

Regulatory T cell therapy as individualized medicine for asthma and allergy

Emmanuel Xystrakis, Zoë Urry and Catherine M. Hawrylowicz

Purpose of review

Regulatory T cells have been identified as key players in the maintenance of peripheral tolerance, which prevents inappropriate immune responses to both self-antigens and innocuous allergens. This review aims to provide an update on our current understanding of the therapeutic potential of naturally occurring and adaptive regulatory T cell subsets in allergic and asthmatic disease.

Recent findings

Evidence is emerging that regulatory T cells control aberrant immune responses to allergens in health and exhibit impaired function in active disease. These data provide a rationale for developing therapeutic strategies that promote regulatory T cell numbers or function in patients.

Summary

Preclinical studies of adoptive transfer of antigen-specific regulatory T cells into mouse models of allergic airway disease ameliorate the inflammatory response and in some studies airway hyperresponsiveness. Although these studies are encouraging this remains an invasive and expensive therapeutic protocol for the treatment of allergic disease in humans and a number of concerns relating to safety and efficacy exist. Existing therapies, both allergen specific immunotherapy and nonspecific treatments such as glucocorticoids, induce IL-10 secreting T regulatory populations in patients. Strategies to combine allergen immunotherapy with add-on treatments or adjuvants with the potential to boost regulatory T cells, safety and efficacy remain a major research focus.

Keywords

allergy, IL-10, regulatory T cells

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King's College London, MRC and Asthma UK Centre in Allergic Mechanisms of Asthma, London, UK

Correspondence to Dr Catherine Hawrylowicz, King's College London, MRC and Asthma UK Centre in Allergic Mechanisms of Asthma, Department of Asthma, Allergy and Respiratory Science, 5th Floor Thomas Guy House, Guy's Hospital, London SE1 9RT, UK
Tel: +44 20 7188 0599; fax: +44 20 7403 8640;
e-mail: catherine.hawrylowicz@kcl.ac.uk

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Abbreviation

Treg regulatory T cell

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Introduction

Allergic diseases, including asthma, are defined as chronic inflammatory disorders originating from an aberrant immune response to innocuous antigens. During allergic sensitization, naive CD4⁺ T cells differentiate into allergen-specific T-helper type 2 (Th2) cells producing cytokines such as IL-4, IL-5, and IL-13 that promote the switch of B lymphocytes to IgE production and the recruitment and activation of inflammatory cells following allergen challenge [1,2]. Current therapies, including glucocorticoids and antihistamines, are effective in cross-sections of patients, but remain mostly palliative. Allergen desensitization, although it represents a patient-specific treatment, is successful in only a proportion of cases, is associated with a significant risk for adverse reactions and requires administration over a prolonged period for maximal efficacy. There remains, therefore, a need for novel or improved strategies to provide long-term relief from disease symptoms. Given the capacity of regulatory T cells (Tregs) to suppress disease-promoting immune responses, enhancing Treg function in patients represents an attractive therapeutic strategy for the treatment of allergic disease.

Regulatory T cells

Two broad categories of suppressive T cells have been described: the naturally occurring CD4⁺CD25⁺ Treg and inducible Treg cells secreting inhibitory cytokines, such as IL-10 (IL-10-Treg, Tr1) and TGF- β (Th3).

Naturally occurring Tregs constitute 1–5% of the CD4⁺ T cell population in unmanipulated animals and healthy humans. They were originally defined by their high constitutive level of the IL-2 receptor α chain (CD25) expression. The CD4⁺CD25⁺ phenotype is not unique to Tregs, however, and this lack of specificity is also observed with other surface antigens that are increased in this Treg population for example CTLA-4, GITR [3–6]. Recently expression of the IL-7 receptor, CD127, was proposed to inversely correlate with FoxP3 and suppressive function of human CD4⁺ Tregs and to discriminate between human regulatory and activated T cells [7,8]. Identification of unique surface markers will facilitate Treg purification and functional characterization in human disease.

