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Review article

Regulatory T Cells in Health and Disease

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Abstract

$CD4^+$ T cells are commonly divided into regulatory T (Treg) cells and conventional T helper (Th) cells. Th cells control adaptive immunity against pathogens and cancer by activating other effector immune cells. Treg cells are defined as $CD4^+$ T cells in charge of suppressing potentially deleterious activities of Th cells. Suggested functions for Treg cells include: prevention of autoimmune diseases by maintaining self-tolerance; suppression of allergy, asthma and pathogen-induced immunopathology; feto-maternal tolerance; and oral tolerance. Identification of Treg cells remains problematic, because accumulating evidence suggests that all the presently-used Treg markers (CD25, CTLA-4, GITR, LAG-3, CD127 and Foxp3) represent general T-cell activation markers, rather than being truly Treg-specific. Treg-cell activation is antigen-specific, which implies that suppressive activities of Treg cells are antigen-dependent. The classification of Treg cells as a separate lineage remains controversial because the ability to suppress is not an exclusive Treg property. Suppressive activities attributed to Treg cells may in reality, at least in some experimental settings, be exerted by conventional Th cell subsets, such as Th1, Th2, Th17 and T follicular (Tfh) cells. Recent reports have also demonstrated that $Foxp3^+$ Treg cells may differentiate *in vivo* into conventional effector Th cells, with or without concomitant down regulation of Foxp3

Keywords: FoxP3, immunity, Autoimmunity

Introduction

$CD4^+$ regulatory (Treg) T cells play a central role in the immune system by potently controlling the responses of other immunocytes. The significance of Treg cells in the modification of the immune response was established in the 1990s Their activity appears to be essential not only for the maintenance of immunological self-tolerance but also for the potential control of all physiological immune responses against normal self-protein, microbes or cancerous cells[1,2]. $CD4^+CD25^+$ $FOXP3^+$ regulatory T (Treg) cells can be divided into two groups: thymus derived natural Treg (nTreg) cells and periphery induced adaptive Treg cells (iTreg). Both populations express FOXP3 and suppress immune responses through contact-dependent mechanisms and the production of soluble factors, including the cytokines TGFβ, IL-10 and IL-35[3]. $CD4^+$ cells are a major effector of autoimmune reactions in the heart tissue in RHD patients. Previously, circulating $CD4^+CD25^+$ T-cell

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count were lower in patients with mitral stenosis compared to control subjects. In addition, the ratios between $CD4^+CD25^+$ T cell count, total WBCs and total lymphocytes were significantly lower in patients compared to controls[4]. Also, regulatory $CD4^+CD25^+$ T-cell count was lower in active stage of the disease compared to remission stage[5]. Moreover, autoimmune disorders were prevented in experimental animals exposed to surgical thymectomy with simultaneous transfer of $CD4^+CD25^+$ T cells at the time of surgery[6]. In the light of these findings, Treg replacement was used to prevent autoimmune diseases and/or alleviate their symptoms[7].

Immune Regulation by Regulatory T cells

Several mechanisms contribute to the capacity of the immune system to discriminate self from non-self, facilitating the maintenance of immunological tolerance to self-antigens and the induction of protective immunity to foreign antigens. Alt hough the removal of immature self-reactive lymphocytes by negative selection in the thymus is considered pivotal to the former process, it is becoming increasingly clear that regulatory T cells (Tregs) are equally important in inducing and maintaining peripheral self-tolerance and thus preventing immune pathologies[2]. Two broad categories of Tregs have been described (Fig. 1). The first is the thymus-derived naturally occurring $CD4^+CD25^+$ Treg subset that comprises 1–10% (estimates vary) of the $CD4^+$ T-cell population in healthy adult humans and mice [8-9]. In addition, inducible antigen-specific populations generated a variety of antigenic stimulatory regimes *in vitro* or *in vivo*, have been described[10-11]. These secrete inhibitory cytokines such as interleukin (IL)-10 and transforming growth factor (TGF)-β Together, these cells are thought to play a specialized role in controlling both innate and acquired immune responses[12].

