Regulatory T Cells in Allergic Asthma

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ABSTRACT: Defective immunological suppression can be a cause of the inflammation that leads to an allergic condition such as asthma. Suppressor regulatory T cells (Tregs) are essential for inducing and maintaining immunological tolerance to foreign and self-antigens, including allergens. Tregs are apparently altered in number and function in allergic asthmatic patients. Some treatments that ameliorate asthma symptoms lead to an increase in the number and functional impairment of Tregs, indicating that these cells play an important role in the anti-inflammatory effect of those medications.

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T cells do not proliferate following exposure to allergens in healthy individuals, but a combination of genetic susceptibility to atopy, exposure to allergens, co-exposure to infectious agents and/or defective immunological suppression can result in inflammation that in turn leads to allergic disease, such as asthma. Inflammation is both the central pathogenic feature and the principal clinical manifestation of asthma and is responsible for airway obstruction and hyper-responsiveness [1]. The prevailing consensus is that the immunological basis of atopic sensitization is due to exacerbated T helper type 2 cytokine production and responses to allergens [2]. Recent advances in both immunological and clinical phenotyping of asthma have raised the possibility that other mechanisms, such as TH1 and TH17 responses, as well as alteration in suppressive regulatory T cells, may trigger pathology in some patients with asthma, or co-exist with TH2 type inflammation [2].

This review reports current evidence on the involvement of Tregs in the development of asthmatic disease.

Suppressor Treg cells are essential for the induction and maintenance of immunological tolerance to foreign and self-antigens, including allergens

SUBTYPES OF REGULATORY T CELLS

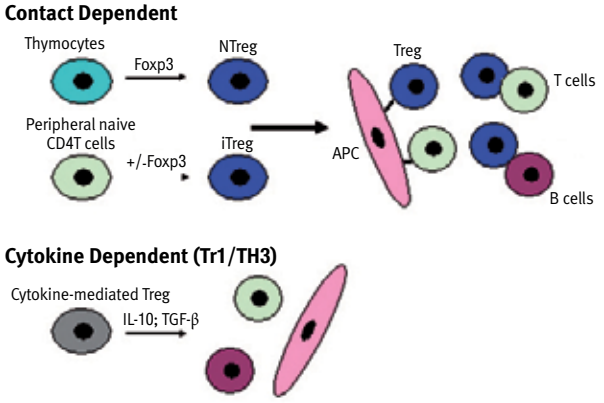
Suppressor Treg cells are important for the induction and maintenance of immunological tolerance to foreign and self-antigens, including allergens. These cells were discovered in the mid-1990s by Sakaguchi et al. [3], who were the first to demonstrate that a minor population of CD4+ T cells, co-expressed with the CD25 molecule (which represents the α-chain of interleukin-2 receptor), functioned as suppressor Tregs in adult mice. Subsequent experiments demonstrated that these cells also manifest regulatory activity in vitro [4], and human CD4+CD25+ T cells with similar functions were identified by several groups [5]. In 2003, work by Hori et al. [6] led to the next leap in the research of Tregs. The authors demonstrated that Foxp3 transcription factor is selectively expressed by Tregs and is required for their development. The discovery of Foxp3 enhanced the ability to characterize this subpopulation [4].

Regulatory T cells can develop in the thymus ("natural" Tregs) or in peripheral lymphoid tissues ("adaptive" or "induced" Tregs) [4]. The majority of nTregs express the transcriptional factor Foxp3, and their development and function are dependent upon it. In contrast, iTregs are usually Foxp3 negative, but they can express this transcription factor in certain antigen presentation situations, such as low doses of antigens or in the presence of transforming growth factor-beta in the periphery [7]. These cells suppress the immune system by cell-cell contact, acting on T and B cells and on antigen-presenting cells [8] [Figure 1].

TH = T helper
Tregs = regulatory T cells

nTregs = natural Tregs
iTregs = induced Tregs

Figure 1. Treg subtypes and function. nTreg = natural Treg, iTreg = induced Treg, IL = interleukin, TGFβ = transforming growth factor-beta, APC = antigen-presenting cells



In addition to CD4+CD25+ Tregs, which function by cell-cell contact, there are other types of regulatory suppressive T cells that act via modulatory cytokines. Regulatory T helper cells type 3 (TH3) and regulatory T cells type 1 (Tr1) are examples of these Tregs. TH3 are TGFb dependent and can be observed in mice models in which oral exposure to low doses of the antigen induces oral tolerization [4]. Tr1 cells are defined by their specific cytokine production profile, which includes the secretion of high levels of IL-10 and TGFb, and their ability to suppress antigen-specific effector T cell responses via cytokine-dependent mechanisms. Tr1 cells clearly play a role in regulating the adaptive immune response in both mice and humans; however, the lack of a specific surface-expressed molecule marker for this subset of regulatory T cells impedes their full characterization [Figure 1].

