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Healthy immune response to allergens: T regulatory cells and more

Mübeccel Akdis

The specific immune response to allergens is decisive in the development of clinically healthy or allergic states. In healthy individuals, the B-cell response varies between there being no response and the production of IgG₄- or IgG₁-dominating allergen-specific antibodies in the presence or absence of low amounts of IgE. If a detectable immune response is mounted, T regulatory type 1 (Tr1) cells specific for common environmental allergens consistently represent the dominant subset in healthy individuals. Exposure to high doses of allergens leads to a high concentration of specific IgG₄, detectable IgE and a Tr1 type of immune response. 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 (SIT).

Addresses

Swiss Institute of Allergy and Asthma Research (SIAF), Obere Strasse 22, CH-7270 Davos, Switzerland

Corresponding author: Akdis, Mübeccel (akdism@siaf.unizh.ch)

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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 [1]. These effector Th2 cells produce interleukin (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 hyperreactivity, as well as allergic tissue homing of Th2 cells (Figure 1) [1,2]. The Th1 component, by contrast, might contribute to chronicity and to the effector phase in allergic disease [2]. Activation and apoptosis of epithelial cells induced by interferon

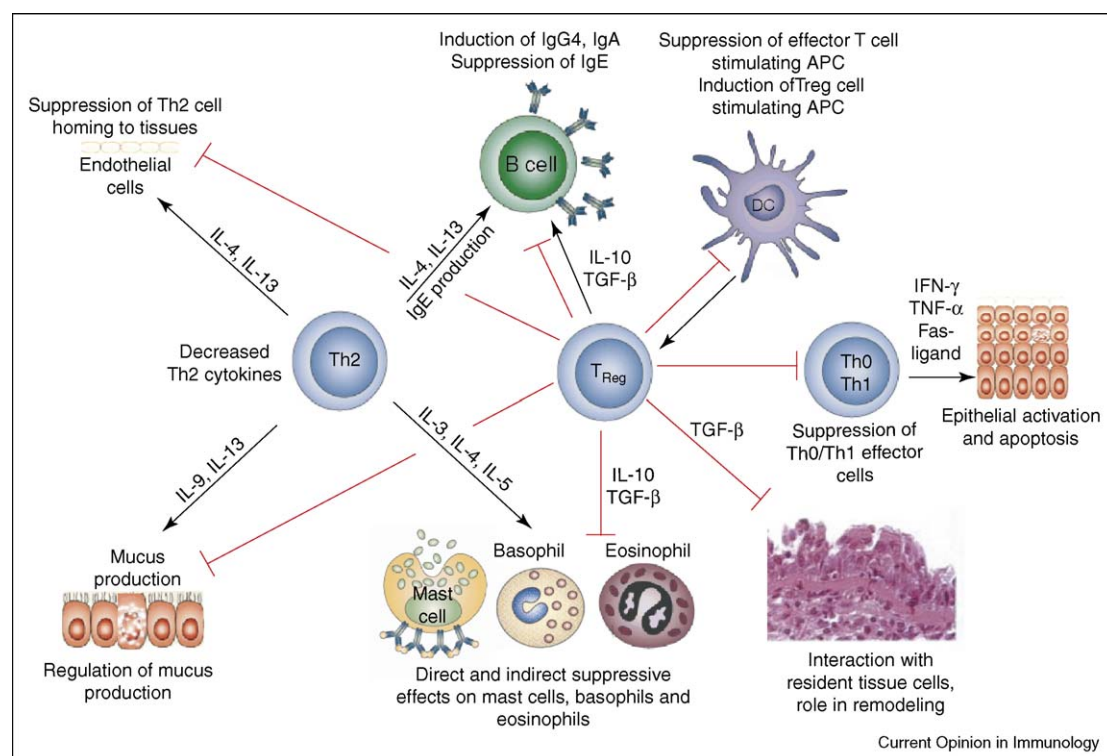
(IFN)- γ , tumor necrosis factor (TNF)- α and Fas-ligand is an essential pathogenetic event in eczematous lesions in atopic dermatitis and bronchial epithelial cell shedding in asthma [2]. Anti-TNF treatments appear to be promising, which suggests the role of TNF in especially severe forms of asthma [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 and 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 role in the healthy immune response to allergens and allergen-specific immunotherapy (SIT) (Figure 1) [7–10,11[•]].

