CD4+CD25+Foxp3+ regulatory T cells: from basic research to potential therapeutic use

Christian Mottet, Dela Golshayan

Division of Gastroenterology & Hepatology, BH-10N-545, Centre Hospitalier Universitaire Vaudois (CHUV), Lausanne, Switzerland
Division of Nephrology & Transplantation Centre, Centre Hospitalier Universitaire Vaudois (CHUV), Lausanne, Switzerland

Summary

Regulatory T cells control immune responses to self- and foreign-antigens and play a major role in maintaining the balance between immunity and tolerance. This article reviews recent key developments in the field of CD4+CD25+Foxp3+ regulatory T (TREG) cells. It presents their characteristics and describes their range of activity and mechanisms of action. Some models of diseases triggered by the imbalance between TREG cells and effector pathogenic T cells are described and their potential therapeutic applications in humans are outlined.

Key words: CD4+CD25+ Foxp3+ regulatory T cells; tolerance; review

Introduction

The existence of cell-subsets specialised in suppressing immune responses was postulated in the early seventies by Gershon and colleagues [1]. Nevertheless, convincing evidence came only in the mid eighties from data obtained by Sakaguchi et al. [2] and by Don Mason and colleagues [3, 4] using experimental autoimmune disease mouse or rat models. Progress was hampered until the mid-nineties by the lack of reliable phenotypical markers. The breakthrough in the modern era of regulatory T cells is almost certainly due to the work of Sakaguchi and colleagues, who in 1995 described a subpopulation of CD4+ T cells with high cell surface expression of the interleukin-2 receptor (IL-2R)α-chain, also called CD25, which was essential for the prevention of autoimmunity [5].

This review focuses on naturally occurring CD4+CD25+ Foxp3+ T (TREG) cells. It stresses recent key progress in defining their characteristics and focuses on the transcription factor Foxp3, IL-2 and the components of the high-affinity IL-2 receptor, as these molecules are essential for the development, function and survival of TREG cells. It also describes the TREG cells' mechanisms of action and their potential therapeutic applications either in enhancing their regulatory activity in inflammatory diseases such as autoimmune diabetes, allograft rejection, graft versus host disease (GVHD) and allergic diseases, or in blocking their suppressive activity in tumour immunity or vaccine development.

A definition according to function

Immunoregulation is an active process in which one population of cells controls the activity of another cell population. So far, various populations of T cells with regulatory properties have been characterised in vitro and in vivo and have...
been implicated in the control of autoimmune diseases, allotransplantation tolerance and anti-tumour immune responses. Naturally occurring regulatory T cells have been identified in non-manipulated rodents and humans, and comprise cells of the adaptive immune system (CD4+CD25+Foxp3+ T cells) and of the innate immune system (natural killer [NK] T cells) [6-8]. Apart from these spontaneously arising regulatory T cells, it has been reported that an uncommitted T cell can be skewed towards a regulatory function by in vitro or in vivo manipulation. Repetitive in vitro stimulation with immature dendritic cells (DC) or in the presence of suppressive cytokines such as IL-10 or TGF-β can induce regulatory T cells. In vivo, these cells have been identified in the course of tolerance induction protocols. Regulatory T cells of the CD4+ subset, such as Th1 and Th3 cells [9-11], CD8+ (CD8+CD28- T cells) [12], double negative CD3+CD4+CD8- T cells [13], and γδ T cell [14] subsets have been functionally characterised in in vitro and in vivo settings. We will not discuss these cells further and will focus on the naturally occurring CD4+CD25+Foxp3+ T cells (TREG).

