

5 Immunologic Responses to Sublingual Allergen Immunotherapy

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INTRODUCTION

The physiopathology of the allergic immune responses is complex and influenced by several factors, including genetic susceptibility, route of exposure, allergen dose, and in some cases the structural characteristics of the allergen (1). The allergic immune response requires sensitization and development of specific immune response toward the allergen. During sensitization to allergen, priming of allergen-specific CD4⁺ T helper 2 (Th2) cells results in the production of Th2 cytokines [such as interleukin-4 (IL-4) and IL-13] that are responsible for class switching to the ϵ heavy chain for IgE production by B cells, mucus production and activation of endothelial cells for Th2 cell, and eosinophil migration to tissues (2,3). IgE sensitizes mast cells and basophils by binding to the high-affinity receptor for IgE (Fc ϵ RI) expressed on their surface. Upon cross-linking of the IgE-Fc ϵ RI complexes by allergen, mast cells and basophils degranulate, releasing vasoactive amines (principally histamine), lipid mediators (prostaglandins and cysteinyl leukotrienes), cytokines and chemokines, all of which characterize the immediate phase of the allergic reaction (2,3).

After the sensitization phase, clinical allergic inflammation and reactions to allergen challenge are observed in the target organ leading to the development of allergic rhinoconjunctivitis, eczema, asthma, and systemic anaphylaxis. T cells constitute a large population of cellular infiltrate in allergic inflammation, and a dysregulated immune response appears to be an important pathogenetic factor. Cardinal events during allergic inflammation can be classified as activation, organ-selective homing, survival and reactivation, and effector functions of immune system cells (4,5). T cells are activated by aeroallergens, food antigens, autoantigens, and bacterial superantigens in allergic inflammation. They are under the influence of skin, lung, or nose-related chemokine network and show organ-selective homing. A prolonged survival of the inflammatory cells in the tissues and consequent reactivation is observed in the subepithelial tissues. Finally, T cells display effector functions, which result in elevated IgE levels, eosinophil survival, and mucus hyperproduction. They also interact with bronchial epithelial cells, smooth muscle cells, and keratinocytes causing their activation and apoptosis (4,6). Peripheral T-cell tolerance to allergens can overcome all of the above pathological events in allergic inflammation because they all require T-cell activation.

Allergen-specific immunotherapy (SIT) is the only treatment that leads to prolonged tolerance against allergens due to restoration of normal immunity. Although very limited studies have been performed, immunological studies on mechanisms of sublingual immunotherapy (SLIT) might show similar immune regulation patterns compared with subcutaneous immunotherapy (SCIT). In addition, animal studies of mucosal tolerance indicate generation of T regulatory (Treg) cells and their cytokines by application of vaccines via sublingual, oral, and other mucosal routes. The induction of a tolerant state in peripheral T cells represents an essential step in SCIT and peptide immunotherapy (PIT) (7). Peripheral T-cell tolerance is characterized mainly by suppressed proliferative and cytokine responses against the major allergens and its T-cell recognition sites. It is initiated by autocrine action of IL-10 and/or transforming growth factor β (TGF- β), which are increasingly produced by the antigen-specific Treg cells. Tolerized T cells can be reactivated to produce either distinct Th1 or Th2 cytokine patterns, thus directing allergen-SIT toward successful or unsuccessful treatment. Treg cells directly or indirectly influence effector cells of allergic inflammation, such as mast cells, basophils, and eosinophils. In addition, there is accumulating evidence that they may suppress IgE production and induce IgG4 and IgA production against allergens. By the

application of the recent knowledge in mechanisms of allergen-SIT, more rational and safer approaches are awaiting for the future of prevention and possibly cure of allergic diseases.

NONINJECTION IMMUNOTHERAPY

Concerns about the safety of subcutaneous administration of allergens have inspired the search for effective noninjection routes of administration for allergen-SIT. These routes include oral immunotherapy (OIT), local bronchial immunotherapy (LBIT), local nasal immunotherapy (LNIT), and SLIT.

First reports on the oral administration of allergen (immediate swallowing of allergen) date back as far as 1927 (8). OIT has been abandoned in clinical practice due to a lack of statistically significant improvement of symptoms (only three studies have shown clinical benefit of OIT (9), high costs, and frequent side effects). However, given the large number of animal studies that show oral tolerance, it still remains a fruitful approach.

