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Regulatory T Cells and Rheumatoid Arthritis

Rupesh K. Srivastava*

School of Biological Sciences, Department of Zoology,
Dr. Hari Singh Gour Central University, Sagar (M.P.)

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Abstract

Regulatory T (Treg) cells play a crucial role in controlling autoimmunity by inflammatory by suppressing auto reactive T cells. Treg cells generated in the thymus are called natural Treg cells. Treg cells could also be generated outside the thymus from peripheral naive $CD4^+CD25^+$ T cells under both in vitro and in vivo conditions and are referred as adaptive or induced Treg cells. The fork-head transcription factor (Foxp3) is the master regulator for the development and function of Treg cells. Treg cells in rheumatoid arthritis (RA) patients have a defect in their ability to suppress proinflammatory cytokine production by activated T cells and monocytes. Also, deficiency of Foxp3 results in the paucity of $CD4^+CD25^+$ Treg cells and leads to severe multiorgan autoimmune diseases in both mice and humans. Thus, the development of functional Treg cells holds promise for the treatment of RA and other autoimmune diseases. In this review, I discuss the potential of Treg cells in treating RA and various other autoimmune diseases.

Keywords: Regulatory T cells, Foxp3, Rheumatoid arthritis, Autoimmunity, Inflammation

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*Corresponding author: rupesh_srivastava13@yahoo.co.in

1. Introduction

The immune system protects us against infectious pathogens and it has evolved to do so while minimizing damage to self tissues. These responses are potent enough to destroy not only pathogens but also the host. It is now widely accepted that various mechanisms involving T cells that actively suppress the activation and the proliferation of various immune cells is important in the maintenance of tolerance to self and thus the prevention of autoimmune diseases. Circulating T cells having specificity to self-antigens are present in healthy individuals but usually do not trigger clinically potent auto immunity [1]. This suggests a dominant inhibitory phenomenon that controlling autoimmunity is permanently in operation. A distinct subset of $CD4^+$ T cells, called as regulatory T (Treg) cells are the mediator of such permanent suppression of autoimmune responses. Studies in the recent past have identified the X-chromosome–encoded fork head transcription factor (Foxp3), as the key player in development of Treg cell function [2-4].

2. Subsets of Treg Cells

There are different types of CD4+ Treg cells with variable mechanism of action and origin constituting 5–10% of naive CD4+ T cells in healthy individuals.

2.1 Natural Treg (nTreg) cells

In mice Treg cells which constitutively express high levels of CD25 and develop in thymus to enter the peripheral circulation as $CD4^+CD25^+T$ cells are called as natural Treg (nTreg) cells [5]. However, identifying and defining nTreg cells as a distinct subset in humans is challenging since a large fraction of $CD4^+$ T cells express CD25 of which a large proportion have no regulatory properties. Also activation of nTreg cells needs TCR activation alone whereas that of induced Treg cells requires both TCR activation and co-stimulation. nTreg cells have a more cell contact dependent mechanism of suppression of effector T cells.

2.2 Inducible Treg (iTreg) cells

iTreg cells are very similar to nTreg cells but are derived from Foxp3- naive T cells in the periphery after stimulation in the presence of TGF-β. Once induced, they express the master transcription factor Foxp3 along with cytotoxic T lymphocyte antigen (CTLA-4) and secrete IL-10 and TGF-β. iTreg cells inhibit the proliferation of effector T cells in a cell contact-independent fashion. Also the secretion of both IL-10 and TGF-β has a potent immuno suppressive effect on both effector T cells and antigen presenting cells (APCs) [6].

2.3 *Adaptive Treg cells*

Adaptive Treg cells are formed from naive CD25- T cells in the periphery under specific conditions of antigen exposure and cytokine stimulation. A variety of adaptive Treg cells exist with differing patterns of cytokine secretion. There is still much debate about the distinctive differences between the cell types within this subset of Treg cells, and can broadly be divided into three groups.

2.3.1 Tr1 cells

Tr1 cells differ from nTreg cells in their ability to produce IL-10 and TGF-β in enormous amounts, thereby suppressing the function of both naive and memory CD4+ T cells [7].Just like iTreg cells,Tr1 cells are also induced by antigen-mediated TCR stimulation, but unlike iTreg cells, they are induced in the presence of IL-10 [8].

