

Immune tolerance in allergy

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Research on the mechanisms of immune regulation in allergy and asthma has shown substantial progress in recent years and has led to a variety of allergen-specific therapeutic and preventive approaches. Studies on the area of allergen-specific immunotherapy (allergen-SIT) have provided substantial knowledge on the mechanisms of allergic disease with novel developments for treatment and prevention. Several studies have demonstrated that increased numbers and the activation of allergen-specific T regulatory cells correlate with successful allergen-SIT. Particularly, targeting of the molecular mechanisms of immune tolerance and reciprocal regulation of effector and regulatory T-cell subsets are foreseen for the development of new strategies for immune intervention. This review focuses on current knowledge of immune tolerance to allergens in healthy immune response to allergens and allergen-SIT.

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Introduction

Molecular and cellular mechanisms of immune tolerance-related conditions, such as allergy, asthma, autoimmunity, tumor tolerance, and organ transplantation rejection are complex and influenced by several factors. Encounters with various new molecules in air, water, and diet, and living in a more polluted world with less exposure to infectious agents are supposed to be major causative factors adding to the genetic propensity to developing IgE antibodies responsible for symptoms and signs of allergic disorders [1,2]. Clinical manifestations are allergic rhinitis, allergic asthma, food allergy, allergic skin inflammation, and ocular allergy as single or combined diseases and anaphylaxis [1]. The activation of allergen-specific CD4⁺ T helper 2 (Th2) cells and the production of interleukin-4 (IL-4) and IL-13 that are responsible for class switching to the ϵ heavy chain for IgE antibody

production by B cells represent the sensitization phase of allergens and IL-5 leads to peripheral and tissue eosinophilia. This concept has led to several treatment approaches. For example, anti-IL-5 monoclonal antibody therapy reduces exacerbations and improves quality of life questionnaire scores in patients with refractory eosinophilic asthma, suggesting that eosinophilia and IL-5 have a role in severe exacerbations of asthma [3]. As Th2-like cytokines secreted from tissue cells, IL-25 and IL-33 have been suggested to play a role in the pathogenesis of allergic diseases with eosinophilia. A novel subset of natural killer T (NKT) cells expresses the interleukin-17 receptor B (IL-17RB) for IL-25 (also known as IL-17E) and plays a role in the induction of airway hyperreactivity [4[•]]. IL-33, a novel IL-1 family member, primarily promotes an allergic phenotype by directly activating basophils and eosinophils, suggesting that IL-33 promotes allergic inflammation and Th2 polarization, mainly by the selective activation of these specialized cells of the innate immune system [5].

The default mechanisms in the development of effector T-cell subsets reflect the immune response to intracellular pathogens, helminths and extracellular pathogens for Th1, Th2, and Th17 cells, respectively [6]. Th17 cells were detected in small proportions in allergic inflammation, but their change in response to treatment and their role in the balance between different allergen-specific T-cell subsets remains to be elucidated [7]. T-cells could independently express IL-22, even with low expression levels of IL-17. This argues for a functional specialization of T-cells such as Th17 and Th22 cells, and may drive different features of epidermal pathology in inflammatory skin diseases, including the induction of antimicrobial peptides for Th17 cells and epidermal hyperplasia for Th22 cells. IL-22⁺CD8⁺ T-cell frequency correlated with disease severity in atopic dermatitis (AD) [8[•]]. Although AD has been framed by the Th1/Th2 paradigm as a Th2 polar disease, there is evidence that the IL-23/Th17 axis is largely absent, perhaps accounting for recurrent skin infections in this disease [9]. Prostaglandin E2 (PGE2), acting on its receptor EP4 on T-cells and dendritic cells, not only facilitates Th1 cell differentiation but also amplifies IL-23-mediated Th17 cell expansion *in vitro*. The administration of an EP4-selective antagonist *in vivo* decreases the accumulation of both Th1 and Th17 cells in regional lymph nodes and suppresses the disease progression in mice subjected to experimental autoimmune encephalomyelitis or contact hypersensitivity [10^{••}].