LBIT has been performed in only two double-blind placebo-controlled studies, both on house dust mite-allergic patients (10,11). Only one of these studies reported clinical improvement and patients suffered from many unwanted effects. Unproven efficacy and high risk levels have essentially stopped research in this area.

More studies have been performed using LNIT during the last few decades. Aqueous allergen extracts were proven highly effective, but frequently induce rhinitis. Dry powder extracts did not cause this problem and showed minimal side effects. Despite this and clinical success, the use of LNIT is in decline. The requirement of highly skilled staff to administer the allergens correctly (without the powder entering the deep airways) and indications that LNIT might not maintain its clinical benefit after it is discontinued favor SCIT over LNIT.

Many studies have been performed with sublingual administration of allergen extracts, either with or without subsequent swallowing of the extract, beginning in the mid-1980s. SLIT has been successfully used to treat allergic rhinitis and asthma, although efficacy varies greatly among studies (12,13). Because of potentially severe, albeit infrequent, side effects associated with SCIT, mucosal routes of administration are being investigated (14). While its safety and efficacy are now largely documented, much remains to be investigated on the immunological mechanisms underlying efficacy of SLIT. A meta-analysis of the double-blind, placebo-controlled trials performed in the past decade show that SLIT is clinically efficacious although, at present, the treatment benefit is approximately half that achieved with SCIT (15). SLIT seems to be effective in the amelioration of clinical symptoms, drug consumption, and bronchial hyperreactivity (16–18), although definitive evidence from large randomized controlled trials is lacking.

A longitudinal, double-blind, placebo-controlled, parallel-group study that included 51 centers from 8 countries, aimed to confirm the efficacy of a rapidly dissolving grass allergen tablet in patients with seasonal rhinoconjunctivitis. Subjects were randomized (1:1) to receive a grass allergen tablet or placebo once daily. A total of 634 subjects with a history of grass pollen-induced rhinoconjunctivitis for at least two years and confirmed IgE sensitivity (positive skin prick test and serum-specific IgE) were included in the study. Subjects commenced treatment at least 16 weeks before the grass pollen season, and treatment was continued throughout the season. The primary efficacy analysis showed a reduction of 30% in rhinoconjunctivitis symptoms and 38% rhinoconjunctivitis medication scores compared with placebo. Side effects mainly comprised mild itching and swelling in the mouth that was, in general, tolerated and led to treatment withdrawal in less than 4% of participants. There were no serious local side effects or severe systemic adverse events. In this multicenter study, SLIT grass allergen tablets were effective in grass pollen-induced rhinoconjunctivitis and represents a safe alternative to SCIT (19).

Various prophylactic or therapeutic sublingual immunization approaches in BALB/c mice with ovalbumin-induced asthma have been studied. Prophylactic sublingual administration of ovalbumin completely prevents airways hyperresponsiveness as well as IL-5 secretion and IgE induction. This effect can be due to prevention of allergen sensitization or induction of oral tolerance. However, therapeutic administration of ovalbumin in solution via either the sublingual or oral route has limited efficacy in already sensitized mice. In contrast, sublingual application of ovalbumin formulated with maltodextrin to enhance mucosal

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adhesion results in a major reduction of established airways hyperresponsiveness, lung inflammation, and IL-5 production in splenocytes. This mucoadhesive formulation significantly enhances ovalbumin-specific T-cell proliferation in cervical, but not mesenteric, lymph nodes and IgA production in the lungs. The availability of a murine SLIT model allows for the investigation of immune mechanisms associated with SLIT (20).

The immunological mechanisms of SLIT seem to be similar to SCIT, although the magnitude of the change in most parameters is modest or no change is observed. It seems likely that the contact of the allergen with the oral mucosa is critical for the success of SLIT (21). It is postulated that most likely oral Langerhans cells are critically involved in this process (22). During SLIT, the allergen is captured within the oral mucosa by oral Langerhans cells and subsequently these cells mature and migrate to proximal draining lymph nodes. Those local lymph nodes may favor the production of blocking IgG antibodies and the induction of T lymphocytes with suppressive function (23,24). Most studies using SLIT report increased levels of serum IgG4 with a relatively modest increase compared with injection immunotherapy (25). There is no consistency in T cell and IgE and effector cell responses, and a significant number of studies fail to detect systemic immunological changes (15,25). This may be related to the different doses of allergen administered in various studies or to the development of more localized immunological changes.

