# Treg Cell Manipulation Has Therapeutic Potential In Autoimmune Diseases And Cancer

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## Abstract

With the support of T-regulatory (Treg) cells, the immune system maintains optimal T-effector (Teff) immunological responses against invading microorganisms and tumor antigens while limiting inappropriate autoimmune responses against self-antigens. As a result of their mutual control, Treg and Teff cells contribute to immunological homeostasis. While Tregs can help tumor immune evasion by reducing anti-tumor Teff responses, Treg depletion can lead to Teff responses against self-antigens, which can lead to autoimmune illness. As a result, a breakdown of homeostatic balance between Teff and Treg cells is frequently linked to cancer and autoimmunity. Immune suppression by Treg cells appears to be a key obstacle to successful anticancer immune responses, and their inactivation or elimination is being considered as a potential treatment option. Despite the lack of adequate techniques for selective Treg cell modification in humans, a variety of medications and biologicals, as well as reprogramming tumor-infiltrating antigen presentation cells, can modify their number and function.

**Keywords:** Treg cells, Autoimmune Diseases, Cancer

## Introduction

The immune system must be able to distinguish between self and non-self-cells while still responding to pathogen attacks. The negative selection in thymus plays an important role in removing self-reactive T cells; some autoreactive cells are discharged into the periphery by *de novo* [1, 2]. Sakaguchi and colleagues [3] discovered that CD4+ T cells expressing IL-2Ra (CD25) might prevent autoimmune illness, reviving the concept of specialized suppressor cells dominating immune responses and protecting against self-reactivity [4]. Regulatory T cells (Tregs) are a diverse population of lymphocytes that play a key role in the control of self-tolerance and tissue homeostasis [5].

Naturally occurring Treg cells (nTregs) arise in the thymus as a result of a negative selection interaction with medullary dendritic cells; they suppress via cell contact-dependent mechanisms involving the granzyme B/perforin or Fas/FasL pathways, and are a major regulatory T cell subset for peripheral tolerance maintenance. Inducible or adaptive Tregs (iTreg, Tr1) are a kind of Treg that is activated in the periphery in response to environmental cues and mediates suppression via a contact-independent route. TGF, IL and -β-10 are produced as a result. T Helper cells were once thought to be a single subset of T lymphocytes1 until the 1980s. A growing body of research demonstrates that at least seven

unique T helper subsets have been identified in reaction to specific cytokine combinations. Different transcription factors also have a role in producing cytokines in their natural environment and perform an effector function against one's own body as well as foreign antigens [6].

**TABLE 1** Subsets of t cells and their roles [6]

| Subsets of t cells | Cytokinins | Transcription factors | functions | Target cells |
|---|---|---|---|---|
| Treg | IL-2, TGFβ | TGFβ,IL-10,IL- 35, FOXP3, STAT5 | Immune suppression | lymphocytes |
| Th 1 | IL2 | IFN-γ,STAT4, TBET,LTα | Chronic inflammation/ acts upon intracellular pathogens | Macrophages, CD8+ lymphocytes |
| Th 2 | IL4 | IL-4,IL-5,IL-13, STAT 6, GATA3 | Allergens / asthma, anti-helminth immunity | Eosinophil, basophil, mast cell |
| Th 17 | TGFβ+IL6+ IL-2 1 | IL-17,IL-21,IL-2 2, STAT3 | Autoimmunity extracellular pathogens | neutrophil |
| Th 9 | TGFβ + IL4 | IL-9, STAT6 PU.1, IRF1, IRF4, BATF | Allergy, anti-helminth immumity | Mast cells, lymphocytes |
| Th 22 | IL-6+ TNF | IL-22, TNFα | Epithelial barrier homeostasis/ inflammation | Epithelial cells, hepatocytes |
| Tfh | IL-6+IL-21+TY PE I | IL-21, IFN-γ, IL-4, IL-9, STAT3, STAT4 | Germinal center, ig class switch. | B lymphocyte |

## T regulatory cells development

During the normal course of T-cell maturation in the thymus, one fraction develops, producing an endogenous, or 'natural', population of antigen-specific TReg cells that survives as a long-lived population in the periphery poised to avoid potentially harmful autoimmune reactions. The activation of mature T cells under specific conditions of sub-optimal antigen exposure and or co-stimulation results in the development of the second fraction of 'adaptive' TReg cells. Although it has been suggested that these are two distinct subsets of regulatory cells, we propose a unifying model in which the adaptive TReg-cell population can either develop from classical naive T-cell populations or differentiate from the naturally occurring TReg-cell subset under specific antigen exposure conditions [7].

