

REVIEW ARTICLE

Immunological mechanisms of allergen-specific immunotherapy

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Abstract

The studies on the mechanisms of specific immunotherapy (SIT) point out its targets that decide on the efficacy of SIT and hence might be used for its further improvement. Several mechanisms have been proposed to explain the beneficial effects of immunotherapy. The knowledge of the mechanisms underlying allergic diseases and curative treatment possibilities has experienced exciting advances over the last three decades. Studies in several clinical trials in allergen-SIT have demonstrated that the induction of a tolerant state against allergens in many ways represents a key step in the development of a healthy immune response against allergens. Several cellular and molecular mechanisms have been demonstrated: allergen-specific suppressive capacities of both inducible subsets of CD4⁺ CD25⁺ forkhead box P3⁺ T-regulatory and IL-10-secreting type 1 T-regulatory cells increase in peripheral blood; suppression of eosinophils, mast cells, and basophils; Ab isotype change from IgE to IgG4. This review aims at the better understanding of the observed immunological changes associated with allergen SIT.

Allergen-specific immunotherapy (allergen-SIT) not only is the most effective therapy for allergies but also provides a unique opportunity to specifically restore normal immunity against allergens in the long-term course of the disease. It represents the only curative and specific approach to the treatment of allergies and provides a prototype model for the development of antigen-specific treatment modalities for other chronic immune regulation-related diseases, such as autoimmunity, certain type of malignancies, organ transplantation, and recurrent abortions. The induction of the persistent specific allergen tolerance is the essential immunological mechanism of SIT. Thus, the long-term (often life-long) desensitization against allergens is achieved by mechanisms, which involve an altered allergen-specific memory T- and B-cell responses leading to the immune tolerance (1–3). Allergen tolerance is the adaption of the immune system characterized by a specific noninflammatory reactivity to a given allergen that in other circumstances would likely induce cell-mediated or humoral immunity leading to tissue inflammation and/or IgE production. Most probably the same mechanisms account for the prevention of new antigen sensitizations (4) and progression of allergic disease, for example, development of asthma in patients with rhinitis (5). In this review, insights into the effects of allergen-SIT on the

reciprocal regulation of the different regulatory and effector components of the immune system are outlined. The understanding of these modalities enables the development of new forms of immunotherapy that might improve efficacy, safety and compliance and achieve more durable results.

The effect of allergen-SIT on specific T cells

The allergen tolerance is combined with peripheral T-cell tolerance, which is characterized mainly by the generation of allergen-specific T-regulatory (T_{Reg}) cells. The effects of Treg cells in allergen-SIT are summarized in Fig. 1. With the new developments in the understanding of the T-cell functions, the former suppressor T cells have now been renamed Treg cells. Two subgroups of T_{Reg} cells have received particular attention: the naturally occurring forkhead box P3 (FOXP3)⁺ CD4⁺ CD25⁺ regulatory T cells (from here on referred to as CD4⁺ CD25⁺ T_{Reg} cells) (6), which develop in the thymus and are present in birth, and the inducible T_{Reg} cells, which are generated in the periphery under various tolerogenic conditions. Among them, T-regulatory type 1 (Tr1) cells have been shown to play a major role in allergen tolerance and can be induced by allergen-SIT in humans (7–10). Analysis of total Treg numbers in peripheral blood

