cells by various methods such as depletion, blocking their recruitment into tumors, reduce differentiation and inhibit their suppressive mechanisms.

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