2.3.2 Th3 cells

The Th3 cells are Treg subsets and are vital for the maintenance of oral tolerance. They secrete large amount of IL-10, IL-4, and TGF-β which along with their suppressive capacity also promote indirect differentiation of antigen-specific $Foxp3^+$ Treg cells in the periphery.Th3 cells are different from Tr1 in their dependence on TGF-β for differentiation from $CD4^+CD25$- T cells [9].

3. Foxp3 and Treg Cell Differentiation

Recent developments in the understanding of Treg cell biology came with the discovery of the X chromosome-encoded gene Foxp3 [10]. Humans and mice with a loss of function mutation in the Foxp3 gene are affected by fatal early onset lympho proliferative immune-mediated disease affecting various organs and tissues. Various subsequent studies revealed the importance of Foxp3 in Treg cell differentiation, function, proliferative potential and metabolic fitness [11-13]. Sustained Foxp3 expression is essential for the maintenance of the Treg cell phenotype and suppressor function and loss of Foxp3 or its diminished expression leads to acquisition of effector T cell properties [12, 14]. Together, these studies established Foxp3 as a master transcription factor in the development of Treg cells.

4. Mechanisms of Treg Cell Differentiation

Treg cell differentiation requires TCR stimulation in the presence of the cytokines TGF-β and IL-2, for both in vitro and in vivo iTreg cell generation. In contrast to the essential role of TGF-β in the differentiation of iTreg cells, its role in nTreg cell differentiation is less clear. Studies with T cell-specific deletion of TGF-βRII have shown that TGF-β is not required for thymic nTreg cell development [15-18]; furthermore, young TGF-β1-deficient mice have normal number of thymic nTreg cells. IL-2 by itself is not necessary for Foxp3$^+$ nTreg cell generation but appears to be essential for iTreg cell generation and/or homeostasis. IL-2 is required in vitro for TGF-β induction of Foxp3 transcription and suppressor activity [19]. IL-2, but not other common-γ chain signaling cytokines, could replace the requirement for CD28 costimulation for the induction of Foxp3 by anti-CD3 and TGF-β [19-20]. Recent studies from our group have demonstrated the role of interleukin-3 (IL-3) in increasing the percentage of Foxp3$^+$ Treg cells through secretion of IL-2 by non-Treg cells [20]. IL-2 signaling activates signal transducer and activator of transcription factor 5 (STAT5) which binds to Foxp3 gene and induces the transcription of Foxp3 [21-22].

5. Mechanisms of Foxp3$^+$ Treg Cell Mediated Suppression

In vitro model systems have identified a long list of molecules and processes that contribute to Treg cell suppressive activities and it remains unclear whether any of the conclusions drawn from these studies shed light on how Treg cells function in vivo. A detailed analysis of Treg cell function is further confounded by the large number of different cell types that are purported to be directly targeted by Foxp3$^+$ Treg cell. The precise molecular mechanisms of suppression by human Treg cells remains to be determined, although in vitro and in vivo mouse studies have implicated several mechanisms. These include modulation of the cytokine micro environment, metabolic disruption of the target cell, secretion of molecules and cytolysis as depicted in Fig.1.

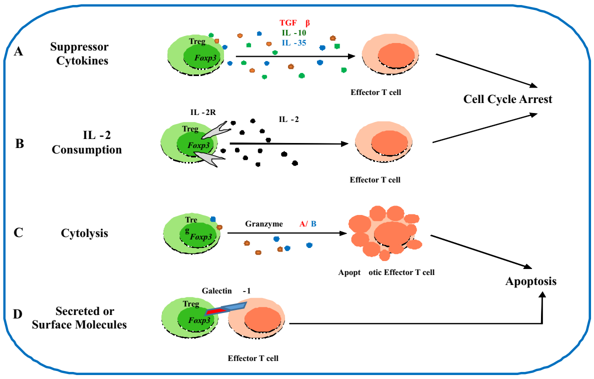

Figure 1: Mechanisms by which Treg cells suppress responder T Cells.