## Natural regulatory T cells

During the early phases of fetal and neonatal cell development, the resident regulatory cells that grow in the thymus are created in a burst of activity. They are polyclonal due to a variety of TCR usage, and they may be capable of recognizing a variety of self-antigens. Kyewski and colleagues discovered that 'islands' of medullary epithelial cells in the thymus express messenger RNA transcripts encoding many tissue-specific proteins. T cells that act as natural regulators [8]. Self-antigens generated by these thymic medullary epithelial cells may be able to eliminate immature self-reactive T cells [9]. TReg cells are members of a family of autoreactive regulatory T cells that form during early T-cell development and serve to maintain immunological homeostasis. They include T cells and NK1.1+ T cells. An important function in the control of immune responses, as

well as a ‘active’ or ‘differentiated’ phenotype, indicating continual exposure to self-antigens, are shared by these cells. However, it is unclear if all natural TReg-cell populations in the thymus are subject to the same induction and selection mechanisms [10].

**Adaptive regulatory T cells**

In several immune situations, additional populations of regulatory cells have been identified. These cells can be activated ex vivo by culturing mature CD4+ T cells with antigen or polyclonal activators in the presence of immunosuppressive cytokines, such as IL-10, in the presence of immunosuppressive cytokines. TReg cells in vivo are cytokine-dependent. As a result, believe that adaptive TReg cells vary from natural TReg cells not by their origin (the thymus), but by their need for additional differentiation as a result of antigen exposure in a different immunological setting [11]. Certain ways of antigen administration, such as intranasal or oral administration, appear to selectively trigger the development of T cells with this regulatory phenotype. Furthermore, unlike normal TReg cells, which are fully functional at the time of thymic export due to high TCR engagement, adaptive TReg cell growth in the periphery may be driven by low-affinity antigen or altered TCR signal transduction. However, that adaptive TReg cells, unlike native TReg cells, may not require CD28 co-stimulation for development or function [12][13].

**Functions of Treg cells**

Treg cells have a broad suppressive effect on all types of immune cells in response to self- and nonself-antigens. Treg cells use a variety of ways to efficiently moderate immune responses, depending on the tissue, microenvironment, and targeted cells. Treg cells’ ability to control APC function is one effective strategy to prevent the start and expression of immunological responses [2]. According to a paper published by Sakaguchi’s group, Treg cells use membrane-bound cytotoxic T lymphocyte antigen 4 (CTLA-4) to effectively shut off Antigen Presenting Cell function. The lymphocyte-activation gene 3 is another membrane-bound protein that appears to be involved in Treg cell-mediated APC inhibition (LAG3) [14] [15]. In addition to membrane-bound substances, Treg cell-derived cyclic adenosine monophosphate (cAMP) and cytokines (TGF) Transforming growth factor- and IL-10 have been implicated in Treg cell-mediated APC inhibition [16][17].

Regulatory T cells (Tregs) suppress effector activity by secreting inhibitory cytokines like IL-10 and TGF-**β**, or by engaging inhibitory checkpoint molecules like TIGIT T cell immunoreceptor with Ig and ITIM domains and (CTLA-4) cytotoxic T-lymphocyte-associated protein-4 through cell-mediated engagement. Th17 cells have been linked to autoimmune, carcinogenesis, and antitumor immunity, whereas Treg cells are required for immunological tolerance and have been proven to suppress autoimmunity and antitumor immunity [18] [19]. IL-4 causes Th2 cell differentiation, which results in cytokine release, which can cause allergies or asthma. TGF-**β**, IL6, and IL-21 all have a role in the formation of Th17 cells. During clonal proliferation, IL-1 and IL-23 keep Th17 cells stable. Th17 cells are most typically characterized by their expression of RORt and STAT3 after differentiation. Th9, Th22, and Tfh cells have only lately been discovered, and the transcription factors that control their differentiation are yet unknown [6].