Allergen-specific antibodies in healthy individuals

The allergen-specific antibody response in healthy individuals ranges from the presence of no detectable antibodies (Abs) to the occurrence of low or sometimes relatively high amounts of specific IgG₄, IgG₁ and IgA response in the presence or absence of low amounts of specific IgE (Table 1) [10,12,13]. One of the key questions has been the reason for and mechanisms behind 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 is 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 [14^{••}]. In principal, no exposure or no presentation (exposure in small amounts) could be responsible for the lack of Ab response. Low amounts of exposure leading to immunological ignorance owing to no accessibility and/or short persistence of the allergens in secondary lymphoid organs, which has been demonstrated in mouse models, still remains as a hypothetical possibility [15]. Short persistence and low dose might not be as demonstrative in humans as they are in mice, because a single bee sting can induce IgE and IgG and relatively small doses of T-cell epitope peptides injected into skin can initiate allergic reactions [16–18]. One honeybee sting releases ~50–140 μ g of venom per sting and contains 5–15 μ g of the major allergen phospholipase A₂. A single honeybee

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Figure 1



Suppression and regulation of B cells, dendritic cells (DCs), and Th1 and Th2 cell-mediated features of allergic inflammation by T_{Reg} cells. T_{Reg} cells utilize multiple suppressor factors to regulate the undesired activity of effector Th2 cells. IL-10 and TGF- β suppress IgE production and induce non-inflammatory Ig isotypes IgG₄ and IgA, respectively. Furthermore, these two cytokines directly suppress allergic inflammation induced by effector cells such as mast cells, basophils and eosinophils. In addition, Th2 cells are suppressed by T_{Reg} cells and can therefore no longer provide cytokines such as IL-3, IL-4, IL-5, IL-9 and IL-13. These cytokines are required for the differentiation, survival and activity of mast cells, basophils, eosinophils and mucus-producing cells, as well as for the tissue-homing of Th2 cells. Suppression of DCs that support the generation of effector Th2 and Th1 cells and the induction of T_{Reg} cell-inducing DC subsets is one of the key features. The Th1 cytokine, IFN- γ , in combination with TNF- α and/or FasL induces activation, chemokine production and apoptosis of skin keratinocytes and bronchial epithelial cells, which represent essential tissue injury events in atopic dermatitis and asthma. T_{Reg} cells might be involved in tissue remodeling via TGF- β in asthma. T_{Reg} cells suppress the stimulation of Th0/Th1 cells, leading to the abrogation of tissue injury mechanisms (red line, suppression; black arrow, stimulation).

sting is sufficient to induce IgE or IgG production against phospholipase A₂ [18]. Wasp venom has ~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 [18].

Non-allergic beekeepers, who are exposed to multiple honeybee stings owing to their profession, represent a suitable model for high-dose allergen exposure and still a healthy immune response. These beekeepers have ~1000-times higher specific IgG₄ versus specific IgE ratio compared with allergic individuals [19]. The level of specific IgG₄ primarily reflects exposure. In beekeepers, the amount of bee venom (BV) specific serum IgG₄ produced correlates with the number of annual stings and with the number of years spent in bee-keeping. High dose of exposure appears to induce clinical tolerance: 45% of beekeepers who were stung <25 times a year had a history

of systemic sting reactions, compared with no allergy in those with >200 stings per year [18]. High-dose exposure causing increased specific IgG₄ and protection from allergy has also been demonstrated in those that have high pet contact, as in this group there is a low occurrence of asthma and allergies in children [20]. IgG₄ has unique structural and functional features among IgG subsets. Its heavy chains might re-pair in a way that leads to generation of functional monomeric antibodies, it has low affinity for certain Fc γ receptors, and it does not activate complement [21]. As such, IgG₄ is uniquely capable of inhibiting immune complex formation by other isotypes and thus might have an anti-inflammatory role in immunity.

An allergen dose controlled model that induces long-term cure in humans is allergen-SIT. Currently allergen-SIT is based on subcutaneous or sublingual administration of disease eliciting allergens or derivatives. Although peripheral tolerance has been demonstrated in specific T

Table 1

Components of immune response to allergens in healthy and allergic individuals.

