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

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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 (T_{REG}) cells. It presents their characteristics and describes their range of activity and mechanisms of action. Some models of diseases triggered by the imbalance between T_{REG} 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 (T_{REG}) 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 T_{REG} cells. It also describes the T_{REG} cells' mechanisms of action and their potential therapeutic applications either in enhancing their regulatory activity in inflammatory diseases such as autoimmunity, allograft rejection, graft versus host disease (GVHD) and allergic diseases, or in blocking their suppressive activity in tumour immunity or vaccine development.

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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

Abbreviations

APC	Antigen-presenting cells
BMT	Bone marrow transplantation
CTLA-4	Cytotoxic T lymphocyte-associated antigen-4
DC	Dendritic cells
EAE	Experimental autoimmune encephalomyelitis
GITR	Glucocorticoid induced tumour necrosis factor
GVHD	Graft versus host disease
IBD	Inflammatory bowel disease
IPEX	Immunodysregulation, Polyendocrinopathy, Enteropathy, X-linked recessive
IL-	Interleukin
mAb	Monoclonal antibody
MHC	Major histocompatibility complex
T1D	Type 1 diabetes
TCR	T cell receptor
TGF-β	Transforming growth factor-β
TLR	Toll like receptor
T_{REG}	$CD4^+CD25^+$ $Foxp3^+$ T
XLAAD	X-linked autoimmunity-allergic dysregulation

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 Tr1 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 (T_{REG}).

T_{REG} cells are defined operationally by their functional ability to regulate/suppress immune responses. Per se, T_{REG} 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, T_{REG} 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 T_{REG} is antigen-specific and T_{REG} 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 T_{REG} 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 T_{REG} cells.

In contrast to their *in vitro* resistance to proliferation upon TCR stimulation, T_{REG} cells show active proliferation *in vivo* following antigenic stimulation [23, 24]. Naturally arising T_{REG} 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, T_{REG} cells have been found to suppress a number of T cell-mediated immune pathologies, including allergic responses and autoimmune 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 allograft 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 $CD4^+CD25^{high}$ 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 T_{REG} population expresses low levels of CD45RB and contains increased frequencies of $CD62L^{high}$ T, indicating an activated/primed or memory state.

The $\alpha_E\beta_7$-integrin (CD103), an integrin which recognises epithelial cadherin responsible for tissue-specific retention of lymphocytes, was shown to identify a subpopulation of T_{REG} cells [54] and to be important in the homing and retention of T_{REG} 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. T_{REG} cells also express specific chemokine receptors such as CCR4, CCR5 and CCR8. Mature DC producing CCL17 and CCL22 were found to preferentially attract T_{REG} cells to sites of antigen presentation in secondary lymphoid organs and peripheral inflamed areas [56]. T_{REG} cells have also been shown to be attracted by CCL4, a chemokine expressed by activated B cells, DC and macrophages [19], and their

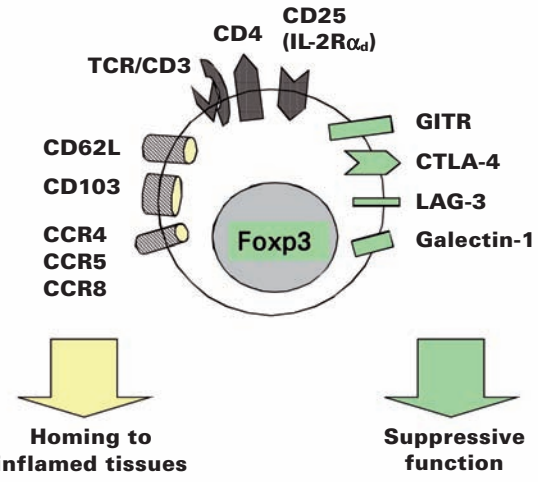

Figure 1

Phenotypic markers characteristic of naturally occurring T_{REG} cells.

homing to allografts was dependent on the CCR4 chemokine receptor pathway [57].

More recently, LAG-3 (CD223) a cell surface CD4-related molecule that binds the major histocompatibility complex (MHC) class II was shown to be selectively expressed on T_{REG} cells up on activation and to be involved in their suppressive function [58]. Lechler's group has also reported that a member of the β-galactoside-binding proteins, galectin-1, was constitutively overexpressed in T_{REG} cells and was an important effector of regulation mediated by these cells [59]. Also, two groups have recently described a critical role for the Wiskott-Aldrich Syndrome Protein (WASP) in the activation and suppressor function of T_{REG} cells which implicates T_{REG} cell dysfunction in the autoimmunity associated with the Wiskott-Aldrich syndrome [60, 61].