CTLA-4 has been identified as an important molecule in Treg function. The loss of CTLA-4 in Treg cells causes deadly autoimmunity to develop. Treg cells may also use granzymes to inhibit their suppressive function, allowing them to perform effector activities through apoptosis [20][21]. Treg cells also lack the ability to produce IL-2, which is required for the proliferation and differentiation of effector T cells. Treg cell binding to the IL-2 receptor prevents other T cells from producing IL-2, and so represents one route of immune-mediated suppression. CTLA-4 overexpression and IL-2 suppression in effector T cells are similar to Treg-mediated suppressive characteristics [22]. Treg cells can directly control T cell function through cell contact-dependent and -independent pathways, in addition to their effect on APC. In other words, the inhibitory cytokines IL-10, IL-35, and TGF-**β**, IL-2 consumption, cytotoxic actions, adenosine synthesis by CD39 and CD73 resulting in de novo generation of cAMP in target cells, and a direct transfer of cAMP from Treg cells to target cells are all established here [23]. All of these mechanisms are influenced by tissue-specific (tumor-specific) variables and develop under the influence of the local microenvironment [24][25].

**Mechanism of suppression in Treg cells**

The normal and adaptive subsets of TReg cells differ in their method of action, in addition to potential changes in TCR repertoire and differentiation stage. Many studies have shown that adaptive TReg cells produce immunosuppressive cytokines such transforming growth factor-(TGF-) and IL-10 to mediate their inhibitory actions [26]. Natural TReg cells, on the other hand, function without the use of cytokines, at least in vitro, and are thought to interact directly with responding T cells or antigen-presenting cells3. CD4+ CD25+ natural TReg cells examined in vitro suppression models most firmly demonstrated this contact-dependent mechanism of suppression, whereas cytokine-mediated suppression has been best proven for peripheral adaptive TReg cells in vivo [27].

**Fox p3**

T Regulators that express the forkhead box P3 (Foxp3) in mice and humans, are a small subset of CD4+ T cells that are essential for immunological homeostasis and the prevention of autoimmunity [33]. The transcription factor Foxp3 is required for the formation, maintenance, and function of these cells. Treg potency is based on their ability to use a variety of immunosuppressive mechanisms depending on the immunological environment, as well as their ability to extend their effect through the pathogenic tolerance process [34]. Foxp3 is widely known for its role in Treg differentiation, maintenance, and function. As a result, research in the Treg field has primarily focused on the control of Foxp3 expression and stability. Foxp3 is governed by two basic layers of regulation: transcriptional and post-transcriptional. Posttranslational, which are both sensitive to positive and negative stimuli elements in the tissue environment, such as cytokines, regulate inflammatory mediators and metabolic mediators [35].

The Foxp3 locus contains numerous conserved non-coding sequences (CNSs) that are required for the initiation and maintenance of Foxp3 transcription for transcriptional control. CNS2 has been shown to prevent autoimmunity among the three CNSs discovered thus far. It's a TCR-responsive enhancer with Runx1–CBF transcription-factor complex binding sites that's critical for Foxp3 stability [36]. Foxp3 stability is dependent on the presence of r complexes. CNS2 also has

a conserved CpG island (TSDR), which is hypomethylated in Treg but hypermethylated in naive or effector T cells. This Treg-specific demethylated region (TSDR) within the Foxp3 enhancer CNS2 is crucial; ablation of CNS2 causes Foxp3 expression to be lost in proliferating Tregs [37][36].

Acetylation is one of these post-translational modifications that helps to maintain Treg stability, whereas phosphorylation and ubiquitination cause Foxp3 to be degraded or unstable by the proteasome. TIP60 induces acetylation-dependent dimerization of Foxp3, and fatal autoimmunity occurs in the absence of TIP60. Stub1, a protein that binds to Foxp3 and promotes Foxp3 ubiquitination, resulting in Treg cell deactivation, could be a viable therapeutic target for the treatment of autoimmune disorders. Expression of the deubiquitinase (DUB) USP7, on the other hand, boosted Foxp3 expression and suppressive activity in Treg [38] These Foxp3 post-translational alterations have a critical role in controlling the plasticity or instability of Treg cells, adding another layer of complexity to the regulation of the Treg functional programme, which could have implications for the onset of autoimmunity. Satb1, a genome organizer, is required for Treg cell-specific super-enhancer activation and subsequent Treg signature gene expression. Due to Treg deficiency, Satb1 deficiency can cause severe autoimmunity [39].