TREG cells are defined operationally by their functional ability to regulate/suppress immune responses. Per se, TREG cells are hyporesponsive to T cell receptor (TCR) stimulation in vitro, but exogenous IL-2, strong co-stimulation with increasing concentrations of anti-CD28 monoclonal antibody (mAb) or stimulation bypassing the TCR (such as mitogens) can overcome their anergic state [7]. On polyclonal or antigen-specific TCR stimulation, TREG cells potently suppress the proliferation and cytokine production of effector CD4+ and CD8+ T cells by inhibiting IL-2 gene transcription [15-18]; they also inhibit the proliferation and antibody production of B cells [19]. The induction of the suppressive function of TREG is antigen-specific and TREG cells appear to have a higher avidity for specific antigens as compared to their CD4+CD25+ counterparts, as their suppressive activity is elicited at a 10-100-fold lower concentration of a specific peptide [18]. Several studies have implied that the regulation mediated by TREG cells was dependent on a continuous supply of allo-antigens [20] or tissue-specific target auto-antigens [21, 22], as removal of the source of tissue antigens may lead to rapid contraction of the pool of tissue-specific TREG cells.

In contrast to their in vitro resistance to proliferation upon TCR stimulation, TREG cells show active proliferation in vivo following antigenic stimulation [23, 24]. Naturally arising TREG cells play a key role in the maintenance of peripheral dominant tolerance to self- and alloantigens in vivo (reviewed in [7, 25]). Depletion of CD4+CD25+ T cells induces effective tumour immunity [26–29], enhances immune responses to invading microbes, triggers allergic responses to innocuous environmental substances and breaks foeto-maternal tolerance during pregnancy [30]. On the other hand, TREG cells have been found to suppress a number of T cell-mediated immune pathologies, including allergic responses and auto-immune diseases such as type 1 diabetes (T1D) [31], experimental autoimmune encephalomyelitis (EAE) [32-34], gastritis, colitis [35], glomerulonephritis, and polyarthritis [5, 36] as well as allo-graft rejection [37–40] and GVHD [41–43].

**Phenotypic characterisation**

Naturally occurring CD4+CD25+ T cells develop in the thymus [44] and constitute some 5–10% of peripheral CD4+ T cells in non-manipulated normal mice and humans [45-47], but in humans only the CD4+CD25+Foxp3+ cells which constitute 2-3% of the CD4+ T cells are really regulatory [48]. To date the best surface marker correlating with suppressive activity is CD25 (Figure 1). However, this marker is not specific as its up-regulation following cell activation does not confer regulatory properties, and it has been demonstrated that cells with regulatory properties can also be found in the CD4+CD25+ T cell pool [49, 50]. The naturally occurring CD4+CD25+ T cell population also expresses the cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) [35, 51] and the glucocorticoid-induced tumour necrosis factor (GITR) [52, 53], but, like CD25, these molecules are also up-regulated following activation of naïve CD4+CD25- T cells. When compared to the CD4+CD25+ population, the TREG population expresses low levels of CD45RB and contains increased frequencies of CD62Llo T, indicating an activated/primed or memory state.

The αβ-integrin (CD103), an integrin which recognises epithelial cadherin responsible for tissue-specific retention of lymphocytes, was shown to identify a subpopulation of TREG cells [54] and to be important in the homing and retention of TREG cells in inflamed tissues [55]. CD103 is expressed in more than 90% of intestinal and extraintestinal (bronchi, inflammatory skin/breast/salivary glands, tumour epithelium) intraepithelial T lymphocytes, while it is only expressed by 0.5-5% of lymphocytes in peripheral blood and lymphoid organs. TREG cells also express specific chemokine receptors such as CCR4, CCR5 and CCR8. Mature DC producing CCL17 and CCL22 were found to preferentially attract TREG cells to sites of antigen presentation in secondary lymphoid organs and peripheral inflamed areas [56]. TREG cells have also been shown to be attracted by CCL4, a chemokine expressed by activated B cells, DC and macrophages [19], and their
Phenotypic markers characteristic of naturally occurring TREG cells.

Mechanisms of regulation
Contact-dependent versus cytokine-mediated effect
TREG cells’ precise mechanisms of action are still unclear, with divergent conclusions regarding the importance of cell-cell contact versus cytokines in their suppressive function (Figure 2). The discrepant results might be explained by differences in the experimental systems (in vitro versus in vivo), the various disease models studied, the pathogenic effector mechanisms and target organs involved, and the contribution of the genetic background of the mouse strains used.