Treg cells may secrete suppressor cytokines that can directly inhibit the function of effector T cells. Treg cells express high CD25, the IL-2 receptor a chain, and compete with effector T cells for IL-2 resulting in cytokine-mediated deprivation of the effector cells leading to apoptosis of effector T cells. Activated Foxp3$^+$ Treg cells can also function as cytotoxic cells and directly kill effector cells in a manner similar to CD8$^+$ cytotoxic cells via granazyme A/B. Activated Treg cells can also express known (e.g., galectin-1) or unknown molecules on their cell surface that can interact with receptors on effector T cells resulting in cell cycle arrest. All of these mechanisms may also be utilized by Treg cells to inhibit the function of antigen-presenting cells or other cells of the innate immune system.

6. Treg Cells and Rheumatoid Athritis (RA)

RA is debilitating autoimmune disease of unknown etiology characterized by systemic inflammation of the joints with ensuing destruction of cartilage and bone. While the exact mechanism(s) of RA is unknown, clinical symptoms are paralleled by the production of pro-inflammatory cytokines and immune cell activation within the joints [22]. It is believed that a fundamental breakdown in the processes of self-tolerance is responsible for the initiation of RA and subsequent destruction of joint tissue. Data obtained over the last years, however, have shed new light on the role of T cells in RA. This line of research started almost 15 years ago with the discovery of so-called Treg cells[5]. This exciting discovery raised expectations for novel ways of treating arthritis by targeting these Treg cells. As in other autoimmune diseases, Collagen Induced Arthritis (CIA) the experimental model of RA is exacerbated by depletion of Treg cells [23]. However, unlike other diseases, the target tissue in RA-synovium can be obtained easily from patients with disease. This has allowed investigators to analyse the number and function of Treg cells not only in the peripheral blood of these patients but also in the diseased tissue.

7. Causes of Treg Cell Functional Impairment in RA

Several studies on the number of Treg cell in the peripheral blood of patients with RA have produced contradictory results. Despite these differences, there is a general agreement that the percentage of Treg cells is higher in the synovial fluid in patients with RA than in control or healthy individuals [24-26]. Treg cells are malfunctioned in RA patients with respect to their ability to suppress the production of IFNγ and TNFα [27]. This defect is intrinsic to the Treg cells of subjects with RA which is a result of a defect in CTLA-4 mediated inhibition of TCR signalling in Treg cells from patients with RA. This is confirmed by the fact that this defect was reversed by over expression of CTLA-4 in these Treg cells [28].

Synovial macrophages have also been reported to influence the responsiveness of effector T cells to Treg cells through their increased expression of MHC class II and CD86 molecules along with increased production of inflammatory cytokines such as TNF, IL-6 and IL-7, thereby altering the cytokine milieu and the stimulatory conditions in which suppression occurs in the joint [29]. Thus defects in immune regulation in RA are probably due to both Treg cell-intrinsic defects and the inflammatory milieu present in the rheumatoid joint.

8. Treg Cells as Therapeutic Targets in RA

The ultimate goal of therapy for patients with RA and various other autoimmune diseases is to restore normal immune function. Evidence has accumulated over the past decade that Treg cells could be an ideal target for therapies to induce durable remission of autoimmune and inflammatory disease. Treg cells are ideal for this purpose because they suppress inflammation in an antigen-specific manner. Furthermore, short-term therapy with Treg cells can lead to long-term inhibition of autoimmune disease, and immunomodulatory agents can affect the number and functioning of Treg cells in both mice and humans. Thus, approaches that bolster numbers or functioning of Treg cells could achieve selective and durable inhibition of pathologic inflammation without blocking protective immune responses against infection.

9. Effects of Approved Drugs on Treg Cells

Although much still remains to be elucidated about how Treg cell defects might contribute to the pathogenesis of RA, approaches that specifically boost Treg cell activity could be useful in the treatment of RA. High concentrations of TNFα can block the immuno-suppressive functions of Treg in vitro [30].

Infliximab(an anti-TNF agent) treatment in patients with RA has been reported to increase the number of peripheral Treg cells, and this correlated with changes in C-reactive protein levels, a marker of disease activity and inflammation. This increase in Treg cells was not a result of expansion of the nTreg cell population but was due to the induction of TGFβ-producing Treg cells [27].