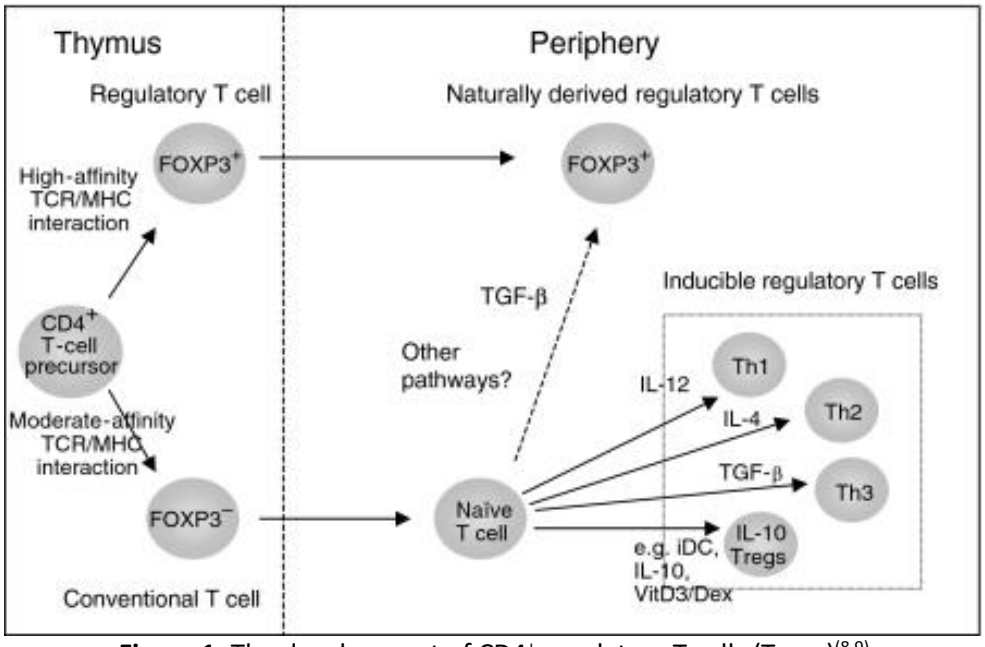

Figure 1: The development of $CD4^+$ regulatory T cells (Tregs)[8,9]

Probably the greatest barrier to fully understanding the function of naturally occurring $CD4^+CD25^+$ Tregs in humans is the lack of specific markers that define these

cells and distinguish them from activated effector T-cell populations and other Treg populations. However, the forkhead (winged helix) transcription factor forkhead box P3 (FOXP3) has been suggested to represent a reliable intracellular marker for naturally occurring Tregs[13]. Patients carrying rare loss-of-function mutations in the *Foxp3* gene develop a range of autoimmune and inflammatory disorders referred to as immunodysregulation, polyendocrinopathy, enteropathy, X-linked syndrome [IPEX; also, known as X-linked autoimmunity and allergic dysregulation (XLAAD) syndrome]. This includes type I diabetes, autoimmune thyroiditis, eczema, bleeding abnormalities and chronic wasting[14]. These individuals also demonstrate an increased susceptibility to infection and an elevated incidence of allergic-type symptoms, including severe eczema, increased serum immunoglobulin E (IgE), eosinophilia and food allergy[15]. The subsequent finding that FOXP3 is a transcription factor selectively expressed in and essential for the development of the $CD4^+CD25^+$ Treg lineage suggests that a defect in the naturally occurring Treg population underlies the clinical manifestations in IPEX patients[16]. More importantly, it implies that the presence and efficient function of Tregs is required to maintain health. However, although FOXP3 in mice seems to be exclusively expressed by $CD4^+$ $CD25^{bright}$ T cells with regulatory function, in humans the situation is less clear, as FOXP3 appears also to be up-regulated upon activation of naïve $CD4^+$ $CD25^-$ T cells and $CD8^+$T cells[17]. Most studies on $CD4^+$Tregs use a combination of CD25, cytotoxic T-lymphocyte-associated antigen (CTLA)-4, FOXP3, IL-10 and/or TGF-β to define Treg populations[14,15]. Ultimately only the demonstration of actual suppressive function confirms the presence of Tregs. In this review, we focus on the evidence for defective (lack of or over-exuberant) naturally occurring and inducible Treg activity in human disease.