Natural Tregs inhibit the activation and proliferation of naive T cells, Th1 and Th2 cells, acting through cell

contact-dependent pathways *in vitro* and do not secrete significant quantities of inhibitory cytokines in culture, for example IL-10, TGF- β , when compared with inducible Tregs. Human thymus-derived natural Tregs inhibit Th2 responses less efficiently than Th1 responses [9]. A second study [10] reported that natural Tregs inhibited Th2 differentiation, but were less effective in the suppression of cytokine production and proliferation of established Th2 cells, although preactivation of the CD4+CD25+ T cells *in vitro* induced strong inhibition of Th2 responses. In-vivo inhibition by natural Tregs via cell-contact, through secretion of inhibitory cytokines (e.g. TGF- β , IL-10) or the induction of inhibitory cytokines by adjacent cell populations, have all been described in different models [5].

The transcription factor forkhead box P3 (Foxp3) is essential for lineage commitment, development and suppressor function of natural Tregs. It ensures proliferative fitness and maintenance of Tregs through amplification of its own expression and inhibition of alternative differentiation pathways [11,12]. Foxp3 may play a critical role in maintaining Treg activity through adult life, since deletion of Foxp3+ T cells in adult animals results in lymphoproliferation, expansion of endogenous dendritic cells and premature death [13]. In humans, however, most T cells turn on FoxP3 during early stages of activation without necessarily developing a suppressive activity [14–17]. Moreover, FoxP3+ CD4 cells with genuine regulatory activity do not always express CD25 [7]. Emerging studies in animal models also suggest that Foxp3 expression is not always associated with regulatory function.

Despite the lack of distinct markers, the role of Tregs in preventing the occurrence of allergic disease is strongly implicated by studies of patients with mutations influencing this cell compartment. Immune dysregulation, polyendocrinopathy, enteropathy and X-linked syndrome (IPEX) result from mutations in the Foxp3 locus, which cause functional impairment of the CD4+CD25+ Treg compartment [18,19^{••},20^{••}]. These individuals suffer from severe allergic manifestations and autoimmune diseases from an early age. CD25 deficiency, due to an autosomal recessive mutation in the CD25 gene, has been described in a patient with a normal Foxp3 gene, but with clinical manifestations of IPEX. This patient exhibited defective IL-10 expression by CD4+ T lymphocytes, whereas a Foxp3 deficient patient expressed normal levels of IL-10 [21^{••}]. A large proportion of Wiskott–Aldrich syndrome patients develop autoimmunity and allergy associated with Wiskott–Aldrich syndrome protein (WASP) deficiency. WASP is proposed to control CD4+ CD25+ Foxp3+ Treg function. Although peripheral natural Tregs are reportedly present at normal levels in these individuals they failed to suppress the function of autologous or allogeneic CD4+ T cells *in vitro* and may also be defective

in secreting immunomodulatory cytokines such as IL-10 [22^{••},23^{••}].

In parallel with these genetic studies, functional analyses [24–27] have demonstrated that depletion of CD4+CD25+ T cells from the peripheral blood of healthy nonallergic donors enhances their in-vitro Th2 responses to allergens. Furthermore, Treg function is reported to be impaired in some allergic patients with active disease. These peripheral blood-based studies have been substantiated by an important recent report [28^{••}] examining pulmonary CD4+CD25high cells in paediatric asthma. Compared with age-matched control subjects, the frequency of those cells was reduced in the bronchoalveolar lavage fluid of asthmatic children. Pulmonary natural Tregs from asthmatic patients had an impaired capacity to suppress both responder T cell proliferation and Th2 cytokine synthesis.

Pro-inflammatory signals abrogate the regulatory function of natural Tregs, directly and indirectly via effects on effector T cells or dendritic cells and this is likely to contribute to the observed impairment of natural Treg function in active disease. The activation of dendritic cells through certain Toll like receptors (TLRs) has been shown to induce signals such as IL-6, which blocked the suppressive activity of murine natural Tregs [29,30]. Other cytokines like tumour necrosis factor [31^{••},32^{••}], IL-7 and IL-15 [33] overcome regulatory activity in human autoimmune diseases. It is important to elucidate the mechanisms involved in the potential loss of function of natural Tregs in an inflammatory environment in order to inform strategies to restore their activity. For example, anti-inflammatory mediators such as glucocorticoids may promote or restore Treg activity both *in vitro* and *in vivo* [34^{••},35,36]. Another example is of the immunosuppressive agent rapamycin that reportedly expands human CD25+Treg *in vitro* as well as in type I diabetic patients in whom a defect in these cells has been reported [37^{••}].