Tocilizumab, an antibody that blocks the human IL-6 receptor, has shown efficacy in the treatment of RA [31]. IL-6 can block the immuno-suppressive activity of Treg cells in mice [32]. By contrast, blockade of the IL-6 receptor with a monoclonal antibody in mice attenuates the severity of graft-versus-host disease and increases the absolute number of Treg cells in the spleens of treated mice through conversion of peripheral $CD4^+$ T cells to Treg cells [33]. IL-6 prevents Foxp3 up regulation in human T cells in vitro [34]. Thus, blockade of the IL-6 receptor is useful in patients with RA, at least in part, by augmenting the conversion of peripheral effector T cells to Treg, by preventing IL-6 driven conversion of $CD4^+$ cells toward a Th17 phenotype [35]. Surprisingly, IL-6 had no effect on the immuno suppressive capacity of Treg cells in the synovial fluid of patients with RA [29].

Both abatacept (a CTLA4–Ig fusion protein), and its higher-affinity derivative, belatacept bind to CD80 and CD86 (CD28 ligands) with a higher affinity than does the CD28 receptor, thereby preventing T-cell co-stimulation and thus T-cell-driven autoimmune processes [36]. Determining the optimal dose of CTLA4–Ig in patients with RA and other diseases will be critical if effector T cells are to be selectively inhibited while retaining Treg cell function.

Rapamycin is an immunosuppressive small-molecule drug with a wide variety of effects on cells of both the innate and adaptive immune systems [37,38]. Importantly, rapamycin and other mTOR (mammalian target of rapamycin) inhibitors promote Treg cell survival and differentiation and also block effector T cell proliferation [39-41]. RA patients treated with everolimus (rapamycin derivative), and methotrexate, showed greater response rate than patients treated with methotrexate alone. Thus, mTOR inhibition ameliorates autoimmunity in part by promoting Treg cell function. However, to what extent does the clinical efficacy of rapamycin results from its effects on Treg cells is not specifically known.

10. Experimental Drugs Targeting Treg Cells

Toll-like receptors (TLRs) respond to the presence of microbial products as well as to human self-molecules such as RNA and DNA [42]. Treg cells express multiple TLRs, and different TLR ligands have been reported to regulate the immunosuppressive capacity of murine Treg cells both in vitro and in vivo[42-43]. Deficiency of TLR9 in mice results in elevated numbers of Treg cells in gut-associated lymphoid tissue highlighting the importance of TLRs in Treg homeostasis [44]. Furthermore TLR3 ligands have been found in synovial fluid from patients with RA involved in inflammatory cytokine production [44]. Hydroxychloroquine, a drug currently used to treat RA works in part by blocking TLR signaling [45]. These studied suggest that TLR antagonists could have therapeutic effects in patients with RA by modulating Treg cell function.

Trichostatin-A, an inhibitor of histone deacetlyases (HDACs) increases Treg cell numbers by increasing thymic output of Treg cells [46]. A recent study demonstrated that the HDAC inhibitors MS-275 and vorinostat (suberoylanilide hydroxamic acid) induce Foxp3 expression in human T cells [47]. Similarly Trichostatin-A also works by increasing the acetylation and functioning of Foxp3 [46]. Thus, promotion of Treg cell differentiation might be a general result of HDAC inhibition suggesting role of HDAC inhibitors as a future therapy for various autoimmune and inflammatory diseases. Recently vorinostat, an HDAC inhibitor has been approved by the FDA for the treatment of cutaneous T cell lymphoma [48].

IL-2 is a principal survival factor for Treg cells [49] as its absence contributes to defective Treg cell functions [51]. Mice treated with a stabilized form of IL-2 demonstrate increased Treg cell proliferation and function [50,51]. IL-2, also known as aldesleukin, is currently used in cancer therapy, and evidence suggests that it also boosts Treg cell numbers in humans [52]. Thus, administration of IL-2 could be useful in the treatment of autoimmune disease.

11. Direct approaches to enhance Treg Cell function

There are several methods available to directly target Treg cells for therapy of various autoimmune diseases. These include but are not limited to the expansion and induction of Treg cells in vitro followed by reinfusion into the patient or in vivo by immunomodulatory agents.

