

Expert Opinion

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General

T cell tolerance to inhaled allergens: mechanisms and therapeutic approaches

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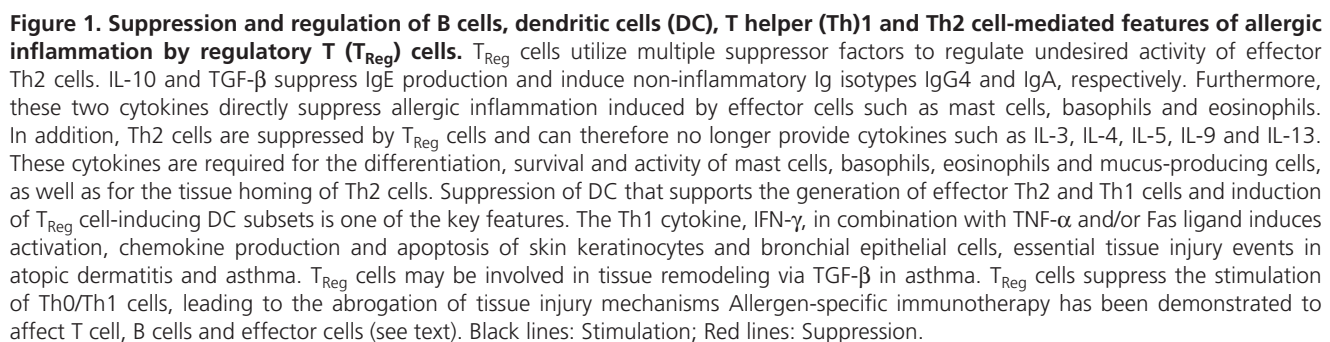
Background: Specific immune response to allergens is decisive in the development of clinically healthy or allergic states. **Objective:** Recent developments in the mechanisms of allergen-specific peripheral tolerance can be used for future treatment modalities. **Methods:** This review focuses on recent developments in allergen tolerance. **Results/conclusion:** The balance between allergen-specific IL-10-secreting T regulatory cells (Tr1) and T helper 2 (Th2) cells appears to be decisive in the development of allergic and healthy immune response against allergens. Induction of IL-10- and TGF- β -producing Tr1 cells, IgG4-isotype-blocking antibodies, and suppressed mast cells, basophils and eosinophils represent major components of a relatively normalized immune response after allergen-specific immunotherapy and healthy immune response to aeroallergens in sensitized individuals.

Keywords: allergens, allergen-specific immunotherapy, allergy, IL-10, suppression, T cell tolerance, TGF- β , T regulatory cells

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1. Introduction

The interaction of environmental and genetic factors can lead to the development of atopic disorders in some individuals but not in others, following allergen exposure. The generation of allergen-specific CD4⁺ T helper (Th) 2 cells is the initial event responsible for the development of allergic diseases. These effector Th2 cells produce IL-4, IL-5 and IL-13, which mediate several regulatory and effector functions. These functions include allergen-specific IgE production by B cells, eosinophil development and recruitment, mucus production and bronchial hyper-reactivity, as well as allergic tissue homing of Th2 cells (Figure 1) [1,2]. The Th1 component, on the other hand, may contribute to chronicity and the effector phase in allergic disease [1]. Activation and apoptosis of epithelial cells induced by IFN- γ , TNF- α and Fas-ligand is an essential pathogenic event in eczematous lesions in atopic dermatitis and bronchial epithelial cell shedding in asthma [1]. Anti-TNF treatments appear to be promising, suggesting that TNF has a role in especially severe forms of asthma [3]. In addition, IFN- γ upregulates several chemokines in epithelial cells such as IFN- γ -inducible protein 10, monokine induced by IFN- γ and IFN- γ -inducible α -chemoattractant, which further attract Th0/Th1 cells towards epidermis by binding to CXCR3 in atopic dermatitis [4]. A further subset of T cells termed T regulatory (T_{Reg}) cells, which have an immunosuppressive function and cytokine profile distinct from that of either Th1 or Th2 cells, has been described [5,6]. T_{Reg} cells are able to inhibit the development of allergic Th2 cell responses, as well as effector Th1 cell responses and therefore play an important