It was also shown in two very recent studies that transforming growth factor- β (TGF- β) in the presence of IL-4

reprograms Th2 cell differentiation and leads to the development of a new population of Th9 cells that produce IL-9 and IL-10 [11*,12*]. Th9 cells seem to lack suppressive function and constitute a distinct population of effector T-cells that promote tissue inflammation [11*,12*]. Furthermore, a key inducer of both Th17 and inducible (i)T_{Reg} cell differentiation, TGF- β , downregulates a transcriptional repressor growth factor independent 1 (Gfi-1) expression, implying a reciprocal negative regulation of CD4 T-cell fate determination. Gfi-1 plays a critical role both in enhancing Th2 cell expansion and in repressing the induction of Th17 and CD103⁺ iT_{Reg} cells [13**]. Peripheral T-cell tolerance induced by different subsets of T_{Reg} cells is seen to be vital for a healthy immune response to allergens and represents a mechanism to overcome chronic inflammation mediated by the aforementioned effector T-cell subsets.

Features of immune tolerance induced by allergen-specific immunotherapy

For almost a century, allergen-specific immunotherapy (allergen-SIT) has been used as a desensitizing therapy for allergic diseases. Allergen-SIT involves repeated administration of the sensitizing allergen (usually by subcutaneous injection or, more recently, by sublingual application) and is the only curative and specific approach to treatment. The induction of an allergen-tolerant state defined as persistence of efficacy following discontinuation of treatment, implying an altered allergen-specific memory T and B cell response, represents a key step in allergen-SIT and healthy immune response to allergens in sensitized individuals [14–19]. In addition, the prevention of new antigen sensitizations [20] and the prevention of progression to more severe disease, such as development of asthma after allergic rhinitis [21] are essential clinical implications of immune tolerance (Box 1).

T regulatory cells in allergy and allergen-specific immunotherapy

In recent years, T_{Reg} cells have become a prime target for strategies aimed at inducing tolerance. Adoptive transfer

of T_{Reg} cells was shown to prevent or cure several T-cell-mediated diseases, including allergy, asthma-like lung inflammation, autoimmune diseases, and allograft rejection, by restoring immune tolerance to allergens, self antigens or alloantigens. Two subgroups of T_{Reg} cells have received particular attention: the naturally occurring forkhead box P3 (FOXP3)⁺CD4⁺CD25⁺ regulatory T-cells and the inducible IL-10 and TGF- β -secreting type 1 T regulatory (Tr1) cells. The CD4⁺CD25⁺ T_{Reg} cells develop in the thymus and are present in normal naive mice and healthy individuals from birth, and the inducible Tr1 cells, which are generated in the periphery under various tolerogenic conditions [22]. The spontaneous development of allergic airway inflammation, hyper IgE, and eosinophilia in addition to various autoimmune diseases in Foxp3 mutant mice is compelling evidence for its importance in allergic inflammation [23]. Humans suffering from immunodysregulation polyendocrinopathy enteropathy X-linked syndrome (IPEX) are similarly affected and mostly develop hyper IgE and eczema due to mutations in the FOXP3 gene [23]. Human FoxP3⁺CD4⁺ T-cells are composed of three phenotypically and functionally distinct subpopulations: CD45RA⁺FoxP3^{low} resting T_{Reg} cells and CD45RA⁺FoxP3^{hi} activated T_{Reg} cells, both of which are suppressive *in vitro*, and cytokine-secreting CD45RA⁺FoxP3^{lo} nonsuppressive T-cells [24*]. Asthmatic patients have decreased FOXP3 expression within their CD4⁺CD25^{high} T_{Reg} cells, and treatment with inhaled glucocorticosteroids in asthmatics increases FOXP3 expression within the CD4⁺CD25^{high} T-cell population [25]. Although most studies have been with natural and inducible CD4⁺ T_{Reg} cells, several other cells with a suppressive or regulatory function such as CD8⁺ T_{Reg} cells and NK_{Reg} cells have been demonstrated [26,27] (Figure 1).