Role of cell surface molecules
In vitro, TREG cells have been shown to inhibit the activation of effector CD4+CD25− T cells by a mechanism which depends on cell-cell contact rather than on soluble mediators [16]. CTLA-4 [35, 51], membrane-bound transforming growth factor-β (TGF-β) [73], GITR [52, 53], and galectin-1 [59] appear to be functionally important molecules since in various models monoclonal antibodies (mAbs) against these molecules have been shown to overcome the suppression mediated by TREG cells in vitro and/or in vivo. Moreover, after activation, human TREG cells were shown to be able to directly kill activated CD4+...
and CD8+ T cells in a perforin or granzyme-dependent way [74].

Direct T-T interactions via surface molecules may mediate suppression in vitro, since activated TREG cells have been shown to suppress the proliferation of effector T cells in response to TCR stimulation in the complete absence of antigen-presenting cells (APC) [7]. TREG cells may also down-modulate the function of APC and render them unable to activate effector T cells. It was shown that co-culture with activated TREG cells leads to reduced amounts of costimulatory molecules on DC and B cells [75], inhibiting their immunogenic properties. “Tolerogenic” DCs may in turn induce other regulatory cells, thus contributing to the maintenance of tolerance.

In vivo, however, it is likely that more complex interactions between TREG cells, effector T cells and APC/DC determine the resulting immune response (“ménage à trois”). Another way in which TREG cells could mediate their immune suppressive properties is to induce differentiation of naïve T cells into cells with a regulatory function rather than into pathogenic effector T cells. This phenomenon has been termed “infectious tolerance”, and obvious candidate molecules mediating this effect may be regulatory cytokines such as IL-10 or TGF-β [76-78].

CTLA-4

A large proportion of TREG cells express CTLA-4 in both mouse and human. CTLA-4 is known to be a negative regulator of T-cell activation but also to play a key role in immunological self-tolerance. Non-activating anti-CTLA-4 mAbs have been shown to block the suppressor activity of TREG cells in vitro and in vivo [35, 51], and mice deficient in CTLA-4 develop a fatal lymphoproliferative disease and multiorgan inflammation [79]. The role of CTLA-4 expression in the homeostasis and function of TREG cells remains however controversial [80].

Role of soluble cytokines (IL-10, TGF-β)

IL-10

In vivo models of colitis [81-83] and infection [84] show that IL-10 is often crucial for the maintenance of immune homeostasis and regulation mediated by TREG cells. In some transplantation models anti-IL-10 or anti-IL-10R mAbs abrogated the induction of peripheral tolerance by otherwise previously proven robust tolerogenic protocols [39]. IL-10 is produced by a large number of immune cells such as T cells, monocytes, macrophages and epithelial cells, and has pleiotropic effects on B, T and NK cells, DC and mast cells. Signalling through the IL-10/IL-10R results in potent inhibition of cell proliferation, of pro-inflammatory cytokine production and of the maturation and antigen presentation of DC [78]. IL-10 also plays a pivotal role in TREG cell function, CD4+CD25+ T cells from IL-10−/− mice having been shown to be significantly less potent than their wild type counterparts. Using a T cell-mediated model of murine colitis, Powrie’s group has shown that intestinal inflammation mediated by the transfer of naïve CD4+CD25+ T cells can be cured by the transfer of TREG cells. In this model, during the cure of colitis, IL-10-secreting Foxp3+ T cells selectively enriched within the colonic lamina propria. The administration of an anti-IL-10R mAb to mice treated with TREG cells completely abolished the cure. These findings were further confirmed by analysis of human colonic samples, where an accumulation of
Foxp3+CD4+CD25+ T cells was found in the lamina propria of patients with IBD, diverticulitis, pseudomembranous and CMV colitis [85, 86].