2. Changes in specific antibodies to allergens in healthy and allergic individuals: a mechanism of immune tolerance

IgE (Table 1, Figure 1) [11,14-16]. One of the key questions has been the reason for- and mechanisms of the existence of normal levels of IgE in all humans. Recently, it has been demonstrated that spontaneously produced IgE in T cell-deficient and germ-free wild type mice is composed of natural specificities and induced by a mechanism independent of MHC class II cognate help. This does not require secondary lymphoid structures or germinal center formation, although some bystander T cell-derived IL-4 is necessary [17]. In principal, no exposure or no presentation (exposure in small amounts) may be responsible for no Ab response. Low amounts of exposure leading to immunological ignorance due to no accessibility and/or short persistence of the allergens in secondary lymphoid organs, which

Table 1. Immune response to allergens and clinical outcome in healthy and allergic individuals.

	Specific antibody response	Allergen-specific T cell response	Clinical outcome
Healthy	No response	No response	Healthy
	Detectable IgG1, IgG4 and IgA	Th0 response in PBMC and specific T cell clones with low frequency	Skin prick test and IgE positivity, clinical disease cannot be induced
	High amounts of IgG4, relatively low amounts of IgG1 and detectable IgA and IgE	Tr1, particularly IL-10-dominating response with relatively high frequency	Skin prick test and IgE positivity, healthy in normal circumstances, clinical disease can be induced by provocation tests and is dose-dependent
Allergic	Relatively high amounts of IgE, together with low or high amounts of IgG1, IgG4 and IgA	Th2 response with varying quantities of IL-4, IL-5 and IL-13, detectable IL-10 and IFN- γ	Intermittent or persistent allergy in various clinical forms

PBMC: Peripheral blood mononuclear cell; Th: T helper; Tr: T regulatory.

has been demonstrated in mouse models, still remains as a hypothetical possibility [18]. Short persistence and low dose may not be as demonstrative in humans as in mice, since a single bee sting can induce IgE and IgG and relatively very small doses of T cell epitope peptides injected into skin can initiate allergic reactions [19-21]. One honeybee sting releases ~ 50 – 140 mg of venom per sting and contains 5 – 15 mg of the major allergen phospholipase A₂. A single honeybee sting is sufficient to induce IgE or IgG production against phospholipase A₂ [21]. Wasp venom has approximately 10 – 20 times less protein content (1.7 – 3.1 μ g), however, again one wasp sting is sufficient to induce IgE or IgG type antibodies against single allergic components, some of which are less than 10% of the protein content [21].

Non-allergic beekeepers, who are exposed to multiple honeybee stings due to their profession, represent a suitable model for high dose allergen exposure whilst still retaining a healthy immune response. These beekeepers have approximately 1000 times higher specific IgG4 versus specific IgE ratios compared with allergic individuals [22]. The level of specific IgG4 primarily reflects exposure. In beekeepers, bee-venom-specific IgG4 correlates to the number of annual stings and to the number of years spent in bee-keeping. High-dose exposure appears to induce clinical tolerance, 45% of beekeepers who were stung less than 25 times a year had a history of systemic sting reactions, compared with no allergy in those with more than 200 stings per year [21]. High dose exposure, increased specific IgG4 and protection from allergy has also been demonstrated for high-pet-contact and the low occurrence of asthma and allergies in children [23]. In atopic children and adults, maintenance of tolerance to cow's milk without cow's milk allergy is associated with elevated levels of specific IgG4, in combination with low specific IgE. The upregulation of specific IgG4 in tolerant atopic individuals may be related to the type of allergen and its regular dose of exposure [24]. IgG4 has unique structural and functional features among IgG subsets. Its heavy chains may repair in a way that leads to functional monomeric antibodies, it has low

affinity for certain Fc γ receptors, and it does not activate complement [25]. As such IgG4 is uniquely capable of inhibiting immune complex formation by other isotypes and thus may have an anti-inflammatory role in immunity. In addition the results on IgG4 pose a challenge to many of the hypotheses about reasons for the increase in asthma. The evidence that children or adults who make a modified Th2 response i.e., immunoglobulin (Ig)G and IgG4 Ab without IgE, are not at increased risk of asthma strongly supports the role of IgE in asthma. Equally the results may give insight into a form of tolerance that could be a target for protecting patients against allergic disease. In principle, allergen dose correlated with IgG4 and protection in cat allergy and bee venom allergy versus tolerance [26].