It has now become clear that peripheral T-cell tolerance is characterized mainly by the generation of allergen-specific T_{Reg} cells, which suppress proliferative and cytokine responses against the major allergen [14–19,28,29]. Different studies have suggested roles for both CD4⁺CD25⁺ T_{Reg} cells and Tr1 cells. Allergen tolerance is initiated by autocrine action of IL-10 and TGF- β , which are increasingly produced by the allergen-specific T-cells [14–19]. The demonstration of local FOXP3⁺CD25⁺ T-cells in the nasal mucosa, and their increased numbers after immunotherapy, supported the role of T_{Reg} cells in the induction of allergen-specific tolerance in humans [30]. Their increased numbers correlated with clinical efficacy and suppression of seasonal allergic inflammation [30].

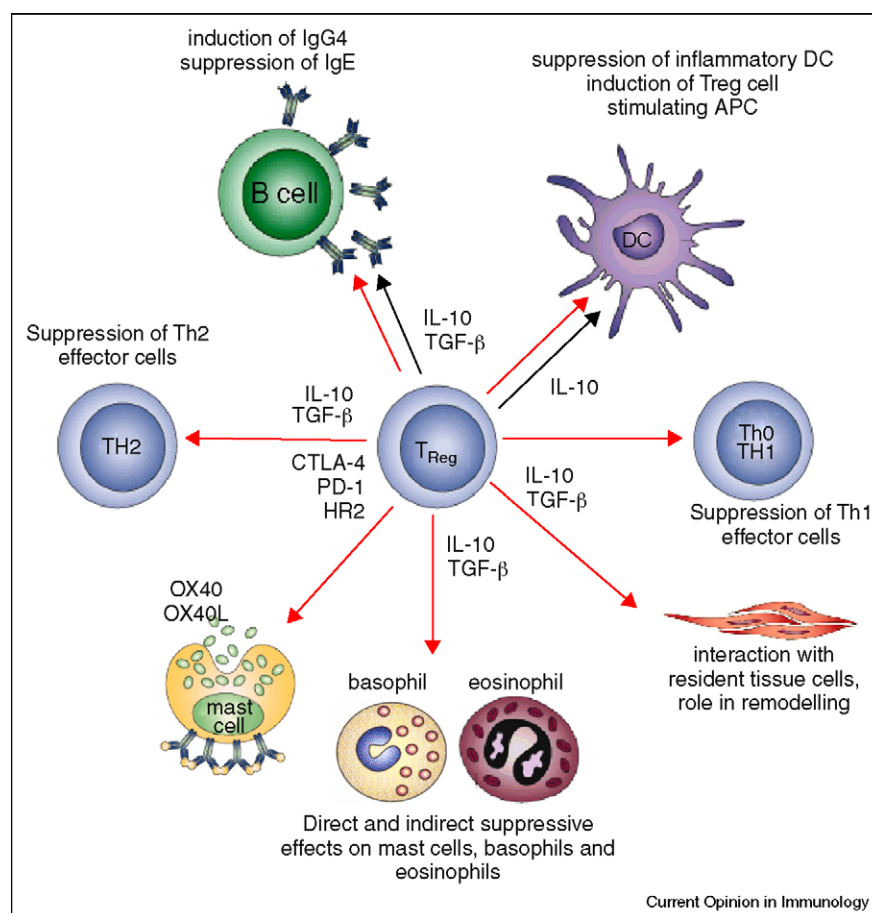
T-cell epitope peptide immunotherapy

Allergen-SIT using peptides, peptide immunotherapy (PIT), is an attractive approach for the induction of peripheral T-cell tolerance in humans. Short allergen peptides, either as native sequences or with amino acid

Box 1 Mechanisms of tolerance induction by allergen-SIT

- Decrease in allergen-stimulated T-cell proliferation [14,58].
- Induction of IL-10 and TGF- β in T-cells [15,16,18,19,58–60].
- Increased suppressive capacity of CD4⁺CD25⁺ T_{Reg} cells [16,29].
- Suppression of Th2 cells and Th1 cells [14,59,60].
- Suppression of allergen-specific IgE production [61].
- Induction of serum allergen-specific IgG4 [62,63] and/or IgA production [16,64].
- Suppression and decreased tissue expression of mast cells, basophils and eosinophils, and their mediators [65–67].
- Allergen presentation and tolerance induction by oral Langerhans cells and in sublingual immunotherapy [68,69].