11.1 Ex vivo expansion of Treg cells

Treg cells can be isolated and expanded *ex vivo* by anti-CD3/anti-CD28 stimulation in the presence of IL-2 [53]. With this protocol up to 3000-fold expansion can be reached without losing the suppressive capacity. Moreover, the cells have a higher inhibitory potential compared with directly isolated Treg cells [53]. Therefore, expanded Treg cells could have enhanced suppressive capacity which is required for successful treatment of autoimmune disease.

11.2 In vitro induction of Treg cells

Treg cells can also be induced *in vitro* from non-Treg cells. This method evades the difficulty of obtaining large number of natural Treg cells required for expansion. Treg cell induction works well in mice in which naïve T cells activated in the presence of TGF-β develop into Foxp3-expressing cells with suppressive capacity that is maintained even after transfer *in vivo*[54]. In contrast, TCR stimulation of human $CD4^{+}CD25^{-}$ cells can also result in transient Foxp3 expression [55]. Furthermore, activation-induced expression of Foxp3 in humans does not coincide with a regulatory phenotype and can even result in IL-2 and IFN-γ production [56]. Therefore, *in vitro* induction of Treg cells is far more complicated in humans compared with mice and needs to be further established which culture conditions to reinforce stable Foxp3 expression and suppressive function resulting Treg cells.

11.3 In vivo expansion and induction of Treg cells

Several immunomodulatory agents can be used to enhance Treg cell function in vivo and the most important ones are discussed below:

11.3.1 Anti-CD3 antibodies

Treatment with humanized CD3 antibodies leads to preserved β-cell function and reduced insulin need in patients with new-onset Type 1 diabetes [57]. Also in RA, efficacy of anti-CD3 treatment was confirmed [58]. Thus, CD3-specific antibodies are capable of inducing Treg cells and have already been proved to be safe and effective in patients with autoimmune disease. As such, they may provide a valuable treatment option for both RA and juvenile idiopathic arthritis (JIA).

11.3.2 Neuropeptides

Vasoactive intestinal peptide (VIP), is capable of enhancing Treg numbers and suppressive function via the induction of tolerogenic dendritic cells [59]. In CIA, VIP administration increases both the absolute number and percentage of Treg cells, leading to lower arthritis scores [60]. Urocortin(another neuropeptide), also reduces disease severity in CIA model via induction of Treg cells [61]. Although clinical trials in human autoimmune disease are still awaiting, neuropeptides could be of valuable therapeutic option, due to their Treg-enhancing capacity.

11.3.3 Retinoic acid

All-trans retinoic acid (ATRA) an active metabolite of vitamin A induces Treg cell generation, while simultaneously inhibiting Th17 development [62]. Thus ATRA restores the balance between Treg and pathogenic Th17 cells involved in various autoimmune diseases [63]. ATRA administration has been shown to reduce both the severity and incidence of CIA. This beneficial effect was also reported to be accompanied by as imultaneous decrease in the production of both pro-inflammatory cytokines and collagen-specific antibodies [64]. Also the induced Treg cells generated in the presence of ATRA are resistant to conversion into Foxp3$^-$ cells [65] and have enhanced suppressive capacity [66]. Thus, ATRA is a potential candidate for optimizing protocols for in vitro expansion and induction of Treg cells.

11.3.4 Histone deacetylase inhibitors

HDAC inhibitors increase Foxp3 gene transcription and prevent protein degradation, thereby enhancing and stabilizing Foxp3 expression. Two HDAC inhibitors, MS-275 and suberoylanilide hydroxamic acid (SAHA), have been shown to induce Foxp3 expression in human CD4$^+$CD25$^-$ cells in vitro [47]. Another HDAC inhibitor, nicotinamide, increases the number and suppressive capacity of Treg cells in vitro [67]. Also in vivo, administration of HDAC inhibitors leads to increased numbers of Foxp3$^+$ T cells with enhanced suppressive capacity. Moreover, treatment with HDAC inhibitors reduces clinical pathology of RA [68], by enhancing Treg function.