An allergen-dose controlled model, which induces long-term cure in humans, is allergen-SIT. Although peripheral tolerance has been demonstrated in specific T cells, the ability of B cells to produce specific IgE antibodies is not eliminated during allergen-SIT [27]. In fact, the serum levels of both specific IgE and IgG4 antibodies increase during the early phase of treatment. However, the increase in antigen-specific IgG4 is more striking and therefore the ratio of specific IgE to IgG4 significantly decreases between 6 months and 3 years of treatment. A similar change in specific isotype ratio has been observed in SIT for various allergies [27-29]. Moreover, IL-10 produced and progressively secreted during allergen-SIT, appears to upregulate synthesis of antigen-specific IgG4 and downregulate IgE antibodies [10]. IL-10 potently suppresses both total and allergen-specific IgE, and simultaneously increases IgG4 production [10,30]. Therefore, IL-10 not only generates T cell tolerance, it also regulates specific isotype formation and skews the specific IgE response towards an IgG4-dominated phenotype. SIT also induces a serum-blocking activity, presumed to be allergen-specific IgG4 that blocks formation of allergen-IgE complexes and binding to CD23⁺ B cells. A likely consequence of this is inhibition of IgE-facilitated allergen presentation to T cells [28]. The practical problem today is that although many laboratories offer assays for

IgG-subgroup antibodies to common allergens, the clinical significance of these measurements needs to be clarified. In addition, the increase in allergen-specific IgA during SIT may imply a role for TGF- β as an IgA isotype switch factor and involvement of regulatory T cells, which needs to be further investigated [11,31,32]. A recent study has shown that the IgA response to SIT (IgE-mediated allergic rhinitis and asthma) is selective for IgA2, correlates with increased local TGF- β expression, and induces monocyte IL-10 expression, suggesting that IgA Abs could thereby contribute to tolerance development in IT-treated allergic patients [33]. Children, with allergic airway disease, who are sensitized to house-dust mites are known to have increased levels of allergen-specific IgE and IgG, and there is an association between the quantity of those immunoglobulins and the clinical features of disease. It was demonstrated that Der p1-specific IgG1, IgG4, IgE and IgA levels of asthmatic children with a duration of disease of more than 4 years were significantly higher than those of children with a disease duration of less than 4 years. The ratio of Der p1-specific IgA/IgE was significantly lower in asthmatic children when compared with children with allergic rhinitis and controls [34].

3. Differences and similarities in specific T cell response to allergens in healthy and allergic individuals

Studies on T cell responses to allergens in healthy individuals have demonstrated a wide range of immune response from no detectable response to involvement of active peripheral tolerance mechanisms mediated by different subsets of T_{Reg} cells (Table 1, Figure 1). Investigation into T-cell-mediated immune suppression strengthened during the 1990s and the concept of T_{Reg} cells suppressing immune responses via cell-cell interactions and/or the production of suppressor cytokines is currently well established. However, many aspects behind these mechanisms remain to be elucidated [6,35,36].

3.1 Immune regulation against allergens and T_{Reg} cells

In a large number of healthy individuals, T cells do not show any proliferative response to allergens in peripheral blood mononuclear cell (PBMC) cultures, which can be due to the low frequency of specific T cells because of life time non-exposure [9,37]. Active suppression against allergens by T_{Reg} cells takes place in cultures from most of the healthy individuals [9,37]. In individuals, who show a detectable IgG response, a balanced allergen-specific immune response due to expansion of Tr1 cells has been demonstrated [9]. Tr1 cells, also known as inducible T_{Reg} cells, are defined by their ability to produce high levels of IL-10 and TGF- β [6,35]. It is now clear that during the early course of allergen-SIT, IL-10- and/or TGF- β -producing Tr1 cells in humans are generated *in vivo*, which implies that Tr1 cells are induced

by high and increasing doses of allergens [10,11,28,38]. Therefore, Tr1 cells could be used as a promising target for the development of new therapeutic agents, as well as in cellular therapy for peripheral tolerance modulation in allergy and autoimmunity.