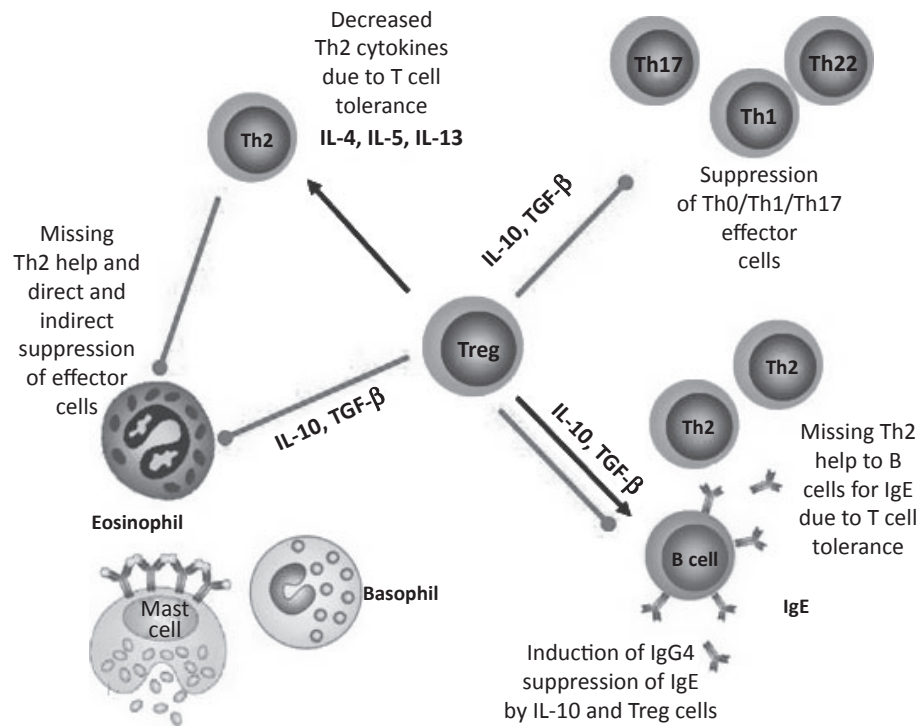


Figure 1 Suppression of features of allergic inflammation by Treg cells. Treg cells utilize multiple suppressor factors to regulate undesired activity of effector Th2 cells. IL-10 and transforming growth factor- β suppress IgE production and induce IgG4 and IgA, respectively. Both cytokines directly suppress allergic inflammation induced by effector cells such as mast cells, basophils, and eosinophils. In addition, Th2 cells are suppressed by Treg cells and can

therefore no longer provide cytokines such as IL-4, IL-5, and IL-13. These cytokines are required for the differentiation, survival, and activity of mast cells, basophils, eosinophils, and mucus-producing cells. In addition, suppression of Th1, Th17, Th22 cell-mediated features of allergic inflammation by Treg cells takes place (red line: suppression, black line: stimulation).

fails to demonstrate significant changes associated with SIT (11–13). However, in vitro allergen-stimulated peripheral blood mononuclear cells (PBMC) showed increased frequency of IL-10-secreting cells. The Tr1 frequency increased progressively from 3–6 months and then decreased at 12 months, although levels were still higher than at pretreatment. Thus, allergen-induced Treg may play a more prominent role in the initial phases of SIT (11, 14, 15). In addition to peripheral blood changes, Treg cells also increased in the tissues of allergic organs after allergen-SIT of allergic rhinitis (16). Grass pollen immunotherapy increased the expression of mucosal and peripheral T-cell IL-10 (17) and transforming growth factor (TGF- β) (18). Increased numbers of local FOXP3⁺ CD25⁺ T cells in the nasal mucosa after SIT, which correlated with clinical efficacy and suppression of seasonal allergic inflammation (16), have been demonstrated.

There are both antigen-specific and general nonspecific roles of Treg cells that tune the threshold of T-cell activation. In allergen-SIT, peripheral T-cell tolerance is initiated by the increased autocrine action of allergen-specific T_{Reg} cells that produce high levels of the anti-inflammatory cytokines IL-10 and TGF- β (1, 7, 8, 8, 9, 9, 10, 10, 19). Identification of Treg

cells is still a problem, because all of the presently used Treg markers (CD25, CTLA-4, GITR, LAG-3, CD127, and Foxp3) represent general T-cell activation markers and are not fully Treg-specific. Cytokine production by Treg cells can vary, depending on the type of the organ they reside in and the way in which they are stimulated. Treg cells may suppress Th cells with different antigen specificities. It is to be speculated that the suppression is more effective when the Treg cell and the suppressed Th cell have the same antigen specificity. However, the 'infectious' expansion of IL-10-producing T-cell populations has been described in immunotherapy with peptides containing T-cell epitopes from Fel d 1, the major cat allergen. Reduced proliferative and cytokine responses, associated with induction of IL-10, to both treatment and nontreatment peptides, which is indicative of linked epitope suppression, have been demonstrated. Thus, the peptide-specific T-cell population was capable of down-regulating an established inflammatory response driven by multiple T-cell epitopes (12, 15). The Tr1 subset has been shown to produce only IL-10, IL-10 and some interferon- γ (IFN- γ), or IL-10 and TGF- β in different experimental and/or clinical settings (20–24). For example, Tr1 cells against mucosal allergens, such as house dust mite, birch pollen, or food antigens, produce IL-10 and TGF- β (9)