Alterations in Regulatory T-Cell Function in Human Disease

Autoimmune disease

Many studies have investigated whether human autoimmune diseases are associated with a defective numbers or function of $CD4^+CD25^+$ Tregs. In patients with multiple sclerosis, purified $CD4^+$ $CD25^{bright}$ Tregs from peripheral blood showed reduced capacity to suppress T-cell proliferation and interferon (IFN)-δ production[18], Similar defects were described in patients with autoimmune polyglandular syndrome type II, type I diabetes, psoriasis and myasthenia gravis[19,20]. Interestingly, the percentage of $CD4^+CD25^+$ or $CD4^+$ $CD25^{bright}$. Tregs in the peripheral blood of these patients was unaltered compared to healthy controls, suggesting that a defect in Tregs "function" rather than "number" contributes to disease[21], this may reflect: i) Inability to distinguish between increased numbers of activated $CD4^+CD25^+$ effector T cells and $CD4^+CD25^+$Tregs. ii) increased migration of $CD4^+CD25^+$ Tregs to the tissues, and/or iii) Refractoriness of effector T cells and/or APCs to regulation. To understand the question of *whether immune suppression by Treg cells is impaired in human autoimmune disease or not?* it is important to recognize the potential means by which such defect may occur. Defects in the number and function of Treg cells, as well as a resistance of effector T cells to Treg cell-mediated suppression, could each contribute to failed T cell regulation[22].

Mechanisms of impaired Tregs function in autoimmune disorders

1- *Inadequate numbers of Treg cells:*
In mouse models, the concept that inadequate numbers of Treg cells may contribute to autoimmunity is supported by the occurrence of aggressive autoimmunity in scurfy mice and the successful treatment of their autoimmunity through the adoptive transfer of wild-type Treg cells[21]. Additionally, there is evidence from mouse models that, under the appropriate conditions, Treg cells can be induced in the periphery to protect from the development of autoimmunity. Multiple factors influence the homeostasis and induction of Treg cells in the periphery, including CD28, IL-2, TGF-β, and DCs[23]. Evidence that an inadequate number of Treg cells leads to autoimmunity in humans is most clearly shown in patients with IPEX, who completely lack Treg cells as a result of a mutation in Foxp3**.** Most patients with autoimmune diseases probably have a more modest reduction in Treg cells[24].

2- Impaired function of Treg cell:
Identifying defects in the function of Treg cells is made difficult both by the multiple mechanisms used by Treg cells to suppress inflammation and by the manner in which suppression is measured. In addition, assessment of Treg cell function in humans requires the use of *in vitro* assays that, owing to the rarity of Treg cells in the peripheral blood, are carried out with low cell numbers, which limits the type and quality of assays that can be done [25]. Current assays of Treg cell function address the ability of Treg cells to inhibit the proliferation of, or cytokine production by, co-cultured effector T cells. Most co-culture assays are carried out with autologous responder T cells and APCs; such studies can define defects in suppression but do not specifically test the function of Treg cells[3]. To determine whether the source of impaired suppression is intrinsic to the Treg cells, investigators have used assays that examined the suppressive function of an individual's Treg cells using effector T cell and/or APC populations isolated from healthy controls. Although these assays provide insight into potential Treg cell defects, they still cannot account for the impact of the local milieu on Treg cell function[25].

3- Resistance of effector T cells to suppression.
The resistance of effector T cells to Treg cells suppressive action has been observed in several animal models of autoimmunity[26]. In these models, inflammation and tissue destruction progress despite the presence of functional Treg cells at the site of inflammation. Such findings suggest that a resistance of effector T cells to Treg cells may contribute to disease progression. This phenomenon has been described in mouse models with diabetes, and in the experimental autoimmune encephalomyelitis (EAE)[27]. There are multiple mechanisms by which effector T cells would become resistant to suppression; this include T cell-intrinsic defects, alterations in the strength of T cell activation and the exposure to T cell growth factors[28].

Allergic Diseases

Studies from at least three separate groups have provided evidence for impaired naturally occurring $CD4^+CD25^+$ Treg-mediated inhibition of allergen-specific T helper type 2 (Th2) responses in allergic patients during active hay fever season[29,30] or in individuals who mount vigorous Th2 responses to allergen[31]. Furthermore, depletion of $CD4^+CD25^+$ T cells from peripheral blood of healthy individuals reveals enhanced proliferative and Th2

cytokine responses to various allergens including milk, nickel and grass[32,33] implying that naturally occurring $CD4^+CD25^+$ Tregs play an active role in suppressing allergen-specific Th2 responses in healthy subjects. Recent evidence also suggests an increased frequency or ratio of $CD4^+CD25^+$ IL-10-secreting T cells in healthy individuals compared with individuals with allergic or asthmatic disease[34-35]. It is unclear whether these cells represent naturally occurring $CD4^+CD25^+$ Tregs or IL-10 Treg that may have been induced to increase CD25 expression upon activation in culture, highlighting the lack of appropriate markers to distinguish the different Tregs in humans. Furthermore, the induction of IL-10-secreting Tregs is impaired in patients with severe asthma who do not show clinical improvement upon steroid treatment (glucocorticoid resistant)[36]. Finally, in situations where tolerance is 'naturally' induced, for example in children who grow out of their allergy to cow's milk or in bee keepers who receive multiple stings, associated increases in IL-10-producing and $CD4^+CD25^+$ Tregs have been reported[37,38]. These studies suggest both naturally occurring $CD4^+CD25^+$ Tregs and IL-10-secreting Treg populations actively control immune responses to allergen in healthy individuals and that their function might be impaired in disease, particularly during chronic antigen exposure, suggesting that novel therapeutic strategies may need to target both Treg populations[39].