Peripheral blood mononuclear cells (PBMCs) were stimulated with pollen allergen extract after one and two years of SLIT in another study. The expression of IL-10 mRNA increased in both high and low doses and showed a positive correlation with TGF- β expression. IL-5 was suppressed with high dose, which negatively correlated with TGF- β (26). Birch pollen (Bet v 1) extract SLIT patients showed improved nasal provocation scores to birch pollen; however, cross-reactive apple-induced oral allergy syndrome was not significantly reduced. Bet v 1-specific T-cell tolerance to all epitopes and those cross-reactive with Mal d 1 from apple was shown. However, neither Mal d 1-specific IgE and IgG4 levels nor Mal d 1-induced T-cell proliferation changed significantly, probably due to non-cross-reactive epitopes (27). These results may explain why pollen-associated food allergy is frequently not ameliorated by any form of pollen immunotherapy. Although SLIT is increasingly being utilized, several points need further investigation, such as its efficacy in asthma, its mechanisms of action, the optimal dose and time to be administered, its combination with injection immunotherapy, age of onset for its safe use in young children, and its preventive role in the development of allergy.

SEQUENTIAL EVENTS IN ALLERGEN-SIT AND THEIR UNDERLYING MECHANISMS

Very Early Immunotherapy Effect

The underlying immunological mechanisms of allergen-SIT are under investigation. Very early effects are related to mast cell and basophil desensitization. Intermediate effects are related to changes in allergen-specific T cells and late effects are related to B cells, IgE, as well as mast cells, basophils, and eosinophils. Although a definite decrease in IgE antibody levels and IgE-mediated skin sensitivity normally requires years of SCIT, most patients are protected against bee stings during the early stages of SCIT. An important observation is the decrease in mast cell and basophil degranulation and tendency for systemic anaphylaxis within hours (Fig. 1) (28). Although the response seems similar to the rapid desensitization to a drug, the mechanism for this effect is unknown. Acute oral desensitization to penicillin V in the mice demonstrates that antigen-specific mast cell desensitization is one of the main underlying mechanisms for oral desensitization (28). Mediators of anaphylaxis (histamine and leukotrienes) are released during SIT and sting challenges without inducing a systemic anaphylactic response (29). Their piecemeal release, not enough to cause anaphylaxis, may decrease the granule content of mediators and also may affect the threshold of activation of mast cells and basophils. Decreased mediator release in these cells is a demonstrated feature in *in vitro* analysis shortly after beginning allergen-SIT (29-31). Although there are fluctuations and risks for developing systemic anaphylaxis during the course of allergen-SIT, the suppression of mast cells and basophils continues to be affected by changes in other immune parameters such as the generation of allergen-specific Treg cells and decreased specific IgE.