[this subclass has also been labeled as Th3 cells (25, 26)]. Tr1 cells induced in the presence of toll-like receptor ligands produce both IL-10 and IFN- γ (22), whereas Tr1 cells induced via skin such as venom allergen-SIT produce predominantly IL-10 (27). These different subsets of Tr1 cells suggest that their secreted cytokine profile differs according to influences of micromilieu (28, 29). In addition to Treg cells, several other cells with a suppressive function such as CD8⁺ T_{Reg} cells and NK_{Reg} cells have been demonstrated (30, 31). Earlier studies emphasized a switch from Th2 cells to Th1 cells in peripheral blood (32, 33), allergic nose (34), and cutaneous late-phase responses (32).

Allergen tolerance in healthy individuals represents a model of induced tolerance. Allergen-specific Tr1 cells are dominant type of T-cell subset in healthy individuals (1, 21). It has been demonstrated in nonallergic healthy beekeepers (35) that after multiple bee stings PLA-specific (phospholipase A-specific) Th1 and Th2 cells switch toward IL-10-secreting Tr1 cells. Histamine receptor H2 (H2R)-dependent signaling plays a role in this process. Histamine interferes with the peripheral tolerance induced during SIT in several pathways (36). Histamine induces the production of IL-10 by dendritic cells (37). In addition, histamine induces IL-10 production by Th2 cells (38). Furthermore, histamine enhances the suppressive activity of TGF- β on T cells (39). All three of these effects are mediated via HR2, which is relatively highly expressed on Th2 cells and suppresses IL-4 and IL-13 production and T-cell proliferation (40). The long-term protection from honeybee stings by terfenadine premedication during rush immunotherapy with honeybee venom in a double-blind, placebo-controlled trial was analyzed (41). After an average of 3 years, 41 patients were re-exposed to honeybee stings. None of 20 patients, who had been given HR1-antihistamine premedication, but 6 of 21 given placebo, had a systemic allergic reaction to the re-exposure by either a field sting or a sting challenge. This highly significant difference suggests that H1-antihistamine premedication during the initial dose-increase phase may have enhanced the long-term efficacy of immunotherapy. Expression of HR1 on T lymphocytes is strongly reduced during ultra-rush immunotherapy, which may lead to a dominant expression and function of tolerance-inducing HR2. This indicates a positive role of histamine in immune regulation during SIT (42). In a double-blind placebo-controlled (DBPC) prospective study, the effect of treatment with L-cetirizine during ultra-rush venom immunotherapy was investigated. Decreased H1R/H2R expression ratio, indicating H2 signal dominance after 21 days, was observed in the placebo group (43). L-cetirizine pretreatment prevented the decrease of HR1/HR2 expression ratio. Consequently, patients treated with L-cetirizine produced significantly more IFN- γ . IL-10 production was induced in both groups but only significantly in L-cetirizine group. The effects of histamine signal on T- and B-cell responses are summarized in Fig. 2.

In addition to Th2-cell suppression, T_{Reg} cells show multiple effects in controlling both allergen-specific immune and allergic inflammation (44). These include the suppression of dendritic cells that support the generation of effector T cells or induction of dendritic cells that support the generation of

T_{Reg} cells (45–47); suppression of Th2 and Th1 cells (48); suppression of allergen-specific IgE and induction of IgG4 and/or IgA (49); suppression of mast cells, basophils, and eosinophils (50); interaction with resident tissue cells and remodeling (51–53).

The effects of allergen-SIT on B cells and antibody synthesis

Although peripheral T-cell tolerance is rapidly induced, there is no evidence for direct B-cell tolerance in the course of allergen-SIT (7). Allergen-specific IgE levels increase initially after the start of SIT and decrease to pretreatment levels during the maintenance phase (54, 55). Allergen-SIT particularly induces allergen-specific antibodies of the IgG4 subclass. Similarly, natural exposure to an allergen in nonallergic individuals, such as beekeepers, is often associated with an increase in specific IgG4. However, there is a poor correlation between the levels of allergen-specific IgG and clinical protection and much better correlation with the dose of allergen that has been administered. Therefore, IgG4 antibodies can be viewed as a marker of introduced allergen dose during SIT. Analysis of the IgG subtypes induced by allergen-SIT has shown increases in allergen-specific IgG4 and IgG1, with 10 to 100-fold increases in their serum levels (56–58). IgG4 antibodies can be viewed as having the ability to modulate the immune response to allergen and thus the potential to influence the clinical response to allergen. In the study using well-defined recombinant allergen mixtures (57), all treated subjects developed strong allergen-specific IgG1 and IgG4 antibody responses. Some patients were not sensitized to Phl p 5 but, nevertheless, developed strong IgG antibody responses to that allergen. It has been suggested that subjects without specific IgE against a particular allergen fail to mount a significant IgG4 response (59), but novel results do not support this view and are consistent with induction of a tolerant immune response (57).