Infectious disease

The immune response to infection represents a complex balance between the successful induction of proinflammatory antipathogen responses and anti-inflammatory responses required to limit damage to host tissues. Tregs undoubtedly play an important role in controlling this balance during infection, and the results can range from highly detrimental to the host to highly beneficial to both host and pathogen. The role of both naturally occurring $CD4^+CD25^+$ Treg and IL-10-secre-ting Treg in infection has been the subject of several excellent recent reviews[40-41] and we highlight here a few examples from studies in humans. Recent work on *Helicobacter pylori*-infected individuals suggests that $CD4^+CD25^+$ Tregs might contribute to chronic infection by suppressing appropriate memory T-cell responses to *H. pylori*[42]. This is further supported by the demonstration that infected patients have increased frequencies of $CD4^+CD25^+$ $FOXP3^+$ T cells in the stomach and duodenal mucosa as compared with uninfected controls[43]. Chronic exposure to pathogens might itself result in the induction of a strong immunoregulatory network, mediated by IL-10, and antigen-specific IL-10-producing Treg cells have indeed been isolated from helminth-infected patients [44,45]. $CD4^+CD25^+$ Tregs also appear to be involved in chronic virus infection. In patients with chronic hepatitis B virus (HBV) and hepatitis C virus (HCV) infection, increases in peripheral $CD4^+CD25^+$ Treg numbers have been described[46,47] Moreover, depletion of $CD4^+CD25^+$ Tregs from peripheral blood from virally infected patients results in increased T-cell responses to HBV, HCV, cytomegalovirus and human immunodeficiency virus (HIV) antigens, implying that the presence of $CD4^+CD25^+$ Tregs prevents effective antiviral immunity[46]. A recent study on HIV-infected patients demonstrated that decreased Treg numbers were associated with immune hyperactivation in these patients[48]. Immune hyperactivation, however, is associated with disease progression, indicating that the presence of Tregs might have some protective effect in HIV infection.

Indeed, a recent study showed that in asymptomatic HIV-infected patients strong HIV-specific Treg function *in vitro* was correlated with lower levels of plasma viraemia and higher $CD4^+$:$CD8^+$ T-cell ratios. These findings suggest that, rather than being detrimental to immunity to infection, intact Treg activity may be beneficial to HIV-infected patients[49].

Cancer

Evidence from cancer patients suggests that increased Treg activity may be associated with poor immune responses to tumor antigens and contribute to immune dysfunction. High numbers of $CD4^+CD25^+$ Tregs have been found in lung, pancreatic, breast, liver and skin cancer patients, either in the blood or in the tumor itself[50,51]. These Tregs were able to inhibit proliferation and IFN-γ production by $CD4^+$ and $CD8^+$ T cells, as well as natural killer (NK) cell-mediated cytotoxicity. A recent study on ovarian carcinoma patients elegantly demonstrated that the presence of $CD4^+CD25^+$ $FOXP3^+$ Tregs that suppress tumor-specific T-cell immunity inversely correlated with survival[52]. These Tregs preferentially moved to and accumulated in the tumor and ascites, but not the draining lymph nodes, with evidence for a role of the chemokine CCL22 in directing Treg homing to the tumor. In addition to $CD4^+CD25^+$ Tregs, IL-10-producing Tregs may also contribute to ineffective anti-tumor responses in cancer patients. Both $CD4^+CD25^+$ and IL-10-producing Tregs are found in Hodgkin lymphoma infiltrating lymphocytes, which suppress mitogen- and antigen-specific peripheral blood mononuclear cell responses[53]. A recent study reported that internalization of primary myeloma cells by dendritic cells (DCs) resulted in IL-10 production, but no IL-12 production and these DCs stimulated the generation of IL-10-producing T cells[54]. Besides IL-10, TGF-β produced by various cell types, including Tregs, may inhibit the development of effective tumor immunity *in vivo*. Thus, for cancer immunotherapy, strategies that deplete Tregs, inhibit their function or block their migration, rather than enhance or restore their function, are likely to be advantageous[55].