The CD4⁺ CD25⁺ T_{Reg} cells, also called constitutive T_{Reg} cells, account for 5 – 10% of peripheral CD4⁺ T cells and inhibit the activation of effector T cells in the periphery [39]. These cells, along with CD4 and CD25 expression, are also associated with the transcription factor forkhead box P3 (FoxP3) [40]. Various allergic and autoimmune phenotypes develop in X-linked immune dysregulation, polyendocrinopathy and enteropathy syndrome (IPEX) spontaneously when CD4⁺ CD25⁺ T_{Reg} cells are non-functional, indicating the presence of a population of professional suppressor (T_{Reg}) cells [41]. IPEX is characterized by an allergic phenotype with dermatitis and hyper IgE and an autoimmune phenotype with enteropathy, type I diabetes, thyroiditis, hemolytic anemia and thrombocytopenia, and results from mutations of FOXP3 [42]. The relevance of immune suppressive function of FOXP3⁺ CD4⁺ CD25⁺ T_{Reg} cells in human allergies has been studied. Impaired skin infiltration of CD4⁺ CD25⁺ FOXP3⁺ T cells was observed in acute atopic dermatitis lesions [12] and FOXP3 mRNA expression was significantly increased in asthmatic patients receiving glucocorticoid treatment [43].

There is some evidence in adults that constitutive CD4⁺CD25⁺ T_{Reg} cells and inducible IL-10- and TGF- β -secreting Tr1 cells represent overlapping populations. CD4⁺CD25⁺ T_{Reg} cells normally inhibit Th2 cytokine expression and proliferation of PBMC from non-atopic donors, in response to allergen. This suppression has been shown to be associated with the control of allergic disease [37]. In addition, in allergen-SIT increased allergen-specific suppressor activity of both allergen-specific IL-10-secreting Tr1 cells and CD4⁺CD25⁺ T_{Reg} cells has been demonstrated [10,11,37]. A recent study using IFN- γ -, IL-4- and IL-10-secreting allergen-specific CD4⁺ T cells that resemble Th1-, Th2- and Tr1-like cells, respectively, showed that both healthy and allergic individuals exhibit all three subsets but in different proportions. In healthy individuals, Tr1 cells represent the dominant subset for common environmental allergens, whereas a high frequency of allergen-specific IL-4-secreting T cells is found in allergic individuals. Therefore, the frequency of effector Th2 cells or T_{Reg} cells is decisive in the development of allergy or a healthy immune response [9]. Allergen-specific Tr1 cells employ IL-10, TGF- β , CTLA-4 and PD1 as suppressor molecules.

In this respect, SIT vaccines that target T cells and induce T cell tolerance, while bypassing IgE binding, represent a novel opportunity for the prevention and treatment of allergy [44]. Immunization of mice with a major allergen fusion protein or fragments containing linear T cell epitopes, but not three-dimensional B cell epitopes, of the major bee

venom allergens have been shown to protect against IgE responses to later encounters with the allergens, representing novel vaccines for the prevention of allergy [45,46].

Peptide-induced, antigen-specific non-responsiveness, also known as tolerance, has been demonstrated in numerous murine models of both autoimmunity and allergy [47]. Initial experiments demonstrated that it was possible to induce systemic tolerance to peptides administered in incomplete Freund's adjuvant during the neonatal period. The majority of peptide immunotherapy studies in subjects with allergic disease have been performed with peptides from the major allergen of cat dander (Fel d 1) or bee venom (phospholipase A). T cell epitope (peptide)-SIT of bee-venom-allergic individuals and cat-allergic individuals induced a Tr1 type allergen-specific immune response with decreased IL-4, IL-5, IL-13 and IFN- γ and increased IL-10 production [48-50].