Allergen-SIT also influences the blocking activity of IgG4 on IgE-mediated responses (60), especially the IgE Fc ϵ -receptor facilitated allergen presentation to T cells (61). Specific IgG antibodies can also interfere with IgE-dependent cytokine secretion from mast cells. Therefore, it seems to be relevant to measure the blocking activity of allergen-specific IgG or IgG subsets, particularly IgG4 and also IgG1 instead of analyzing only their levels in the sera. IgG4 displays unique structural features of its hinge region that results in a lower affinity for certain Fc receptors. Particularly, the ability of a dynamic Fab arm exchange, which leads to bi-specific antibodies that are functionally monomeric, significantly decreases to possibility for cross-linking by allergens and enhances the allergen-blocking activity (62, 63). Furthermore, IgG4 does not induce the complement cascade and is capable of inhibiting immune complex formation by other IgG subtypes, giving this IgG subtype anti-inflammatory characteristic. In a study analyzing specific antibody affinities, high-affinity IgG4 was found the dominant binding factor for Bet v 1 in sera of birch pollen-allergic patients. SIT did not show any effect on antibody affinity of allergen-

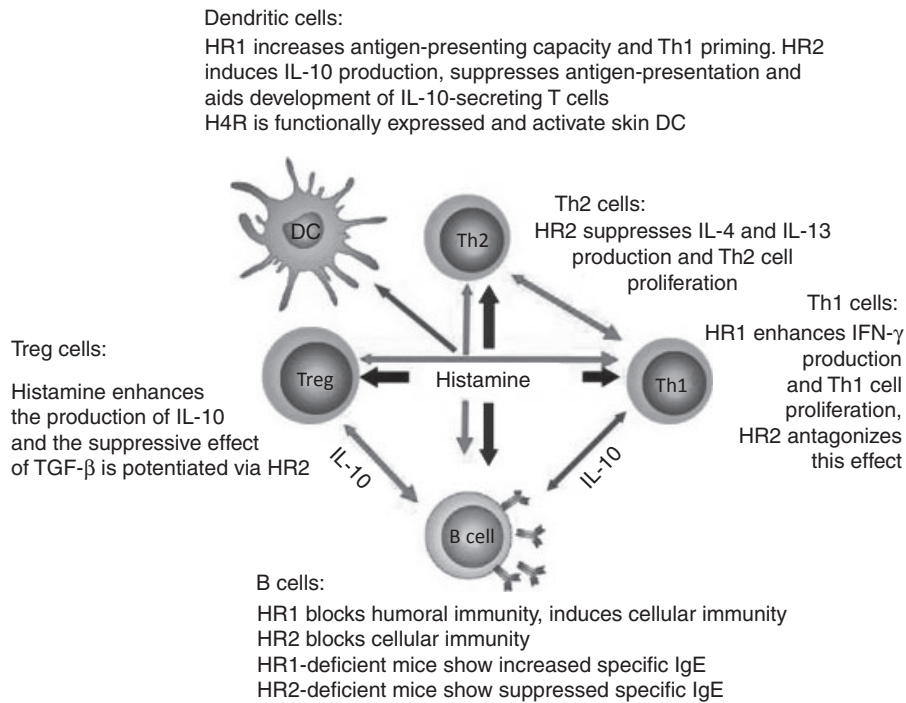


Figure 2 Role of histamine in immune regulation. HRs. HR1 and HR3 induce proinflammatory activity and increased APC capacity, whereas HR2 plays a suppressive role on monocytes and monocyte-derived dendritic cells (DC). Th1 cells show predominant, but not exclusive, expression of HR1, whereas Th2 cells show upregulation of HR2. Histamine induces increased proliferation and IFN- γ production in Th1 cells. Th2 cells express predominant HR2, which acts as the negative regulator of proliferation, IL-4 and IL-13

production. Histamine enhances Th1-type responses by triggering the HR1, whereas both Th1- and Th2-type responses are negatively regulated by HR2, showing an essential role for immune regulation for this receptor. Histamine directly affects B-cell antibody production as a costimulatory receptor on B cells. Allergen-specific IgE production is differentially regulated in HR1- and HR2-deficient mice. HR1-deleted mice show increased allergen-specific IgE production, whereas HR2-deleted mice show suppressed IgE production.

specific IgE, IgG1, or IgG4. Nevertheless, allergic patients with high-affinity IgG1 and IgG4 antibodies showed less symptoms compared to patients with low-affinity antibodies (64, 65).