**TGF-β**

TGF-β is the prototype of a family of polypeptides involved in growth control, extracellular matrix production and development. Three isoforms of TGF-β exist in mammals (TGF-β1, -β2, and -β3) and TGF-β1 has been identified as an immunoregulatory molecule with both immunogenic and immunosuppressive properties depending on the cellular environment [77]. Most models of immune regulation showing a role of IL-10 also implicate TGF-β, the production and action of these two cytokines being interrelated and likely to involve positive feedback loops in which IL-10 enhances expression of TGF-β and vice versa. IL-10 may act locally at the site of inflammation, while TGF-β seems to have a more systemic effect on the immune response. The suppressive activity of TGF-β is best highlighted by the fact that TGF-β-deficient mice develop a lethal lymphoproliferative disease [87]. The results concerning the requirement for TGF-β expression by T REG in vitro and in vivo are controversial [73, 88, 89], and it appears that regulation is dictated primarily by the responsiveness of the effector T cell to TGF-β [90, 91].

---

**Models of diseases triggered by imbalance between T REG and effector T cells**

**Autoimmune and allergic diseases**

There is now a mass of evidence that T REG actively suppress the activation and expansion of self-reactive T cells in vivo and thereby the development of autoimmune diseases. Neonatal thymectomy or depletion of T REG cells from normal animals result in spontaneous development of various organ-specific autoimmune diseases (such as gastritis, IBD, T1D, EAE, myocarditis, depending on the genetic background of the strains of mice used) and increase allosensitivity to skin allografts [5, 7] (Figure 3). In these cases, autoimmunity is prevented by reconstitution of the mice with wild type T REG cells. In the regulation of allergy, T REG cells have been shown to be effective in suppressing the production of antigen-specific IgE in TCR transgenic murine models [92].

In humans, a study by Danke et al. illustrates the fact that T cells reactive to target self-antigens, such as glutamic acid decarboxylase, which is involved in T1D, could be easily expanded in vitro from peripheral blood of normal individuals only after depleting the CD25+ subset [93]. These results indicate that self-reactive T cells are present in most individuals but their pathogenic potential is kept in check by coexisting T REG cells. IPEX as a prototype of disease induced by an intrinsic defect of T REG cells is thus far the clearest example showing that an abnormality in the pool of naturally arising T REG cells is a primary cause of autoimmune diseases in humans, and that T REG cells are crucial in the maintenance of dominant self-tolerance. Alterations in the numbers or functional activity of T REG cells, in peripheral blood or
target organs, have also been associated with several human chronic inflammatory and autoimmune diseases such as multiple sclerosis [94], rheumatoid arthritis [95-97], T1D [98], IBD [99], systemic lupus erythematosus [100], polyglandular syndrome type II [101], myasthenia gravis [102], allergic asthma [103], cow’s milk allergy [104], nickel allergy [105] and atopic dermatitis [106].

**Infectious diseases**

TREG cells probably participate in the immune response to all infectious agents, since apart from regulating adaptive immune responses they have been shown to be also capable of directly suppressing innate immune responses in a model of murine pathogen-mediated colitis [84]. How TREG cell activity is regulated to allow effective immune responses towards pathogens without pathological anti-self reactivity has baffled immunologists [107].

Activation of DCs through Toll like Receptor (TLR) signalling can overcome dominant TREG cell suppression in vitro and enhance effector T cell responses [108]. TREG cells appear however to restrain too vigorous immune reactivity, which in many chronic infections will benefit the host by limiting tissue damage. Current evidence suggests that in the course of an infection TLR signalling would directly regulate the suppressive function of TREG cells by augmenting TREG cell proliferation with a temporal loss of their suppressive activity. The TREG cells would recover their suppressive activity when the infection has subsided, in time to limit potential autoimmunity that could result from overactivated effector mechanisms. It therefore appears that the balance between TREG cells and effector T cells depends on the activation status of the innate immune system and particularly the DC.