3.2 Role of histamine and histamine receptors

Human CD4⁺ Th1 cells predominantly express histamine receptor1 and CD4⁺ Th2 cells histamine receptor 2, which results in their differential regulation by histamine [51]. Histamine induces the production of IL-10 by dendritic cells [52]. In addition, histamine induces IL-10 production by Th2 cells [53], and enhances the suppressive activity of TGF- β on T cells [54]. All three of these effects are mediated via histamine receptor 2, which is relatively highly expressed on Th2 cells and suppresses IL-4 and IL-13 production and T cell proliferation [51]. Apparently, these findings suggest that histamine receptor 2 may represent an essential receptor that participates in peripheral tolerance to allergens.

4. Mechanisms of immune suppression by IL-10 and TGF- β

Antigen-specific T cell suppression of both Th1 and Th2 type immune responses by IL-10, a known suppressive cytokine of T cell proliferation and cytokine production is essential in peripheral tolerance to allergens, autoantigens, transplantation antigens and tumor antigens. In mice, IL-10 administration before allergen treatment induced antigen-specific T cell unresponsiveness and demonstrated the pivotal role of IL-10 in establishment of peripheral T cell tolerance [55]. Moreover, inhibition of graft-versus host disease by IL-10 and allograft rejection in human-leukocyte-antigen-mismatched, bone-marrow-transplanted severe combined immunodeficient patients gives further evidence for a key role of this cytokine in the induction and maintenance of peripheral tolerance [56]. Similarly, inappropriate stimulation of tumor-reactive human T cells was shown to result from increased endogenous IL-10 production by these cells, indicating a role for IL-10 in tumor-specific tolerance [57]. IL-10-producing antigen-presenting cells, such as B cells [58] and dendritic cells [59] as well as clonally expanded IL-10-producing allergen-specific Tr1 cells [9,10] all contribute

to the suppressive effects of IL-10. IL-10 may also modulate tolerance to food antigens in experimental models; a micro-organism bioengineered to deliver IL-10 in the gut can decrease food-induced anaphylaxis and provide an option to prevent IgE-type sensitization to common food allergens [60]. Interestingly, the same features of peripheral tolerance were found in the T cells of healthy beekeepers, who had previously been stung by large numbers of bees. These naturally anergized individuals show increased numbers of IL-10-producing CD4⁺ CD25⁺ T cells and monocytes, similar to allergic patients after bee-venom-SIT [10].

IL-10 inhibits the proliferative T cell response of PBMC to various allergens, however, does not suppress the proliferative responses of T cells that were stimulated by anti-CD3 [61]. IL-10 inhibits T cell proliferation within a certain range of low numbers of triggered TCRs, indicating that T cells require co-stimulation. IL-10 suppresses T cells by blocking CD28 and inducible co-stimulator (ICOS) co-stimulatory signals in a rapid signal transduction cascade [62]. Upon binding to its receptor, IL-10 activates two tyrosine kinases, Jak1 and Tyk2. Tyk2 acts as a constitutive reservoir for Src-homolog-containing phosphatase-1 (SHP-1) tyrosine phosphatase in resting T cells. Upon activation, SHP-1 rapidly binds to CD28 and ICOS and dephosphorylates them within 1.5-3 min. In consequence, the binding of phosphatidylinositol 3-kinase to either co-stimulatory molecule no longer occurs and downstream signaling is inhibited [62]. Supporting these findings, spleen cells from SHP-1-deficient mice showed increased proliferation with CD28- and ICOS-stimulation in comparison to wild type mice, which was not suppressed by IL-10. Generation of dominant negative SHP-1-overexpressing T cells or silencing of the SHP-1 gene by small inhibitory RNA (siRNA) both altered SHP-1 functions and abolished the suppressive effect of IL-10 [62]. Interestingly, the suppressive effect of IL-10 was not observed in other IL-10 family cytokines IL-19, IL-20, IL-22 and IL-24 [63].