Induced regulatory T cells

Naïve or memory peripheral T cells are able to acquire regulatory characteristics as a consequence of repetitive exposure to antigens (microbial, tumour, transplantation antigens as well as allergens) in concert with signals from dendritic cells and immunoregulatory cytokines, for example TGF- β , IL-10, IL-4 [5]. Adaptive Tregs have many features in common with natural Tregs but exhibit marked cytokine-dependent suppressive mechanisms *in vitro*. Selective tissue expression may render them more critical to suppression than natural Tregs [38]. Thus, although natural Tregs are important in controlling immune homeostasis, the ability to induce adaptive Tregs may have important implications for regulating ongoing immune responses.

Pathways for the induction of CD4+CD25+Foxp3+Treg from CD25-FoxP3-T cells in the periphery have been recently described. They may be relevant for the generation of environmental antigen-specific Treg cells as very recent studies in the gut elegantly imply. Experimental protocols for conversion into CD25+Foxp3+Tregs that are phenotypically indistinguishable from natural Tregs involve the delivery of sub-immunogenic peptide by osmotic pumps, by peptide containing DEC 205 antibodies, by in-vitro culture with TGF- β [39–41] and most recently by mesenteric lymph node CD103+ dendritic cells in a process that is dependent on TGF- β and the dietary metabolite retinoic acid [42–44,45]. Naive T cells induced to express FoxP3 in the presence of TGF- β *in vitro* prevented house dust mite-induced allergic inflammation in a murine model of asthma [46]. TGF- β , however, in addition to its immunosuppressive activity has well documented effects on tissue repair [47], and is likely to promote remodelling during prolonged allergen exposure. Interestingly, a murine study [48] has shown that TGF- β -secreting cells can induce IL-10-secreting Tregs, which complement the anti-inflammatory activity of TGF- β and ameliorate TGF- β -mediated fibrosis. Thus, a complex, but as yet relatively unexplored, concept is whether strategies that combine relatively low TGF- β levels with IL-10-promoting therapies may have synergistic activity in controlling human asthmatic and allergic disease, whilst minimizing potential adverse side effects.

Studies [49–51] in both murine models and humans have proposed that IL-10 plays a central role in maintaining immune homeostasis in the lung in health. An inverse correlation exists between the presence of IL-10 and the incidence and severity of allergic and asthmatic disease.

IL-10+ T cells represent the dominant subset specific for environmental allergens in the peripheral blood of healthy individuals. Strikingly, individuals who have acquired natural tolerance to allergen (such as bee keepers) demonstrate a similar allergen reactive IL-10 phenotype. In contrast, there is a high frequency of allergen-specific IL-4-secreting T cells in allergic individuals. These data suggest that increased levels of IL-10 synthesis, including allergen-specific production, are associated with a lack of allergic symptoms [52]. Finally, therapies associated with the amelioration of allergic and asthmatic symptoms, including allergen immunotherapy [53] and glucocorticoids [54], are associated with induction of IL-10 synthesis [51].

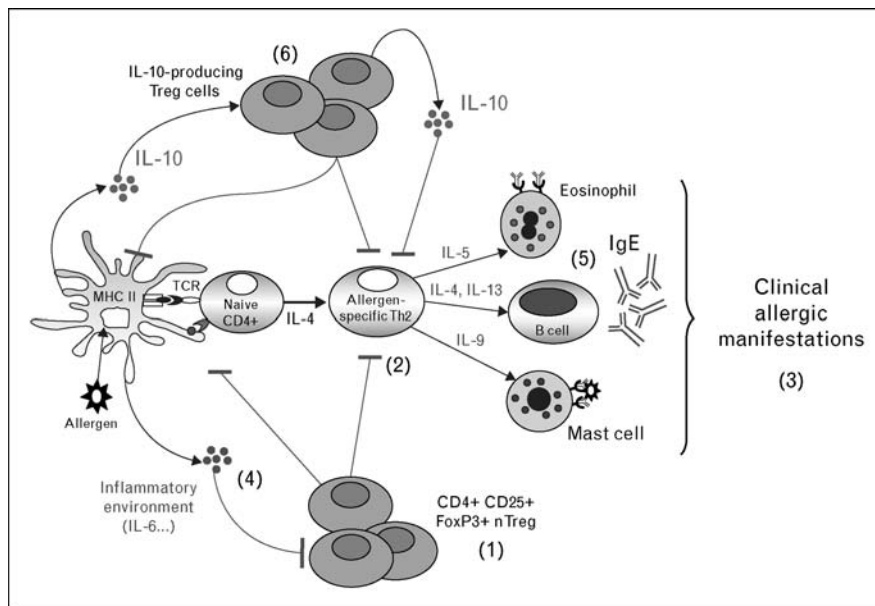
A number of experimental regimens have been described for the induction of IL-10-secreting Treg populations both *in vitro* and *in vivo* [27]. A common feature of many