12. Indirect Approaches to Enhance Treg Cell Function

In addition to the above-described strategies that target the Treg cells directly, indirect approaches can also be used to enhance Treg function in patients with various autoimmune diseases. These include viz. reducing the proinflammatory environment and enhancing responsiveness of effector cells to suppression.

12.1 Inhibition of pro-inflammatory cytokines

The in vivo pro-inflammatory environment at the site of inflammation in patients with autoimmune disease negatively effects Treg cell function. Thus, inhibiting pro-inflammatory cytokines, can indirectly lead to better Treg-cell mediated suppression. This is clearly shown by a recent study which examined Treg cell function in RA patients before and after anti-TNF-α (infliximab) therapy in which impaired Treg cell function was completely restored after infliximab treatment [30]. Thus, neutralization of high TNF-α levels in these patients directly reduced the down-regulating effect of TNF-α on Treg cells, thereby restoring their suppressive function [30].

12.2 Enhancing the responsiveness of effector cells to suppression

Indirect improvement of Treg cell function can also be achieved by enhancing responsiveness of effector cells to suppression. This can partially be achieved by blocking the production of pro-inflammatory cytokines. The increased resistance of effector cells at the site of inflammation in experimental autoimmune encephalomyelitis (EAE) model of mice is caused mainly by TNF-α and IL-6 [69]and IL-6 has been reported to act on effector cells rather than on Treg cells [70]. Similarly IL-7, which is known to reduce Treg-mediated suppression also targets effector cells [29]. Therefore, blocking these pro-inflammatory cytokines reduces the resistance of effector cells to suppression and thus enhance control of inflammation by Treg cells.

13. Combination therapy

Clinical outcome can be enhanced by a genuine combination of both direct and indirect strategies. Both nasal administration of heat shock protein (HSP)-60 peptide as well as a single dose of anti-TNF-α (etanercept) treatment, has been reported to reduce arthritis scores. However, combining the two therapies resulted in a highly significant improvement of disease with reduced joint destruction [71]. Also in humans there is evidence for enhanced effectiveness of Treg cell induction, when combined with anti-inflammatory treatment. Together, these data undoubtedly demonstrate that combining Treg induction with anti-inflammatory treatment regimens enhances clinical outcomes. In addition to increased effectiveness, dampening ongoing inflammation is also crucial in preventing adverse effects, since in a pro-inflammatory environment TGF-β produced by Treg cells drives Th17 differentiation [72]and Treg cells can convert into Th17 cells themselves [70, 73].

14. Pitfalls of Cellular Therapy

Several pitfalls can be possible in the treatment of patients with Treg cells. First, incomplete lineage commitment would enable some human $CD4^+Foxp3^+$ cells to express nuclear factor receptor-related orphan receptor (Ror)-γt and to develop into IL-17-producing Th17 effector cells [70,74].Also some fully differentiated Treg cells may be unstable and could be able to trans-differentiate in vivo into effector memory T cells that produce pathogenic cytokines such as IFN-γ, and lead to exacerbation rather than attenuation of the autoimmune process. Second, Treg cell may be less effective in suppressing effector T cells in an autoimmune setting, and may even augment IL-17 production in vitro [75]. Third, it is unclear whether a large polyclonal population of Treg cell would be able to interrupt an autoimmune process that targets antigens found in specific tissues. Fourth, polyclonal Treg cell therapy could result in generalized immuno-suppression and increased susceptibility to various infections. Finally, some patients with autoimmune disease may have poorly appreciated intrinsic defects in Treg cell function that cannot be overcome by infusion of large numbers of these defective cells. In spite of these potential concerns, however, clinical trials are underway to test the therapeutic potential of Treg in graft-versus-host disease [76].

15. Conclusions

Although existing drugs significantly reduce the morbidity and mortality associated with RA, these therapies are not curative. Several drugs that affect Treg cell numbers or function have shown efficacy in the treatment of RA. These results imply that direct administration of Treg cells could be an ideal therapy to induce durable remission of RA, as these cells persist *in vivo* and act in an antigen-specific manner. Thus, approaches that bolster Treg cell numbers and functions could be a fruitful means of selectively and durably inhibiting pathologic inflammation without blocking protective immune responses against infection.

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