The TGF- β superfamily consists of more than 35 members, with TGF- β 1 being the prototypic member. TGF- β 1-deficient mice develop the phenotype, due to activated CD4⁺ T cells, of a rapid wasting syndrome leading to death by the age of 3 or 4 weeks [64,65]. Although controversial, TGF- β has been implicated in the conversion of naive CD4⁺CD25⁻ T cells into CD4⁺CD25⁺ T cells by the induction of FoxP3 [66]. In contrast, several recent studies have demonstrated that TGF- β plays an essential role in the development of IL-17-secreting T cells, which promote neutrophilic inflammation [67]. TGF- β signaling has been demonstrated to be required for *in vivo* expansion and immunosuppressive capacity of CD4⁺CD25⁺ T cells [68]. However, studies with TGF- β -deficient mice have shown that CD4⁺CD25⁺ T cells can develop for up to 2 weeks and autocrine TGF- β production was not essential for these cells to exhibit suppressive activity *in vivo* [69]. In contrast to its known T-cell-suppressive activity, some reports imply a role for

TGF- β in the pathogenesis of asthma, particularly in the remodeling of injured lung tissue in humans because TGF- β is one of the main fibrotic cytokines [70]. The exact suppressive mechanisms behind TGF- β receptor pathways remain to be elucidated. Recently, a distinct small fraction of NK cells, which display regulatory functions involving IL-10 has been identified in humans [71]. Small molecular drugs that target these pathways, as well as those that induce IL-10 in tissues represent future drug development modalities.

5. Conclusion

In sensitized individuals, peripheral T cell tolerance represents the key mechanism in healthy immune responses to allergens. It is characterized by T_{Reg} cells, suppressive cytokines and also non-inflammatory Ab isotypes. There is increasing evidence to support Tr1 cells and immunosuppressive cytokines as key players in mediating successful allergen-SIT and a healthy immune response to allergens. In addition to allergy, these mechanisms may have implications in autoimmunity, graft-versus-host disease, tumor cell growth, parasite survival, chronic infections and the development of acquired immune deficiency syndrome. Suppression of an immune response may have beneficial effects in the case of hypersensitivity reactions but it may also be harmful in other cases such as cancer development, chronic infection and tissue remodeling. The overexpression of T_{Reg} cells and suppressive cytokines may be responsible for chronicity and tumor tolerance and therefore does not always support a healthy response. Taking this into account, along with the recent advances in knowledge of peripheral tolerance mechanisms; developments of safer approaches and treatment of immune-mediated diseases are expected soon.

6. Expert opinion

Changes in the fine balance between allergen-specific Treg and Th2 and/or Th1 cells are very crucial in the

development and treatment of allergic diseases. In addition to the treatment of established allergy, it is essential to consider prophylactic approaches before the initial sensitization takes place. Preventive vaccines that induce Treg responses could be developed, and allergen-specific Treg cells, which will become predominant, may in turn dampen both the Th1 and Th2 cells and their cytokines, ensuring a well-balanced immune response. By the application of recent knowledge regarding peripheral tolerance mechanisms, more rational and safer approaches are awaited for the treatment, prevention and cure of allergic diseases. Allergen-SIT has been used for almost a century as a desensitizing therapy for allergic diseases and represents the only curative and specific treatment. Administration of appropriate concentrations of allergen extracts has been shown to be reproducibly effective when patients are carefully selected. However, current allergen-SIT vaccines and treatment protocols have several disadvantages related to the content of the vaccine, type of adjuvant, route of application, long duration of treatment, side effects and sometimes limited efficacy. Several strategies have been developed to tackle these issues and it became possible to produce recombinant allergen-SIT vaccines with reduced allergenic activity. The use of immunomodulatory and antigen-directing adjuvants and mucosal SIT routes are being intensely investigated and fruitful results have been reported. Current understanding of immunological mechanisms of allergen-SIT, particularly the role of T regulatory cells in allergen-specific peripheral tolerance may open a new future for novel treatment strategies. With the application of the recent knowledge, more rational and safer approaches for the future of prevention and cure of allergic diseases are expected.

Declaration of interest

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