protocols is the production not only of IL-10 or TGF- β , but also Th1 and Th2 associated cytokines, with the potential to exacerbate inflammatory reactions. Additionally, since many exhibit a limited potential to expand, they may have a restricted clinical application. A strategy designed to generate Tregs secreting IL-10, in the absence of Th1 and Th2-associated cytokines, employed in-vitro stimulation of CD4+ cells in the presence of glucocorticoids, either alone or more effectively, together with the active form of vitamin D, 1 α 25-dihydroxy vitamin D3. These drug-induced IL-10-Tregs have similar functional suppressive properties to the natural Tregs *in vitro* and are able to inhibit naive T cells via cell contact dependent pathways, even though they did not express Foxp3 [55]. In humans these drug-induced IL-10-Tregs inhibited the proliferation and cytokine responses of naive T cells, as well as established Th1 and Th2 cells, including allergen-specific Th2 cell lines [54]. It may be possible to use other drug combinations to effectively generate IL-10-secreting Treg populations which act via effects on T cell or the dendritic cell compartment [56,57]. For example, rapamycin has been shown to selectively promote expansion of human Tregs *in vitro* as well as in renal transplant patients [37,58].

Therapeutic applications of regulatory T cells in allergic and asthmatic disease

Two major Treg-based strategies for the treatment of allergic disease are the enhancement of Treg frequency or function directly in patients, and the adoptive cell transfer of Tregs into patients. In both, it is highly desirable to promote allergen-specific Treg populations with the potential to provide disease-specific immunosuppression and so limit the potential for undesirable bystander inhibition (Fig. 1). A number of other approaches involving the use of monoclonal antibodies (e.g. anti-CD3, anti-CD28) and chimeric proteins (e.g. CTLA4-Ig) are aimed at inducing therapeutic tolerance in human autoimmune diseases and transplantation but not, to date, in allergic disease. They have recently been reviewed and are not further discussed here [59].

The phenotypic and functional characterization of Tregs suggests that the adoptive transfer of these cells could be used to treat allergic disease. Many issues remain to be clarified, however, regarding the feasibility and efficiency of this approach [60]. Firstly, the yield of natural Tregs from peripheral blood is very low and likely to be limiting. Methodologies for the ex-vivo expansion of natural Tregs have now been described. Theoretical concerns, however, regarding their potential to also expand effector cells with pathogenic potential or to introduce transforming viruses during the expansion procedure exist. Secondly, the broad antigen specificity of natural Tregs

Figure 1 Control of allergic responses by regulatory T cells

Allergic manifestations represent the clinical expression of an inappropriate Th2-driven immune response to, otherwise, harmless environmental antigens or allergens. Both CD4⁺ CD25⁺ FoxP3⁺ naturally occurring regulatory T cells (nTregs) and IL-10-producing T cells induced in the periphery have been shown to regulate allergic sensitization *in vivo* through suppression of pathogenic Th2 effectors, allergen presenting dendritic cells and effector cells associated with disease for example mast cells, eosinophils [48]. Although the adoptive transfer of nTreg cells may be a promising way to treat allergy, potential issues that may affect the feasibility of such a protocol include (1) low number in the peripheral blood, since protocols for the expansion of regulatory T cells (Tregs) have been described, but not yet validated in humans; (2) lack of allergen specificity in order to efficiently suppress the disease-specific Th2 effector cells; (3) survival, longevity and homing capacity of transferred Treg cells to the site of inflammation and draining lymph nodes; (4) ability to suppress in an inflammatory environment; and (5) inhibition of an established (memory B cell-dependent IgE production) allergic response, have yet to be demonstrated. Therefore, the *in-vivo* induction or expansion of cell subsets with regulatory activity (e.g. IL-10-producing Tregs) may currently represent a more realistic approach for the treatment of allergy (6).