Potential Therapeutic Use of Tregs

The study of Treg cells has progressed into a new level where the need to demonstrate their existence has been replaced by the need to understand their potential therapeutic use. The alterations in distribution and function of $CD4^+CD25^{high}$ Tregs in autoimmune and rheumatic diseases suggest a role for the therapeutic use of these cells. In mice with collage-induced arthritis, depletion of $CD4^+CD25^{high}$ Tregs causes rapid progression, and the transfer of isolated and *ex vivo*-proliferated $CD4^+CD25^{high}$ Tregs can reverse early joint damage[56] Administration of $CD4^+CD25^{high}$ regulatory T cell also yields improvement in murine models of colitis, autoimmune encephalomyelitis, diabetes, and allogeneic transplantion[57]. Human research has shown that some established therapies may promote $CD4^+CD25^{high}$ regulatory T cell development and survival *in vivo*. For instance, monoclonal antibody to CD20 (rituximab), which depletes B cells, leads to a selective increase in $CD4^+CD25^{high}$ Tregs[58]. Polyclonal antibody therapies, such as anti-lymphocyte serum (ALS) and anti-thymocyte globulin (ATG), have been shown to preferentially deplete T effector cells, and induce $CD4^+CD25^{high}$ Tregs. Rapamycin preferentially expands $CD4^+CD25^{high}$ Tregs, therefore, a major therapeutic effect of rapamycin may be the induction of tolerogenic $CD4^+CD25^{high}$ Tregs *in vivo*[59] Besides these established

therapies, research has focused on cytokine related therapies to modulate $CD4^+CD25^{high}$ regulatory T cell function. Among candidate cytokines are growth factors in the IL-2 family. Several studies have reported that these cytokines enhance immune regulation by $CD4^+CD25^{high}$ Tregs. For instance, IL-7 and IL-15 are involved in the preservation of optimal suppressive function by $CD4^+CD25^{high}$ Tregs [60]. In addition, IL-15 administration alone induces *de novo* generation of $CD4^+CD25^{high}$ Tregs[61]. In contrast to T cell growth factors, pro-inflammatory cytokines have been shown to inhibit function of $CD4^+CD25^{high}$ Tregs, possibly *via* the promotion of Th17 development. Therefore, anti-TNF-α, anti-IL1, anti-IL-6, and anti-IL-21 therapies may affect inflammation not only by direct inhibition of the pro-inflammatory cytokines but also by reestablishment of immune regulation by $CD4^+CD25^{high}$ Tregs. On the other hand, short term treatment with high dose CTLA-4Ig (abatacept), which has been shown to have anti-inflammatory properties in arthritis, leads to a quick loss of $CD4^+CD25^{high}$ Tregs and, in some animal models, exacerbation of autoimmunity[62]. Direct transfusion of $CD4^+CD25^{high}$ regulatory T cell in humans is being explored as a therapy in patients with stem cell transplantation. Despite encouraging data from animal models and early human trials, a number of issues must be resolved for optimal use of $CD4^+CD25^{high}$ Tregs as a therapy[63]. Firstly, there are likely to be differences in the specific role of Tregs in particular diseases. Secondly, regulatory T cell-specific surface markers remain elusive, which delays the isolation of pure populations of $CD4^+CD25^{high}$ Tregs. Third, the use of autologous $CD4^+CD25^{high}$ regulatory T cell clones for particular autoantigens would increase the effectiveness and decrease potential side effects of "bystander" suppression. This will require techniques for identifying and expanding antigen specific clones of $CD4^+CD25^{high}$ Tregs. Successes with $CD4^+CD25^{high}$ regulatory T cell expansion using rapamycin are promising in this regard[64]. Lastly, the fate of transfused $CD4^+CD25^{high}$ Tregs *in vivo* is not fully known. In the unlikely event that $CD4^+CD25^{high}$ Tregs expand into tumor/effector cells or simply become broadly immunosuppressive, there needs to be a way to eliminate them from the body. Future therapies may require the use of "designer" $CD4^+CD25^{high}$ Tregs that have been modified by gene transfer to selectively express preferred proteins including antigen specific TCR, homing receptors, cytokines, and "suicide" genes. Nevertheless, the manipulation of $CD4^+CD25^{high}$ Tregs function shows great promise as a novel therapeutic option in autoimmune and rheumatic diseases[63]

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