In many chronic infections TREG cells appear to restrain immune reactivity in order to limit host tissue damage, but this may handicap the efficacy of protective immunity and clearance of pathogens [107]. Studies on persistent chronic infections, such as herpes simplex virus (HSV), hepatitis C virus (HCV) and HIV, have shown that the presence of TREG cells at the time of infection may affect the magnitude of protective immunity and the outcome of infection [109-113]. When TREG cells were depleted, animals developed memory responses and could subsequently mount better recall responses. TREG cells were increased in number and activity in chronic HCV patients compared to controls who resolved their infections. Such TREG cells isolated from peripheral blood, and in one instance from the liver itself, modulated peptide-specific proliferative responses and maturation of HCV-specific CD8+ T cells. Similarly, reduction of tissue-damaging immunopathology by TREG cell function has been observed in some parasitic infections such as Pneumocystis carinii, Leishmania major and Schistosoma masoni [114, 115].

**Transplantation**

In the transplant setting, circulating alloreactive T cells are crucial in the initiation and the coordination of the rejection response and, to promote tolerance, it is important to deplete or minimise the alloreactive effector T cell pool while enhancing regulatory mechanisms. Various strategies, targeting T cell activation, expansion and/or effector function, have been described as a means of achieving robust peripheral transplantation tolerance in experimental protocols [116]. More recent studies have shown that in many of these protocols immunoregulatory mechanisms dependent on TREG cells were critical in the induction and maintenance of peripheral tolerance. Thus, after a short course of immunomodulatory drugs, such as non-depleting anti-T cell mAbs or costimulatory blockade, donor alloantigen-specific TREG cells are generated which are capable, on adoptive transfer, of suppressing rejection of donor allografts mediated by naïve recipient CD4+ or CD8+ T cells [37-40]. We have also reported that *in vivo* allo-responses can be harnessed by donor alloantigen-specific TREG cells selected and expanded *in vitro* [117].

In murine models of allogeneic bone marrow transplantation (BMT), it has similarly been shown that freshly isolated or *ex vivo* expanded donor-derived TREG cells can delay or even prevent GVHD, and that the selective depletion of TREG cells in the transplant results in increased severity of acute GVHD [41-43]. Patients who developed acute or chronic GVHD after allogeneic BMT had a decreased number of peripheral and tissue infiltrating TREG cells [118, 119]. Intestinal mucosal specimens without histological signs of GVHD showed increased ratios of FOXP3+/CD8+ T cells compared to samples with histological signs of acute and chronic GVHD, providing further evidence that GVHD lesions are associated with lack of regulation by TREG cells.

**Tumour immunity**

Depletion of TREG cells using CD25-specific mAbs has been shown to promote rejection of several transplantable murine tumour cell lines, including melanoma, fibrosarcoma, leukaemia and colorectal carcinoma. These studies imply that TREG cells normally inhibit the generation of effective T cell-dependent anti-tumour immune responses [27-29]. These finding have been confirmed in the clinical setting, where the prevalence of TREG cells was found to be increased in the peripheral blood and tumour microenvironment of cancer patients [26, 120-123].
Therapeutic potential of TREG cells

Approaches to prevention or treatment of T cell-mediated diseases, such as autoimmunity and transplant rejection, have focused historically on potent immunosuppressive drugs non-specifically targeting T cell responses. However, the improved survival rates of allografts and the better outcome of autoimmune diseases have come at a cost, with increased frequencies of drug-related adverse effects. A better understanding of the role of naturally occurring TREG cells in the maintenance of immune homeostasis has prompted researchers to investigate their therapeutic potential. TREG cells could be used as an immunotherapeutic tool, either by enhancing their activity in inflammatory diseases such as autoimmunity, allograft rejection or GVHD, or by blocking their suppressive activity in tumour immunity or vaccine development.

If TREG cells are to be used in immunotherapy aiming at the prevention or treatment of autoimmune diseases or allograft rejection, antigen-specific cells are needed which can specifically control immune responses to the relevant auto- or allo-antigens, while allowing protective immune responses to pathogens. Furthermore, because of the low precursor frequency of alloantigen cross-reactive TREG cells expected in a normal individual without prior antigen exposure, this specific population needs to be expanded in vivo or generated in vitro and their dynamical properties, as demonstrated by adoptive transfer experiments [117, 124–126]. Importantly, we have shown that in vivo manipulations did not modify their in vivo homeostasis, migration patterns or suppressive functions, as demonstrated by adoptive transfer experiments [117]. Alternatively, immunomodulatory drugs, such as rapamycin, could be used to selectively expand TREG cells in vivo while controlling the effector T cell pool [127].