Key roles of Foxp3 and of the IL-2/IL-2 receptor components

In addition to CD25 expressed on the cell surface, the transcription factor Foxp3 was shown to be a highly specific intracellular marker for T_{REG} cells [62-64]. Foxp3, the cytokine IL-2, and CD25 as a component of the IL-2 receptor, are essential for the development, function and survival of T_{REG} cells because mutation or polymorphisms in the genes encoding these molecules are causative of, or predispose to, autoimmune diseases in both rodents and humans. The *Foxp3* gene was identified as the defective gene in the scurfy mouse strain, whose phenotype is an X-linked recessive mutant with lethality in males within a month after birth due to excessive activation of $CD4^+$ T cells and overproduction of pro-inflammatory cytokines [65]. Mutations of the human ortholog *Foxp3* gene were subsequently found to be the cause of the immune dysregulation, polyendocrinopathy, and enteropathy, X-linked recessive (IPEX) and the X-linked autoimmunity-allergic dysregulation (XLAAD) syndromes. Patients carrying these mutations develop organ-specific autoimmune diseases such as T1D, thyroiditis, inflammatory bowel disease (IBD), allergic dermatitis, food allergy, haematological disorders, and serious infections [66–68]. The specific role of Foxp3 in the function of T_{REG} cells was highlighted by the fact that retroviral transduction of *Foxp3* into $CD4^+CD25^-$ T cells can convert them to functional T_{REG} cells able to suppress proliferation of other T cells *in vitro* and inhibit the development of autoimmune diseases, such as colitis or T1D, mediated by pathogenic effector T cells in *in vivo* experimental models [7, 63].

IL-2 has been shown to be essential for the generation of T_{REG} cells in the thymus and their survival, expansion and suppressive function in the periphery [69]. IL-2-, and IL-2R-deficient mice develop T-cell lympho-proliferation and lethal autoimmunity, very probably due to lack of activation-induced cell death (AICD) and lack of T_{REG} cells [70, 71]. Furthermore, *in vivo* IL-2 neutralisation by use of an IL-2 blocking antibody also induces autoimmune diseases in mice [72]. The detailed molecular mechanisms of the effects of IL-2 in the homeostasis and suppressive function of T_{REG} cells have still to be clarified.

Mechanisms of regulation

Contact-dependent versus cytokine-mediated effect

T_{REG} 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, T_{REG} 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 T_{REG} cells *in vitro* and/or *in vivo*. Moreover, after activation, human T_{REG} cells were shown to be able to directly kill activated $CD4^+$

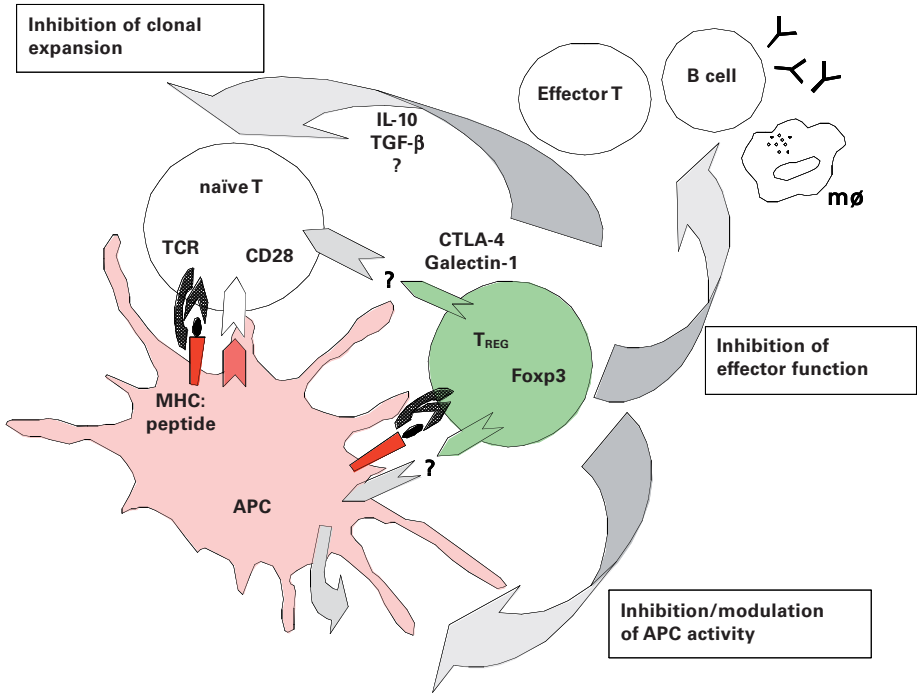

Figure 2

Mechanisms of regulation of immune responses mediated by T_{REG} cells. T_{REG} cells can suppress the activation, clonal expansion and/or the effector function of auto- or alloreactive pathogenic T cells, B cells and macrophages (mϕ), as well as modulate the immunogenicity of APC. The precise mechanisms of action of T_{REG} cells are still unclear, with divergent conclusions regarding the importance of cell-cell contact (via surface-bound molecules) versus secreted cytokines in their suppressive function.