Tregs are necessary for controlling inflammation in asthma, thereby establishing them as a potential tool for the treatment of this disease

REGULATORY T CELLS IN ASTHMA

Recent studies have reported an alteration in the number and/or functions of Tregs in individuals with autoimmune diseases, such as diabetes, psoriasis, myasthenia gravis, sarcoidosis, graft-versus-host disease and multiple sclerosis [9]. Modification can also occur in the regulatory T cell population in allergic patients [2]. The development of animal models of allergic airway diseases has improved our understanding of the mechanisms involved in allergic asthma. These experiments showed that depletion of CD4+CD25+ Treg cells increased airway hyper-responsiveness in C3H mice, which are relatively resistant to the development of airway hypersensitivity [Table 1] [10]. The transfer of antigen-specific Treg cells from DO11.10 mice (transgenic mouse with T cell receptor specific to ovalbumin) could prevent and suppress established AHR- and TH2-mediated airway inflammation in an ovalbumin-inhaled animal allergic model [Table 1] [11]. Furthermore, CD4+CD25+ could prevent development of airway hypersecretion of mucus, smooth muscle hypertrophy and collagen synthesis, but it failed to reverse established remodeling in a long-term challenge model [Table 1] [12].

Several in vitro studies have been performed with the aim of clarifying the role of Tregs in allergic asthma. CD4+CD25+ Tregs and IL-10-producing Tregs were suggested to suppress TH2 responses to allergens. Peripheral blood CD4 cells from non-atopic individuals that were removed from CD25+ cells proliferated and released cytokines following allergen stimulation, and this process could be reversed by adding CD4+CD25+ T cells [13]. In addition, CD4+CD25+ from atopic individuals were not as effective in suppressing allergen-stimulated effector T cells [13]. IL-10-producing cells were able to suppress human allergen-driven TH2 cytokine production of IL-4-producing effector cells. The amount of these IL-10-producing Treg cells decreased and that of IL-4-producing cells increased in blood from atopic allergic donors [2].

Studies have investigated the behavior of Treg cells in allergic asthmatic patients. The results in the literature reveal variability between children and adults and between peripheral blood and airway tissue. Analysis of the behavior of Tregs in allergic asthmatic children's blood and broncheoalveolar lavage samples demonstrated fewer CD4+CD25+ T cells than in healthy control subjects [Table 2] [14,15]. Among the allergic patients, those with persistent severe allergy (rhinitis and/or bronchial asthma) present higher numbers of these cells, as well as of blood Foxp3 and IL-10 mRNA [Table 2]. Examination of the suppressive function of blood Tregs from patients with severe

TGFb = transforming growth factor-beta
IL = interleukin
AHR = airway hyper-responsiveness

Table 1. Findings in animal models of allergic airway diseases that improve our understanding of the involvement of Tregs in the development of allergic asthma

Author, year [ref]	Findings
Lewkowich et al., 2005 [10]	Depletion of CD4+CD25+ Treg cells increased AHR in mice relatively resistant to development of airway hypersensitivity
Kearley et al., 2005 [11]	Transfer of antigen-specific Treg cells from DO11.10 mice could prevent and suppress established AHR- and TH2-mediated airway inflammation in an ovalbumin-inhaled animal allergic model
Kearley et al., 2008 [12]	CD4+CD25+ prevent development of airway hypersecretion of mucus, smooth muscle hypertrophy and collagen synthesis, but failed to reverse established remodeling in a long-term challenge model

ARDS = acute respiratory distress syndrome

Table 2. T regulatory cells in asthmatic patients

Authors, year [ref]	Subjects	Tissue	Treg findings
Lee et al., 2007 [14]	Children	Blood	Fewer CD4+CD25+ T cells than in healthy controls
			Higher numbers of CD4+CD25+ Foxp3 and IL-10 mRNA T cells in allergic patients with persistent severe allergy
			Normal suppressive function range, per cell basis, on Tregs from patients with severe asthma
Hartl et al., 2007 [15]	Children	BAL	Fewer CD4+CD25+ T cells than in healthy controls
Abdulamir et al., 2009 [16]	Adults	Blood	Low numbers of Tregs in patients with moderate-to-severe disease compared to those with mild disease and to healthy controls
			Higher numbers of Tregs in patients with mild disease compared to moderate-to-severe and healthy individuals
Smyth et al. [17]	Adults	BAL	Increased number of CD4+Foxp3+ Treg cells in patients with moderate-to-severe asthma compared to mild patients and healthy controls
Maszumoto et al. [19]	Adults	Blood	Higher number of CD4+IL-10- in patients with mild asthma compared to those with moderate to severe disease

asthma revealed that it was within the normal range per cell [Table 2] [14]. In adult patients, the results differ in cases of Tregs taken from tissue compared to Tregs taken from blood. Abdulamir and collaborators [16] demonstrated significantly low numbers of Tregs in the blood of patients with moderate-to-severe disease compared to those with mild disease and to healthy controls [Table 2], whereas patients with mild disease had higher numbers of Tregs than the other two groups [Table 2]. In contrast, Smyth et al. [17] observed an increase in the number of CD4+Foxp3+ Treg cells in the BAL of adult patients with moderate-to-severe asthma compared to patients with mild disease and to healthy controls [Table 2]. In addition, Thunber and co-researchers [18] demonstrated that allergen provocation in patients with mild asthma increases Foxp3 expression and TH2 cytokines obtained from their BAL. Finally, atopic adult patients with mild asthma have a higher number of CD4+IL-10-producing cells compared to patients who have severe atopic and moderate-to-severe non-atopic asthma [19]. Based on those studies [14-17,19], it would appear that Treg cells are increased in children and decreased in adults with severe allergic states. BAL was not studied in severely asthmatic children, precluding any comparison to adult BAL Treg cells. Taken together, the above data leave little doubt about the importance of Tregs in controlling inflammation in asthma, establishing them as a potential tool for the treatment of this disease.