Cytokines produced by T-helper cells and dendritic cells determine the isotype to which B cells switch, such as IgE induced by IL-4 and IL-13 secreted from Th2 cells (66). IL-10 that is secreted by Tr1 cells during allergen-SIT counter-regulates antigen-specific IgE and IgG4 synthesis (8, 49). Interestingly, T-cell tolerance, induction of Tr1 cells, and increased IL-10 production positively contribute to the B cell and immunoglobulin arm of allergen tolerance. IL-10 is a potent suppressor of both total and allergen-specific IgE, while it simultaneously increases IgG4 production. The role of Treg in the down-regulation of type 1 hypersensitivity reactions is summarized in Fig. 3. During the course of allergen-SIT to desensitize patients allergic to house dust mite, no significant change in specific IgE levels was detected after 70 days of treatment; however, a significant increase in specific IgA, IgG1, and IgG4 was observed (9). The increase in specific IgA and IgG4 in the serum coincided with increased TGF- β and IL-10, 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 (9, 49).

However, while T-regulatory cells may contribute to the suppression of allergic diseases by suppression of IgE and induction of IgG4, it has been demonstrated that the production of IgA is influenced neither by IL-10 nor by IL-10-secreting Tr1 cells nor by CD4⁺ CD25⁺ Treg cells, whereas it was highly induced by direct B-cell activation via TLR7 and 9. Thus, this antibody isotype is particularly regulated by activation of the innate immune system. Thus, the decrease in IgE/IgG4 ratio during allergen-SIT might reflect a change from allergen-specific Th2 to Treg-cell predominance. A change in IgE/IgG4 ratio takes place within weeks because of an early increase in IgG4 (7, 67). However, although Treg-cell generation happens within days, a significant decrease in IgE occurs within years. This discrepancy is difficult to explain. This might be attributed to the bone marrow-residing IgE-producing plasma cells with very long life span, which could serve as a target for the development of other ways of immunomodulation to fight with allergen-specific IgE in the future (68).

The effects of SIT on eosinophils, mast cells, and basophils

After successful SIT, both immediate-type and late-phase responses are reduced in the skin and in the nasal and bron-