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 T_{REG} 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]. T_{REG} 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 T_{REG} 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 T_{REG} cells, effector T cells and APC/DC determine the resulting immune response ("ménage à trois"). Another way in which T_{REG} cells could mediate their immune suppressive properties is to induce differentiation of naive 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 T_{REG} 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 T_{REG} 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 T_{REG} 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 T_{REG} 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 T_{REG} 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 T_{REG} 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 T_{REG} 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 alloresponses 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

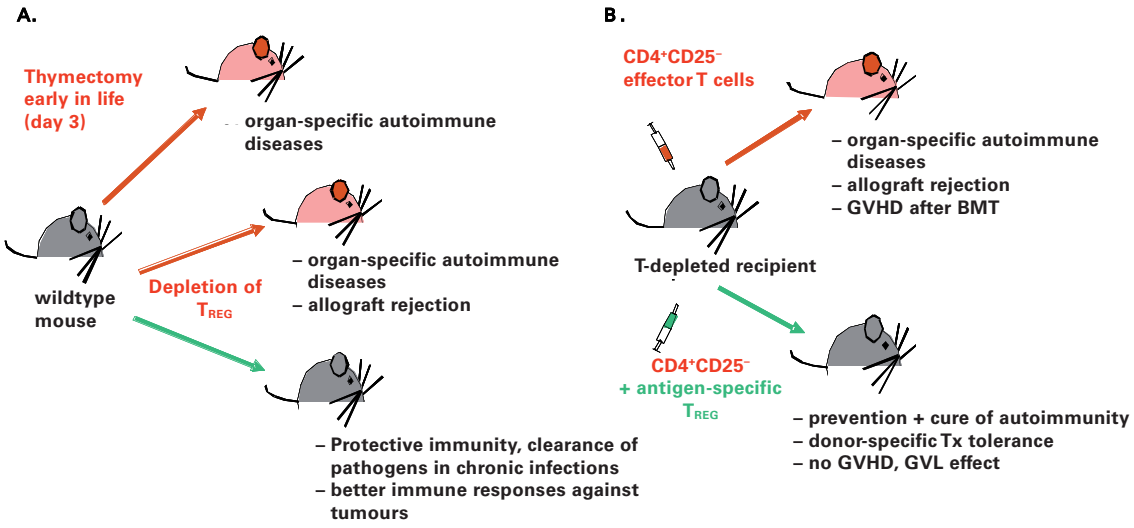

Figure 3

T_{REG} cells regulate immune pathology in vivo.
A. Neonatal thymectomy or depletion of T_{REG} cells from normal animals, results in spontaneous development of autoimmune diseases and increased alloresponses to solid organ allografts, while it enhances immune responses against tumours.
B. In experimental models the co-transfer of T_{REG} cells in lymphopenic hosts protects against immune pathologies induced by effector CD4+CD25- T cells.
GVL: graft versus leukaemia. Tx: transplantation.

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

T_{REG} 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 T_{REG} 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 T_{REG} cell suppression in vitro and enhance effector T cell responses [108]. T_{REG} 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 T_{REG} cells by augmenting T_{REG} cell proliferation with a temporal loss of their suppressive activity. The T_{REG} 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 T_{REG} cells and effector T cells depends on the activation status of the innate immune system and particularly the DC.

In many chronic infections T_{REG} 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 T_{REG} cells at the time of infection may affect the magnitude of protective immunity and the outcome of infection [109–113]. When T_{REG} cells were depleted, animals developed memory responses and could subsequently mount better recall responses. T_{REG} cells were increased in number and activity in chronic HCV patients compared to controls who resolved their infections. Such T_{REG} 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 T_{REG} 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 T_{REG} 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 T_{REG} 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 T_{REG} 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 T_{REG} cells can delay or even prevent GVHD, and that the selective depletion of T_{REG} 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 T_{REG} 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 T_{REG} cells.

Tumour immunity

Depletion of T_{REG} 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 T_{REG} 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 T_{REG} 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 T_{REG} cells in the maintenance of immune homeostasis has prompted researchers to investigate their therapeutic potential. T_{REG} 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 T_{REG} 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 T_{REG} cells expected in a normal individual without prior antigen exposure, this specific population needs to be expanded *in vivo* or generated in large numbers *in vitro* for adoptive transfer. New experimental evidence has shown, in various experimental settings, that antigen-specific T_{REG} cells can be generated and expanded in sufficient quantities *in vitro* without loss of their characteristic phenotype and regulatory properties [117, 124–126]. Importantly, we have shown that *in vitro* 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 T_{REG} 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 T_{REG} cells is deleterious in cancer patients and contributes to the unsuccessful immune responses in some chronic persistent infections. Therefore, depletion of T_{REG} cells in combination with other anti-tumour therapies could optimise eradication of malignancies. Depletion of T_{REG} cells can also be used to boost immune responses in vaccine development or to enhance protective immune responses against invading microbes. This may, however, prevent the induction of long-term infectious immunity or favour the development of autoimmune diseases.

Conclusion

These novel therapeutic approaches are not without risks. The key to success will be to establish the correct balance between T_{REG} 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 T_{REG} cell function *in vivo* and their dynamics with pathogenic T cells provides the rationale for a novel form of individualised "tailored" medicine using T_{REG} cell-based therapies in the prevention and treatment of autoimmune diseases and in transplantation.

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