**T regulatory cells in cancer**

Treg's involvement in cancer therapy, like their prognostic usefulness, is still up for debate. Although in vivo and in vitro research in cancer-prone mice and cancer patients have revealed that Treg hinder the host's anti-tumor immunity, more recent evidence suggests that their role in cancer therapy is complex and varied. Nonetheless, it is widely considered that eliminating Treg in vivo could improve tumor anti-tumor immunity. The immunomodulatory characteristics of low-dose cyclophosphamide are well established, and Treg depletion by cyclophosphamide has been associated with the recovery of T-cell immune responses in many experimental animal cancer models [40]. Treg cells are abundant in tumor tissues from a variety of malignancies, including breast, lung, liver, pancreatic, gastrointestinal, and malignant melanoma.

Large proportions of CD4+ Treg cells among tumor-infiltrating lymphocytes (TILs), as well as lower ratios of CD8+ T cells to FOXP3+ CD25+CD4+ Treg cells among TILs, have been linked to a poor prognosis in ovarian, breast, and gastric malignancies. These data imply that FOXP3+ Treg cells in tumor tissues inhibit tumor-reactive CD8+ CTLs. High infiltration of FOXP3+ Treg cells, on the other hand, has been linked to a better prognosis in colon and head/neck malignancies, as well as Hodgkin lymphoma. Healthy people and cancer patients both have potentially tumor-reactive T cells whose activation and expansion are suppressed by natural Treg cells, and that Treg-cell depletion can activate and expand NY-ESO-1-specific high-avidity T cells from naive T-cell precursors, allowing differentiation into potent anti-tumor effector T cells [7] canceling the effect of Treg-cell depletion to augment anti-tumor immunity. Because activated effector T cells express CD25 and their production of IL-2 is required for the expansion of CD8+ CTLs, CD25-based cell depletion may reduce activated effector T cells as well, canceling the effect of Treg-cell depletion to augment anti-tumor immunity.

The age of the patients can be considered an interfering factor in cancer immunotherapy. Surprisingly, results of Treg-targeted therapy may be more promising in older cancer patients than

in younger individuals. This is owing to the fact that younger cancer patients have a higher number of Tregs within the tumor stroma, and their FOXP3 activity is higher as well. Another element to consider when it comes to cancer immunotherapy is the type of malignancy. Different cancers have a wide range of Tregs. Tregs in ovarian cancer and melanoma, for example, express co-inhibitory and costimulatory receptors in distinct ways. When comparing ovarian cancer Tregs to melanoma Tregs, the rate of expression for FOXP3, PD1, and CD25 is higher in ovarian cancer Tregs. Furthermore, various malignancies cause different mediators to trigger the formation of FOXP3+ Tregs in the TME. BRAF, for example, is the inducer of such cells in melanoma [41].

## T regulatory cell in autoimmunity

The role of Tregs in a variety of human autoimmune diseases has paved the way for new treatments, such as Treg-based cellular therapies and IL-2 therapies that aim to restore the balance of Treg and Teff cells. Treg cells as a cell-based therapy approach was initially proven in mouse models of EAE and CIA, in which Treg cells were shown to be involved in pathogenesis. Treg cell transplantation may help to alleviate the symptoms of the condition [42]. Chronic immune responses against the host's own cells, tissue, and organs generate autoimmune disorders such as multiple sclerosis (MS), type 1 diabetes (T1D), and systemic lupus erythematosus (SLE), which result in tissue death, malfunction, and pathology. These autoimmune illnesses are caused by a complex network of poorly understood interactions between environmental stimuli and polymorphic genetic elements, which results in a loss of self-tolerance. The complexity and heterogeneity of the autoimmune response has made developing targeted treatments difficult, as they must sufficiently purge the immune system of autoreactivity while keeping the immune system's normal functional side intact [20][45]. Recent therapeutic targets for autoimmunity relief have centered on substances that increase Treg induction and growth in vivo. Rapamycin, a mTOR inhibitor, and biologicals including IL-10, low-dose IL-2, TNF receptor 2 (TNFR2) agonists, and the FMS-like tyrosine kinase 3 ligand Flt3L have all been Investigated [46].