Specific antibody response in serum	
Healthy	No response Detectable IgG ₁ , IgG ₄ and IgA High amounts of IgG ₄ , relatively low amounts of IgG ₁ and detectable IgA and IgE
Allergic	Relatively high amounts of IgE, together with low or high amounts of IgG ₁ , IgG ₄ and IgA
T-cell response in allergen-specific cells and in PBMCs	
Healthy	No response Th0 response in PBMCs and specific T-cell clones with low frequency Tr1, particularly IL-10-dominating response with relatively high frequency
Allergic	Th2 response with varying quantities of IL-4, IL-5 and IL-13, in the presence of detectable IL-10 and IFN- γ
Clinical outcome	
Healthy	Healthy Skin-prick test and IgE positivity; clinical disease cannot be induced Skin-prick test and IgE positivity and healthy in normal circumstances; clinical disease can be induced by provocation tests and it is dose-dependent
Allergic	Intermittent or persistent allergy in various clinical forms

cells, the ability of B cells to produce specific IgE antibodies is not eliminated during allergen-SIT [22]. 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 six months to three years. A similar change in specific isotype ratio has been observed in SIT of various allergies [22–24]. Moreover, IL-10 that is produced and progressively secreted during allergen-SIT appears to counter-regulate synthesis of antigen-specific IgE and IgG₄ antibodies [9]. IL-10 potently suppresses both total and allergen-specific IgE, and it simultaneously increases IgG₄ production in cultures [9,25]. Therefore, IL-10 not only generates T-cell tolerance but 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 mediated by allergen-specific IgG₄ that blocks formation of allergen-IgE complexes and binding to CD23⁺ B cells. A probable consequence of this is inhibition of IgE-facilitated allergen presentation to T cells [23]. 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. Affinity maturation of allergen-specific antibodies during natural exposure or allergen-SIT might have an important role, which remains to be elucidated. In addition, increase in allergen-specific IgA during SIT might imply a role for transforming growth factor (TGF)- β as an IgA isotype switch factor and involvement of T_{Reg} cells, which needs to be further investigated [10,26,27].

Allergen-specific T cell response in healthy individuals

Studies of the T cell response to allergens in healthy individuals have demonstrated a wide range of immune

responses, from no detectable response to involvement of active peripheral tolerance mechanisms mediated by different subsets of T_{Reg} cells (Table 1). Investigation into T cell mediated immune suppression increased during the 1990s and the concept that T_{Reg} cells can suppress 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 [6,28].

In a large number of healthy individuals, T cells do not show any proliferative response to allergens in peripheral-blood mononuclear cell (PBMC) cultures; this could be owing to the low frequency of specific T cells because of lifetime non-exposure. In cultures, T_{Reg} cells are responsible for active suppression against allergens in most healthy individuals [8,29]. In individuals who show a detectable IgG response, a balanced allergen-specific immune response caused by expansion of Tr1 cells has been demonstrated [8]. Tr1 cells, also known as inducible T_{Reg} cells, are defined by their ability to produce high levels of IL-10 and TGF- β [6,28]. 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 [9,10,23,30]. 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 [31]. As well as being associated with CD4 and CD25 expression, these cells are associated with the transcription factor FoxP3 [32]. Various allergic and autoimmune phenotypes develop in X-linked immune

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dysregulation, polyendocrinopathy and enteropathy syndrome (IPEX) spontaneously when CD4⁺CD25⁺ T_{Reg} cells are nonfunctional, which indicates the presence of a population of professional suppressor (T_{Reg}) cells [33]. 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 [34]. 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 [11^{*}], and FOXP3 mRNA expression was significantly increased in asthmatic patients receiving glucocorticoid treatment [35].

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 PBMCs from non-atopic donors in response to allergen. This suppression has been shown to be associated with the control of allergic disease [29]. 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 [9,10,29]. 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 [8]. Allergen-specific Tr1 cells utilize IL-10, TGF- β , cytotoxic T lymphocyte antigen 4 and programmed death-1 as suppressor molecules.

Human CD4⁺ Th1 cells predominantly express histamine receptor (HR)1 and CD4⁺ Th2 cells express HR2, which results in their differential regulation by histamine [36]. Histamine induces the production of IL-10 by dendritic cells [37]. In addition, histamine induces IL-10 production by Th2 cells [38] and enhances the suppressive activity of TGF- β on T cells [39]. All three of these effects are mediated by HR2, which is relatively highly expressed on Th2 cells and suppresses IL-4 and IL-13 production and T-cell proliferation [36]. These findings suggest that HR2 might represent an essential receptor that participates in peripheral tolerance to allergens.