Many of the current treatments for asthma and allergy affect those regulatory T cells

ASTHMA TREATMENT AND TREGS

Some drugs are known to increase Tregs [20]. In cases of asthma, the most common drugs used to treat and prevent asthma are glucocorticoids, which are usually used in combination with reliever medications such as beta-agonists and occasionally with other anti-inflammatory drugs [1,21]. Glucocorticoids mediate potent anti-inflammatory actions via inhibition of transcription factors involved in cytokine regulation, such as nuclear factor of activated T cells, activator protein-1 and nuclear factor kappa B [22]. Glucocorticoids have recently been recognized as beneficially influencing Foxp3+ and IL-10+ Treg function. Treatment with glucocorticoids increased Foxp3mRNA, albeit transiently, and closely correlated with the increase of IL-10 mRNA, although TGFβ expression remained unchanged [15,23]. A number of studies have demonstrated that glucocorticoids induce IL-10 syntheses in vitro as well as in vivo. CD4 T cells that had been stimulated in the presence of glucocorticoids, such as dexamethasone, showed a dose-dependent induction of IL-10 [24]. CD4 T cells from patients with steroid-refractory asthma (i.e., those who gain little or no clinical benefit from glucocorticoid therapy), however, failed to induce IL-10, suggesting a role for this cytokine in the immunosuppressive effect of this medication [25]. This treatment was shown to increase Foxp3mRNA and IL-10 in the peripheral blood of adult asthmatic patients, and to restore CD4+CD25 cell numbers, Foxp3mRNA and suppressive functions in the BAL of pediatric asthmatic patients [15,26].

Another well-recognized treatment for patients with allergic rhinitis, asthma and venom allergy is allergen-specific immunotherapy [27]. Several studies have shown that the injection of gradually increasing quantities of the allergen to which the patients are sensitized in order to induce tolerance achieves hyposensitization and reduces both early and late responses that occur during the natural exposure to the allergen [28]. This is a unique approach used today for human immunological diseases. Successful immunotherapy is associated with fewer symptoms, less use of medications and improvement in quality of life [28]. This treatment, after completion, usually confers long-term remission of symptoms and prevents the onset of new sensitization in children for a number of years [28]. Although the administration of specific allergens has been shown to be effective for rhinitis [29] and insect venom allergy [30,31], its benefit in asthma remains controversial. Immunotherapy has proven efficacious in treating mild asthma, as well as in preventing the progression to asthma in patients suffering from rhinoconjunctivitis [32,33], but it is not yet recommended for the treatment of moderate-to-severe asthmatic patients [34].

The decreased proliferative response of peripheral blood T cells to allergens that is observed after immunotherapy is consistent with anergy and/or depletion of allergen-specific T cells following stimulation with high doses of antigens [35]. After discovery of the regulatory T cells, their immunosuppression was emphasized as the main mechanism for the clinical efficacy of immunotherapy [28]. Increased proportions of allergen non-specific Tregs have been described after immunotherapy [36], and IL-10-secreting Treg cells have been detected in skin biopsies as well as in the nasal mucosa of patients undergoing such treatment [37,38]. Increased numbers of local Tr1 have been associated with elevated serum immunoglobulin G4 and block of allergen presentation by IgE [39]. As noted earlier, Tregs have also been associated with TGFβ, which modulates antigen presentation and expression of co-stimulatory molecules on antigen-presenting cells and T cell proliferation [28]. TGFβ is also involved in the mechanisms of immunotherapy. It promotes a switch to IgA in humans, and increased levels of allergen-specific IgA have been associated with an increase in Tregs and successful immunotherapy [40]. Other interesting data showed increases of Treg cells and of IL-10 and TGFβ mRNA expression in a 2 year trial of immunotherapy in chil-

BAL = broncheoalveolar lavage

Ig = immunoglobulin

dren [28]. These findings support the importance of the various Treg subtypes and cytokines for effective immunotherapy.

In conclusion, various Treg subtypes participate in the suppression of airway inflammation. Impaired function or reduced numbers of these suppressors can promote inappropriate immunological responses, which could lead to allergic diseases such as asthma. Many of the current treatments for asthma and allergy also affect those cells. As such, better understanding of the methods that are capable of increasing Treg numbers and of improving their function could be useful in achieving the optimal treatment. Manipulation and investigation of regulatory T cells should be considered when designing future therapeutic approaches.

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