Figure 1



T_{Reg} cells in allergic immune response. T_{Reg} cells and regulatory cytokines, such as interleukin-10 (IL-10) and transforming growth factor-β (TGF-β) may contribute to the control of allergen-induced immune responses in several different ways. T_{Reg} cells utilize multiple suppressor factors such as IL-10, TGF-β, CTLA-4, PD-1, and histamine receptor 2 (HR2) to regulate the immune response in allergic inflammation. IL-10 and TGF-β suppress IgE production and IL-10 induces IgG4. These two cytokines directly suppress allergic inflammation induced by effector cells such as mast cells, basophils, and eosinophils. Mast cells are suppressed by T_{Reg} cells via OX40–OX40L interaction. T_{Reg} cells promote the development of IL-10-producing dendritic cells. In addition, T_{Reg} cells inhibit Th2 cells, which can no longer provide cytokines such as IL-3, IL-4, IL-5, and IL-9. These cytokines are required for the differentiation, survival and activity of mast cells, basophils, eosinophils, and mucus-producing cells, as well as for tissue homing of Th2 cells. Moreover, suppression of the induction of Th0/Th1 cells abrogates tissue injury mechanisms, such as apoptosis of keratinocytes and bronchial epithelial cells (red arrows: suppression; black arrows: induction).

substitutions, do not contain epitopes that allow for IgE crosslinking and therefore reduce the risk of inducing anaphylaxis. To date, clinical trials of PIT have been performed for two different allergies, namely the major cat allergen Fel d 1 and major bee-venom-allergen phospholipase A₂ (Api m 1) [31–33]. T-cell tolerance was demonstrated as an essential mechanism also in PIT, with downregulation of systemic Th1 and Th2 cell responses to allergen, together with concomitant induction of IL-10 production. In a recent study, treatment with selected epitopes from a single allergen resulted in the suppression of responses to other (linked) epitopes within the same molecule. The study demonstrates *in vivo* evidence for linked epitope suppression and IL-10

induction in both allergic patients and a mouse model designed to closely mimic PIT in humans [32[•]].

Regulation of IgE versus IgG4 and IgA in allergen tolerance during allergen-SIT

IL-10 that is induced and increasingly secreted by Tr1 cells during allergen-SIT, counter-regulates antigen-specific IgE and IgG4 synthesis [15,34]. It is a potent suppressor of both total and allergen-specific IgE, while it simultaneously increases IgG4 production. Thus, IL-10 not only generates tolerance in T-cells, but also regulates specific isotype formation and skews the specific response from an IgE-dominated to an IgG4-dominated phenotype. During the course of house dust mite allergen-SIT,

there was no significant change in specific IgE levels after 70 days of treatment; however, there was a significant increase in specific IgA, IgG1, and IgG4 [16]. The increase of specific IgA and IgG4 in the serum coincided with increased TGF- β and IL-10 from allergen-specific T-cells, respectively. This may account for the role of IgA and TGF- β , as well as IgG4 and IL-10, in peripheral mucosal immune responses to allergens in healthy individuals [16,34]. It is likely that the decrease in IgE/IgG4 ratio during allergen-SIT reflects a change from allergen-specific Th2 to T_{Reg} cell predominance. A change in IgE/IgG4 ratio takes place within weeks as a result of an early increase in IgG4 [14,35]. Similarly, nonallergic beekeepers with very strong IL-10-secreting Tr1 cell-mediated T-cell tolerance have approximately 1000 times higher specific IgG4/IgE ratio compared to allergic individuals [36]. However, although T_{Reg} cell generation happens within days, a significant decrease in IgE occurs over years. The reason for the long time-gap between the change in T-cell subsets, but not IgE levels, is not easily explainable by the half-life of antibodies. In this context, the role of bone marrow-residing IgE-producing plasma cells with very long life spans could serve as a target for immunomodulation [37].