In this respect, SIT vaccines that target T cells and that induce T-cell tolerance, while bypassing IgE binding, represent a novel opportunity for the prevention and

treatment of allergy [40]. Immunization of mice using a major allergen fusion protein or fragments that contain 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 of the allergens, representing novel vaccines for the prevention of allergy [41,42^{*}]. Similarly, T-cell epitope peptide-SIT of bee venom-allergic individuals and cat-allergic individuals induced a Tr1-type allergen-specific immune response in which there was decreased IL-4, IL-5, IL-13 and IFN- γ and increased IL-10 production [43,44,45^{*}].

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, auto-antigens, transplantation antigens and tumor antigens. In mice, IL-10 administration before allergen treatment induces antigen-specific T-cell unresponsiveness, which demonstrates the pivotal role of IL-10 in establishment of peripheral T-cell tolerance [46]. 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 provides further evidence for a key role of this cytokine in the induction and maintenance of peripheral tolerance [47]. Similarly, inappropriate stimulation of tumor-reactive human T cells was shown to result from increased endogenous IL-10 production by these cells, indicating also a role for IL-10 in tumor-specific tolerance [48]. IL-10-producing antigen-presenting cells, such as B cells [49] and dendritic cells [50] as well as clonally expanded IL-10-producing allergen-specific Tr1 cells [8,9], all contribute to the suppressive effects of IL-10. Interestingly, the same features of peripheral tolerance were found in the T cells of healthy beekeepers who had previously been stung by high 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 BV-SIT [9].

IL-10 inhibits the proliferative T-cell response in PBMCs to various allergens, however does not suppress the proliferative responses of T cells that are stimulated by anti-CD3 [51]. IL-10 suppresses T cells by blocking CD28 and inducible co-stimulator (ICOS) co-stimulatory signals in a rapid signal transduction cascade. Upon binding to its receptor, IL-10 activates two tyrosine kinases — Jak1 and Tyk2. Tyk2 acts as a constitutive reservoir for SH2-domain-containing protein tyrosine phosphatase 1 (SHP-1) in resting T cells. Upon activation, SHP-1 binds rapidly to CD28 and ICOS and dephosphorylates them within 1.5–3 min [52^{**}]. As a consequence, the binding of phosphatidylinositol 3-kinase to either co-stimulatory

molecule no longer occurs and downstream signaling is inhibited. In support of these findings, spleen cells from SHP-1-deficient mice showed increased proliferation induced by CD28 and ICOS stimulation in comparison with wild-type mice; this proliferation 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 both altered SHP-1 functions and abolished the suppressive effect of IL-10 [52^{••}].

The TGF- β superfamily consists of more than 35 members, with TGF- β 1 being the proteotypic member. TGF- β 1-deficient mice develop the phenotype, caused by activated CD4⁺ T cells, of a rapidly wasting syndrome leading to death by the age of 3 or 4 weeks [53,54]. Although controversial, TGF- β has been implicated in the conversion of naïve CD4⁺CD25⁻ T cells into CD4⁺CD25⁺ T cells by the induction of FoxP3 [55]. By contrast, several recent studies demonstrated that TGF- β plays an essential role in the development of IL-17-secreting T cells, which promote neutrophilic inflammation [56^{••}]. TGF- β signaling has been demonstrated to be required for *in vivo* expansion and immunosuppressive capacity of CD4⁺CD25⁺ T cells [57]. However, studies with TGF- β -deficient mice have shown that CD4⁺CD25⁺ T cells can develop after up to 2 weeks and autocrine TGF- β production was not essential for these cells to exhibit suppressive activity *in vivo* [58]. 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 [59]. The exact suppressive mechanisms behind TGF- β receptor pathways remain to be elucidated.

Conclusions

In sensitized individuals, peripheral T-cell tolerance represents the key mechanism in healthy immune responses to allergens. It is characterized by the presence of T_{Reg} cells, suppressive cytokines and also non-inflammatory Ab isotypes IgG₄ and IgA. Although it is detectable in healthy immune response and shows small increases in allergen-SIT, the complement activating and proinflammatory role of IgG₁ has to be kept in mind. 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 might 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 might have beneficial effects in the case of hypersensitivity reactions, but it might also be harmful in other cases such as cancer development, chronic infection and tissue remodeling. The overexpression of T_{Reg} cells and suppressive cytokines might 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 awaited.

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