is thought to be skewed towards preferential recognition of autoantigens [61], and may limit their allergen-specific regulatory potential. This broad antigen specificity could also conceivably result in a generalized bystander immunosuppression with, for example, the potential to promote tumour growth or chronic infection. In animal models antigen-specific Tregs are more effective than polyclonal Tregs in treating disease [62,63]. Thus, it is likely that antigen-specific Tregs will be required for optimal efficacy. New technologies for the rapid *in-vitro* generation of antigen-specific T cells by transduction of appropriate receptor (TCR) have been pioneered to treat malignancy [64] and could be applied in allergy. Other issues to be addressed are whether adoptively transferred Tregs will home to draining lymph nodes and the disease site *in vivo*. Encouragingly, published studies indicate that transferred Tregs preferentially localize to sites of inflammation where antigen is expressed. Studies in a mouse model of allergic airway disease suggest that the maintenance of protective Treg activity depends on continuing allergen stimulation [65], although one potential negative aspect is that pro-inflammatory signals are reported to overcome the suppressive activity of natural Tregs [29,30,31^{••},32^{••},33]. Finally, once transferred, the half-life of Tregs *in vivo* needs to be addressed [66,67[•]],

and needs to be of sufficient, but not indefinite, duration to allow resolution of inflammation.

Studies of adoptive transfer of Tregs in experimental animal models, including allergic airway disease, have yielded encouraging results [50]. To date, however, no published studies exist in humans. Initial clinical studies are likely to be in transplantation since, uniquely, the timing of sensitization to the disease-promoting antigen (the transplant) is fully controlled. Transfer of expanded natural Tregs or of induced Tregs could be performed under cover of immunosuppressive drugs such as rapamycin, which have the potential to deplete or inhibit effector T cells whilst maintaining or even enhancing Treg activity. A combinatorial therapy including IL-10 and rapamycin has been shown effective in preventing allograft rejection in a preclinical model of allogeneic islet transplantation and in inducing long-term tolerance in nonobese diabetic mice via induction of both Tr1 cells and natural Treg cells [37^{••}]. In these settings rapamycin increased or at least maintained Foxp3 expression and inhibited the simultaneous expansion of contaminating T effector cells that exacerbate the inflammatory process that is supposed to be treated.

Promoting regulatory T cell populations in patients

Several existing treatments for allergic disease already have documented capacity to promote regulatory T cells in patients. The clearest example of this is allergen immunotherapy, which represents a unique disease-specific treatment with clear disease modifying properties [68,69]. Allergen immunotherapy is associated with the induction of IL-10 secreting Tregs and less clearly established effects on the natural Treg compartment. The induction of TGF- β secreting T cells post allergen immunotherapy may also occur. Nevertheless as discussed above, this therapy is not effective in all patient groups, is associated with significant risk of adverse events and for maximal efficacy requires administration over a period of several years. A number of strategies are currently being investigated to improve both the safety and efficacy of allergen immunotherapy including use of allergen-derived peptide [70] that were predicted to not cause early allergic responses, and genetically engineered recombinant allergens with reduced allergenicity [71], combination of allergen immunotherapy with anti IgE to reduce IgE mediated adverse events all with some degree of efficacy. Strategies to promote efficacy of immunotherapy include the use of adjuvants for example TLR agonists to deviate immune responses away from Th2 (reviewed elsewhere in this volume), components of microorganisms, including helminths that predispose to Treg induction [72], and anti-inflammatory drugs with the capacity to better promote IL-10 and Treg induction [27,54*].

Conclusion

Evidence from preclinical models and humans demonstrates a central role for Tregs in controlling allergic disease. Adoptive transfer of Tregs represents a potential tailor-made, donor-specific therapeutic approach that does not currently meet the requirements of a simplified and cost-effective approach to treat millions of patients. The results of adoptive transfer of Tregs to treat any human immune-mediated disease have yet to be reported. Safety considerations and practical limitations mean it is as yet difficult to rationalize adoptive Treg cell transfer for the treatment of allergy. Therefore, we believe that in-vivo induction or expansion of endogenous Tregs in patients remains an attractive alternative Treg-based therapy. Modification of existing treatments, specifically allergen immunotherapy, through co-administration with immunomodulatory drugs, helminth or pathogen derived antigens or other immunomodulatory molecules may present a more realistic approach, to improve allergen-specific Treg induction and provide significant benefits to allergic and asthmatic patients.

Acknowledgements

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Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
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Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 596).

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