**TABLE 2** New autoimmune disease- therapeutic methods [46]

| Cell based therapies | Non cell-based therapies |
|---|---|
| Polyclonal Treg | Low dose IL2 |
| Autoantigen specific TCR Treg | Rapamycin |
| Autoantigen specific CAR Treg | TNFR2 Agonist |
| Autoantigen-specific chimeric Treg | Auto antigen -whole protein, -peptide -loaded-peptide |
| Tolerogenic DC | rituximab |
| Faecal transplantation | Combination therapy |

New autoimmune disease therapeutic methods in the treatment and prevention of autoimmune illnesses, a range of cell-based and non-cell-based therapies are currently being investigated. Ex vivo-expanded polyclonal Tregs or Tregs transduced with an autoantigen-specific T cell receptor (TCR), chimeric antigen receptor (CAR), or other chimeric receptor such as peptide MCH are examples of cell-based treatments. Tregs can

suppress B cells and natural killer T cells and influence the biological functions of antigen-presenting cells in autoimmunity, in addition to their role in T cell responses. Tolerance-regulating plasticity and stability Tregs have been shown to have an impact on autoimmunity has been associated with disease progression [46]. Ex vivo expanded Treg cells have been used in several phase I and phase II clinical trials for the treatment of autoimmune illnesses such as autoimmune hepatitis, GvHD, type I diabetes, SLE, kidney, and liver transplantation [47]. Apart from the development of Treg cell-based therapeutics, several existing medications, such as rapamycin, anti-CD3, CTLA-4Ig, or anti-CD25, target both Treg and Teff cells [48].

Rapamycin, a PI3K akt-mTORC1 signaling inhibitor, promotes Treg cell expansion and survival while suppressing Th1 and Th17 cell proliferation. In a type 1 diabetic mouse model, treatment with CD3 antibody enhanced Treg population and stabilized Treg function. In type 1 diabetic patients, anti-CD3 therapy protected remaining beta-cell function [49]. Klatzmann et al. looked into the potential of low-dose IL-2 therapy as a new therapeutic approach in 11 autoimmune diseases, including Rheumatoid arthritis, Ankylosis spondylitis, systemic lupus erythematosus (SLE), psoriasis, Behcet's disease, granulomatosis with polyangiitis (GPA), Takayasu's disease, Crohn's disease (CD), ulcerative colitis, autoimmune hepatitis, and sclerosing cholangitis Low doses of IL-2 were generally well tolerated and resulted in Treg growth and activation. These findings suggest that similar therapeutic techniques could be used to treat a variety of other autoimmune and inflammatory illnesses [50].

## Conclusion

Treg cells are an appealing target in the development of tumor immunotherapeutics because of their important function in immune control. Tregs are currently thought to be a stumbling block to efficient antitumor immunity. Because of their vital function in immunological homeostasis and avoiding autoimmunity, systemic Treg depletion is not recommended [51]. The discovery of new cell surface chemicals on Treg may also aid in the identification of the as-yet-unidentified suppressive mechanism [4]. Further development of Treg-cell depletion or dysfunction by biologicals or chemicals, as well as increasing the tumor-killing activity of effector Tconv cells, would presumably make cancer immunotherapy more successful with reduced side effects in the future [52]. There are certain concerns to consider while applying these therapies in the clinic. One is how to avoid the potentially harmful autoimmunity that comes with Treg-cell depletion. Furthermore, it is important to optimize the degree and duration of depletion.

It's crucial to target a Treg-cell subset rather than the entire population. entire FOXP3+ cells, in order to elicit effective anti-tumor effects While avoiding autoimmunity, you can gain immunity [53]. This is eventually required for the innovative and interesting treatment techniques that target Treg cells directly, employing polyclonal Treg cells, or as a Treg cell-targeted therapy. For future successful treatment and monitoring of patients with Treg targeted medicines, a clear identification of patients with functional or numerical Treg deficiencies will be required. Within the next several years, a better knowledge of the precise role of Treg cells under varied inflammatory conditions will aid in the development

of a tailored therapy approach [20]. Integrating Tregs' significant anti-inflammatory effects with antigen specificity achieved through transduction of a specific receptor offers a lot of hope for future medicines to be even more precise and effective. The discovery and manufacture of an effective Treg therapy remains an interesting and hard enterprise, with the potential to improve the prognosis of autoimmune disease patients [54].

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