The noninflammatory role for IgG4 is due to the unique structural features of its hinge region, that result in a lower affinity for certain Fc (fraction crystallizable) receptors and the ability of a dynamic Fab (fraction antigen-binding) arm exchange leading to bi-specific antibodies that are functionally monomeric [38,39]. Furthermore, IgG4 does not induce the complement cascade and is capable of inhibiting immune-complex formation by other isotypes, giving these isotype anti-inflammatory characteristics. IgG4 antibodies can be viewed as a marker of introduced allergen dose during SIT.

Mast cell, basophil, and eosinophil regulation by T_{Reg} cells during allergen-SIT

Immune tolerance induced by allergen-SIT also involves increased thresholds for mast cell and basophil activation and decreases IgE-mediated histamine release [40]. There is direct and indirect evidence that this threshold is controlled by T_{Reg} cells. IL-10 produced by increased numbers of Tr1 cells during allergen-SIT may play a significant role. IL-10 was shown to reduce proinflammatory cytokine release from mast cells [41]. In addition, IL-10 downregulates eosinophil function and activity, and suppresses IL-5 production by human Th0 and Th2 cells [42]. Moreover, although demonstrated in a model of myocarditis, IL-10 gene transfer significantly reduces mast cell density, local histamine concentration and mast cell growth, and prevents mast cell degranulation [43]. Recently, allergen-specific basophil suppression was associated with clinical tolerance in patients with milk allergy. Heat-denatured milk-tolerant subjects' basophils were signifi-

cantly less responsive to milk allergen stimulation at all doses than were basophils from reactive (allergic) individuals [44]. A direct effect of T_{Reg} cells was shown by the inhibition of the Fc ϵ RI-dependent mast cell degranulation through cell-cell contact involving OX40–OX40L interactions between T_{Reg} cells and mast cells, respectively [45]. When activated in the presence of T_{Reg} cells, mast cells showed increased cyclic adenosine monophosphate concentrations and reduced Ca²⁺ influx. The *in vivo* depletion or inactivation of T_{Reg} cells enhanced the anaphylactic response. In this way, several aspects of type I hypersensitivity reactions are inhibited by T_{Reg} cells.

Healthy immune response to allergens and immune tolerance

If a detectable immune response is mounted, Tr1 cells specific for common environmental allergens, such as house dust mite, birch pollen, bee venom, and food antigens (hazelnut and pear) consistently represent the dominant subset in healthy individuals [46,47^{••}]. Healthy and allergic individuals exhibit the three allergen-specific T-cell subtypes (i.e. Th1, Th2, and Tr1) in different proportions [46]. Accordingly, a change in the dominant subset and the balance between Th2 and Tr1 cells may lead to either allergy development (when Th2 cells are dominant) or recovery (when T_{Reg} cells are dominant). There are two models of high-dose tolerance to allergens: bee venom and cat. Repeated exposure of nonallergic healthy beekeepers to bee venom antigens during beekeeping season represents a valuable model to ascertain mechanisms of immune tolerance to venom antigens [47^{••}]. After multiple bee stings, venom major-allergen Api m 1-specific Th1 and Th2 cells show clonality and switch toward IL-10-secreting Tr1 cells, which occurs in parallel to suppressed cutaneous late-phase responses and suppressed allergen-specific Th1 and Th2 cells. This regulation is marked as long as venom exposure persists and returns to initial levels within two to three months of the end of the beekeeping season. Exposure to a high dose of cat allergen induces an IgG4 antibody response and IL-10-producing Tr1 cells without sensitization or risk of asthma [48,49]. The results may also explain the observation that animals in the house can decrease the risk of asthma (Box 2).

Another role for T_{Reg} cells in protection from allergy was demonstrated by the exposure of lactating mice to an airborne allergen, which then affects asthma development in progeny [50^{••}]. Airborne antigens were efficiently transferred from the mother to the neonate through milk and that tolerance induction did not require the transfer of immunoglobulins. Breastfeeding-induced tolerance relied on the presence of TGF- β during lactation, and was mediated by regulatory CD4⁺ T-lymphocytes and depended on TGF- β signaling in T-cells.