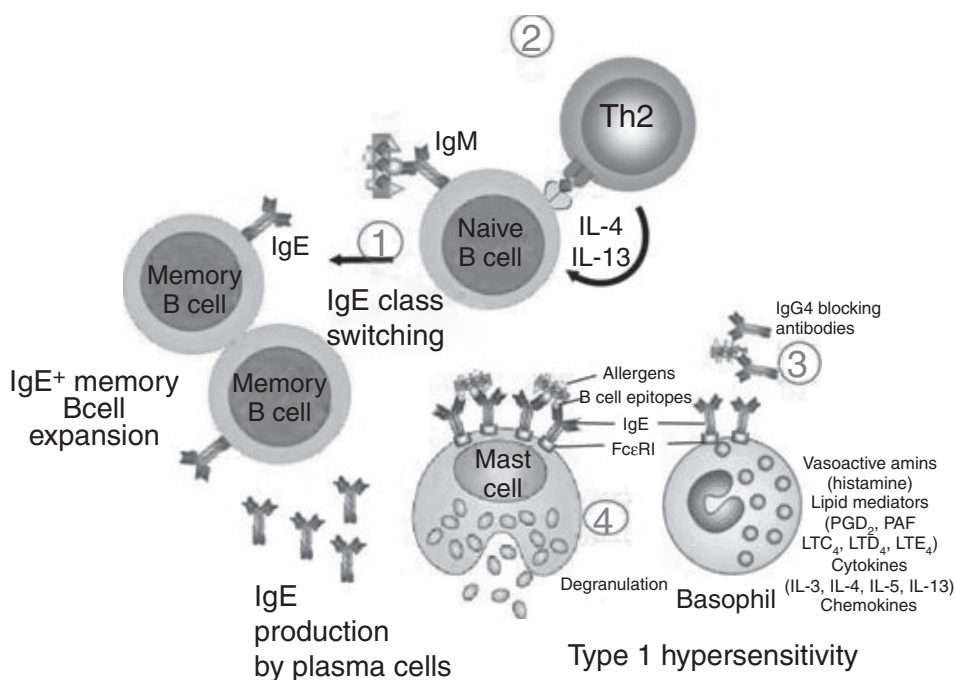


Figure 3 Type 1 hypersensitivity reactions and role of Treg cells that down-regulate them. 1, Suppression of IgE by IL-10; 2 Suppression of Th2 cells that activate mast cells and basophils by IL-10

and transforming growth factor- β . 3, Induction of blocking antibodies by IL-10; 4, Suppression of mast cell, density, growth, degranulation by IL-10.

chial mucosa. The allergen-induced late-phase reactions (LPR) in these target organs are virtually abolished. The mechanism of LPR is different from mast cell-mediated immediate reaction and involves the recruitment, activation, and persistence of eosinophils and activated T cells at the sites of allergen exposure (2). Successful allergen-SIT results in the increase in the threshold of allergen concentration, which is necessary to provoke immediate or LPR in the target tissue. In addition, it decreases the amount and severity of responses to allergen-nonspecific stimulation. Bronchial, nasal, and conjunctival hyper-reactivity to nonspecific stimuli, which seems to reflect underlying mucosal inflammation, decreases after SIT and correlates with clinical improvement (69). The threshold of overall allergen-induced and nonspecifically induced inflammatory response of the tissues shows an increase. During birch pollen SIT, reduced plasma levels of eosinophil cationic protein, a marker of eosinophil activation, and reduced adhesion (70) as well as chemotactic factors for eosinophils and neutrophils correlated with decreased bronchial hyper-reactivity and clinical improvement (71).

Allergen-SIT efficiently modulates the thresholds for mast cell and basophil activation and decreases IgE-mediated histamine release (72). The decrease in allergen-induced *in vitro* basophil degranulation occurs much earlier than a significant decrease in serum allergen-specific IgE and skin test reactivity (73).

There are several lines of evidence suggesting that IL-10 and FoxP3⁺ Treg cells play roles in the regulation of this third arm of the allergic responses. IL-10 produced by increased numbers

of Tr1 cells during allergen-SIT down-regulates eosinophil function and activity and suppresses IL-5 production by human Th0 and Th2 cells (74). In a rodent myocarditis model, IL-10 gene transfer leads to a general increase in IL-10 levels, significantly reduces mast cell density, local histamine concentration and mast cell growth, and prevents mast cell degranulation (75). Moreover, T_{Reg} cells directly inhibit the Fc ϵ RI-dependent mast cell degranulation through cell–cell contact involving OX40/OX40L interactions between T_{Reg} cells and mast cells, respectively (50). The *in vivo* depletion or inactivation of T_{Reg} cells caused the enhancement of the anaphylactic response.

It has been demonstrated that high IL-17 secretion by allergen-specific T cells may be associated with a poor therapeutic outcome of sublingual immunotherapy (SLIT) (76). This is in line with the observation that an indirect effect of Treg cells on neutrophilic inflammation is observed via the suppression of Th17 cells. IL-17A secreted from Th17 cells plays an important homeostatic role in regulating neutrophil generation and blood neutrophil counts (77).

Conclusion

Intensive studies are being performed to improve efficacy and safety of allergen-SIT, such as the use of recombinant proteins, peptides, fragments, and hybrid allergens are promising, but are only in an early stage of human clinical trials. There are several essential requirements underlying novel strategies for the development of safe and more efficient aller-

gen-SIT vaccines. A basic requirement for an allergen vaccine in achieving successful SIT without risk of anaphylaxis is to induce T-cell tolerance. T-cell tolerance seems to be central and different types of Treg cells control several facets of allergic inflammation. They regulate IgE vs IgG4 and favor the allergen-specific antibodies toward the noninflammatory and nonanaphylactic constellation. In addition, they directly or indirectly affect mast cells, basophils, and eosinophils, so that these effector cells are not anymore contributing to allergic inflammation.

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Acknowledgments

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Conflicts of interest

None.

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