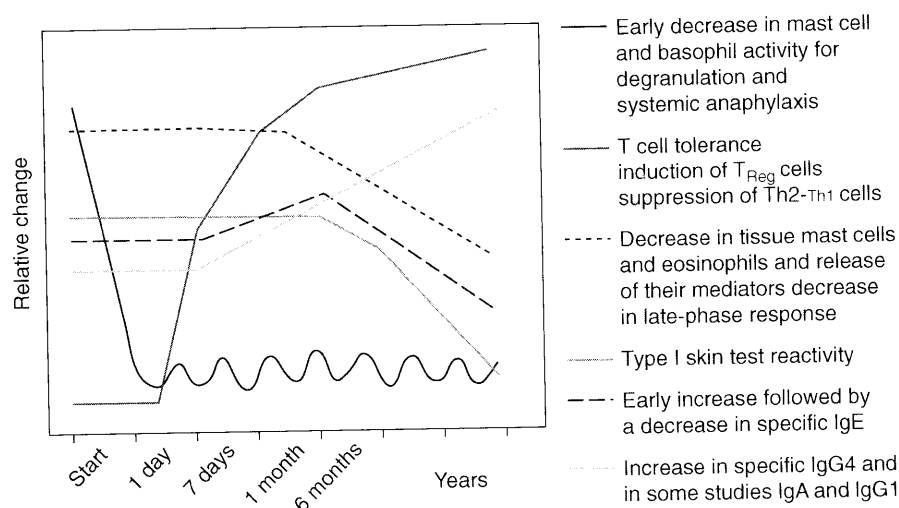


Figure 1 Immunological changes during the course of allergen-SIT. Although there is significant variation between individuals and protocols, with the first injection, an early decrease in mast cell and basophil degranulation and tendency for systemic anaphylaxis is observed. This is followed by generation of allergen-specific Treg cells and suppression of both allergen-specific Th1 and Th2 cells. An early increase of specific IgE and late decrease is observed. This is in parallel to an increase particularly of IgG4 and in some studies IgG1 and IgA. A significant decrease in allergen-specific IgE/IgG4 ratio occurs after several months. A significant decrease in type I skin test reactivity is also observed relatively late in the course of SIT. A decrease in tissue mast cells and eosinophils and release of their mediators and decrease in late-phase response is observed after a few months. The listed effects demonstrated in SLIT were relatively weak compared with injection SIT. *Abbreviations:* SIT, specific immunotherapy; Treg, T regulatory.

Generation of Treg Cells and Peripheral T-Cell Tolerance

The induction of a tolerant state in peripheral T cells represents an essential step in allergen-SIT (Fig. 2). Peripheral T-cell tolerance is characterized by generation of allergen-specific Treg cells and suppressed proliferative and cytokine responses against the major allergen (32–34). Subsets of Treg cells with distinct phenotypes and mechanisms of action include the naturally occurring, thymic-selected CD4+CD25+ Treg cells and the inducible type 1 Treg cells (Tr1) (35). In allergen-SIT, peripheral T-cell tolerance is initiated by the autocrine action of IL-10 and TGF- β , which is increasingly produced by the antigen-specific T cells (33,36,37). The suppression by these cells could partially be blocked by the use of neutralizing antibodies against secreted or membrane-bound IL-10 and TGF- β (34). These cells do, however, express CD4 and CD25, raising the question whether these are inducible Tr1 cells that have upregulated CD25 or naturally occurring CD4+CD25+ Treg cells that produce suppressive cytokines (35). In agreement, CD4+CD25+ Treg cells from atopic donors have a reduced capacity to suppress the proliferation of CD4+CD25– T cells (38). Therefore, upregulation of CD4+CD25+ Treg cells may play a role in allergen-SIT. TGF- β plays a dual role in allergic disease. It suppresses allergen-specific T cells and plays a role in remodeling of the tissues. It remains to be determined whether the remodeling and suppressive role of TGF- β in allergic inflammation show an imbalance, which aggravates instead of controlling the immune response (39).

Studies on the mechanisms by which immune responses to nonpathogenic environmental antigens lead to either allergy or nonharmful immunity demonstrate that Treg cells are dominant in healthy individuals (5,40). If a detectable immune response is mounted, Tr1 cells specific for common environmental allergens, consistently represent the dominant subset in healthy individuals. They use multiple suppressive mechanisms, IL-10 and TGF- β as secreted cytokines, and cytotoxic T lymphocyte antigen 4 (CTLA-4) and programmed death 1 (PD1) as surface molecules. Healthy and allergic individuals exhibit all three, i.e., Th1, Th2, Tr1 type allergen-specific subsets in different proportions (40). Accordingly, a change in the dominant subset and the balance between Th2 and Treg cells may lead to either allergy development or recovery.

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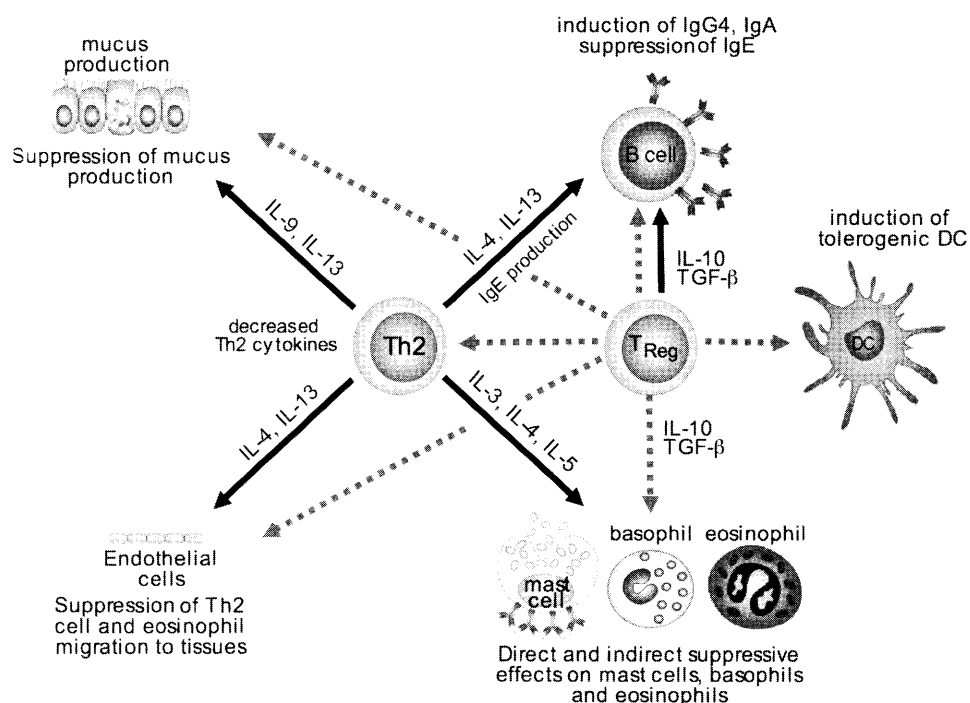