The other side of the coin is that there is now substantial evidence to show that the presence of TREG cells is deleterious in cancer patients and their dynamical properties, as demonstrated by adoptive transfer experiments [117, 124–126]. Importantly, we have shown that in vivo manipulations did not modify their in vivo homeostasis, migration patterns or suppressive functions, as demonstrated by adoptive transfer experiments [117]. Alternatively, immunomodulatory drugs, such as rapamycin, could be used to selectively expand TREG cells in vivo while controlling the effector T cell pool [127].

Conclusion

These novel therapeutic approaches are not without risks. The key to success will be to establish the correct balance between TREG cells and effector cells. These exciting new therapeutic concepts will undoubtedly act as a spur to the translational research community over the next few years. Indeed, steady progress in our understanding of TREG cell function in vivo and their dynamics with pathogenic T cells provides the rationale for a novel form of individualised “tailored” medicine using TREG cell-based therapies in the prevention and treatment of autoimmune diseases and in transplantation.

References


Correspondence:
Dela Golshayan, MD
Division of Nephrology and Transplantation Centre,
Centre Hospitalier Universitaire Vaudois (CHUV)
CH-1011 Lausanne
Switzerland
E-Mail: dela.golshayan@chuv.ch


38. Stephens LA, Mason D. CD25 is a marker for CD4+ thymocytes that prevent autoimmune diabetes in rats, but peripheral T cells with this function are found in both CD4+CD25− and CD4+CD25− subpopulations [In Process Citation]. J Immunol. 2000;165:1105–10.


The many reasons why you should choose SMW to publish your research

*What Swiss Medical Weekly has to offer:*

- SMW’s impact factor has been steadily rising. The 2005 impact factor is 1.226.
- Open access to the publication via the Internet, therefore wide audience and impact
- Rapid listing in Medline
- LinkOut-button from PubMed with link to the full text website http://www.smw.ch (direct link from each SMW record in PubMed)
- No-nonsense submission – you submit a single copy of your manuscript by e-mail attachment
- Peer review based on a broad spectrum of international academic referees
- Assistance of our professional statistician for every article with statistical analyses
- Fast peer review, by e-mail exchange with the referees
- Prompt decisions based on weekly conferences of the Editorial Board
- Prompt notification on the status of your manuscript by e-mail
- Professional English copy editing
- No page charges and attractive colour offprints at no extra cost

*International Advisory Committee*

Prof. K. E. Juhani Airaksinen, Turku, Finland  
Prof. Anthony Bayes de Luna, Barcelona, Spain  
Prof. Hubert E. Blum, Freiburg, Germany  
Prof. Walter E. Haefeli, Heidelberg, Germany  
Prof. Nino Kuenzli, Los Angeles, USA  
Prof. René Lutter, Amsterdam, The Netherlands  
Prof. Claude Martin, Marseille, France  
Prof. Josef Patsch, Innsbruck, Austria  
Prof. Luigi Tavazzi, Pavia, Italy

We evaluate manuscripts of broad clinical interest from all specialities, including experimental medicine and clinical investigation.

We look forward to receiving your paper!

Guidelines for authors:  
http://www.smw.ch/set_authors.html

*Editorial Board*

Prof. Jean-Michel Dayer, Geneva  
Prof. Peter Gehr, Berne  
Prof. André P. Perruchoud, Basel  
Prof. Andreas Schaffner, Zurich  
(Editor in chief)  
Prof. Werner Straub, Berne  
Prof. Ludwig von Segesser, Lausanne

All manuscripts should be sent in electronic form, to:

EMH Swiss Medical Publishers Ltd.  
SMW Editorial Secretariat  
Farnburgerstrasse 8  
CH-4132 Muttenz

Manuscripts: submission@smw.ch  
Letters to the editor: letters@smw.ch  
Editorial Board: red@smw.ch  
Internet: http://www.smw.ch