Box 2 Role of T_{Reg} cells in healthy immune response to allergens

1. Nonallergic beekeepers show T-cell tolerance, induction of Tr1 cells and 1000 times higher specific IgG4 levels [34,36].
2. Nonallergic cat owners show increased IL-10-secreting Tr1 cells and high specific IgG4 [48,49].
3. Children who outgrow milk allergy have increased CD4⁺CD25⁺ T-cells [51].
4. IPEX syndrome has a strong allergy component with hyper IgE, food allergy and atopic dermatitis [23].
5. Breast milk-mediated transfer of an antigen to the neonate resulted in oral tolerance induction leading to antigen-specific protection from allergic airway disease and mediated by T_{Reg} cells and TGF- β signaling [50**].
6. Successful allergen-SIT correlates with the induction of allergen-specific T_{Reg} cells [15,16,18,19,58–60].

Children who outgrew their allergy (tolerant children) had higher frequencies of circulating CD4⁺CD25⁺ T_{Reg} cells and decreased *in vitro* proliferative responses to bovine beta-lactoglobulin in peripheral blood mononuclear cells (PBMCs) compared with children who maintained clinically active allergy. The data suggest that mucosal induction of tolerance against dietary antigens is associated with the appearance of circulating CD4⁺CD25⁺ T_{Reg} cells that are capable of suppressing the effector T-cells generated one week after the reinduction of cows' milk [51].

Role of histamine receptor 2 in allergen tolerance

Immune regulatory functions of histamine are exciting and new data are emerging [52,53]. It has been demonstrated that differential patterns of histamine receptor expression on Th1 and Th2 cells determine reciprocal T-cell responses following histamine stimulation. Th1 cells show predominant, but not exclusive expression of H₁R, while Th2 cells show increased expression of H₂R. Histamine enhances Th1-type responses by triggering the H₁R, whereas both Th1-type and Th2-type responses are negatively regulated by H₂R [54]. In a model of immune tolerance, high-dose bee venom exposure of nonallergic healthy beekeepers led to the upregulation of H₂R on venom allergen-specific Th2 cells. H₂R shows a dual effect by suppressing allergen-stimulated T-cells and increasing IL-10 production.

The rationale for the use of H1-antihistamines during SIT is diverse. There was a recent double-blind placebo-controlled study on 54 bee-venom-allergic patients treated with antihistamine levocetirizine or placebo during dose increase (the first three weeks) of immunotherapy [55]. Levocetirizine reduced side effects without influencing the efficacy of allergen-SIT. Decreased T-cell expression

of histamine receptor HR1/HR2 ratio on day 21 on placebo was prevented by levocetirizine, and levocetirizine group showed significantly high IL-10 production from specific T-cells, suggesting an immunomodulatory effect *in vivo* [55]. In addition, human CD4⁺ T-cells express a functional H₄R [56]. H₄R is upregulated under Th2 conditions, and its stimulation leads to the induction of AP-1 and IL-3, and the induction of pruritus. Research on H₄R shows important evidence for its immunomodulatory role representing an important drug target [55].

Conclusion

Insights into the reciprocal regulation of the different components of the immune system can be translated into improved therapies for immune tolerance-related diseases. Peripheral tolerance to allergens induced by allergen-SIT involves control of allergen-specific immune response in multiple phases, including specific T-cell suppression; generation of noninflammatory antibody isotypes such as IgG4; and suppression of IgE and type 1 hypersensitivity responses; and suppression of late-phase responses, mast cells, eosinophils, and basophils. Combination therapies with allergen-SIT represent a future approach to be tested in clinics and their immunological basis remains to be elucidated. The co-administration of adjunct therapies such as antihistamines, anti-cytokines, and appropriate adjuvants for the induction of T_{Reg} cell response offer promise for clinically more effective and safe allergen-SIT vaccines. So far strategies have been developed for general immune suppression such as anti-dendritic cell, anti-B cell, anti-integrin, anti-cytokine mAbs, or soluble receptors such as anti-CD83 antibodies that target and lyse activated dendritic cells are promising new therapeutic approaches [57*]. Whether they can augment allergen-specific tolerance in humans is not yet known. In addition to allergy, these mechanisms may have implications in autoimmunity, graft-versus-host disease, tumor cell growth, parasite survival, chronic infections, and acquired immune deficiency syndrome.

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