Figure 2 (See color insert.) Immune deviation toward Treg-cell response is an essential step in SIT and natural allergen exposure of nonallergic individuals. Treg cells utilize multiple suppressor factors, which influence the final outcome of SIT. Treg cells suppress proliferation, tissue infiltration, proinflammatory cytokine production, and injury/apoptosis of epithelial cells by both Th1 and Th2 cells. IL-10 and TGF- β induce IgG4 and IgA, respectively, from B cells as noninflammatory Ig isotypes and suppress IgE production. These two cytokines directly or indirectly suppress effector cells of allergic inflammation such as mast cells, basophils, and eosinophils. In addition, IL-10 induces tolerogenic dendritic cells in experimental models (solid line: activation, dotted line: suppression). Abbreviations: SIT, specific immunotherapy; Treg, T regulatory.

Although based on a limited number of studied individuals, a longitudinal study demonstrates that successful SLIT induces IL-10-producing Treg cells and that different immune mechanisms are operative during early and later phases of treatment. In this study, lower allergen doses administered during the early phase might promote the induction of allergen-specific IL-10-producing CD4+CD25+ Treg cells, whereas allergen doses cumulating during the further course of SLIT might foster the induction of deletion/anergy and immune deviation of allergen-reactive T cells, respectively. Active suppression provided by IL-10-producing Treg cells is predominant during the early course of therapy followed by other tolerance mechanisms during the later phase of therapy. Thus, SLIT modulates the allergen-specific T-cell response in a similar way as SCIT (41).

Another study on healthy immune response to allergens demonstrated that CD4+CD25+ Treg cells are associated with the spontaneous remission of cow's milk allergy. Children who outgrow their allergy (tolerant children) have higher numbers of circulating CD4+CD25+ T cells and decreased in vitro proliferative responses to bovine β -lactoglobulin in PBMCs compared with children who maintained clinically active allergy (42). Several studies have been reported in other diseases along the same line. The in vitro proliferative response of human CD4+ T cells to nickel from healthy, nonallergic individuals is strongly augmented when CD4+CD25+ Treg cells are depleted (43). Furthermore, a human in vivo study on immunotherapy of rheumatoid arthritis also showed a marked increase in the number of Foxp3+CD4+CD25+ Treg cells in peripheral blood (44). CD25+ Treg cells are characterized by the expression of the transcriptional regulator *Foxp3* (*FOXP3* in humans), which appears to be master switch gene for Treg cell development and function. The spontaneous development of allergic airway inflammation, hyper IgE, and eosinophilia in addition to various autoimmune diseases in *Foxp3* mutant mice provide compelling evidence for its importance in allergic

inflammation (45). Humans suffering from immunodysregulation polyendocrinopathy enteropathy X-linked (IPEX) syndrome are similarly affected and mostly develop hyper IgE and eczema due to mutations in the *FOXP3* gene (45). Also, a dysregulation of disease-causing effector T cells is observed in atopic dermatitis lesions, in association with an impaired CD4(+)CD25(+)FoxP3(+) T-cell infiltration in the dermis (46).

Allergen-specific T-cell suppression 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. The mechanism of how IL-10 directly inhibits T cells is reported (47). IL-10 suppresses T cells by blocking CD28 and inducible costimulator (ICOS) costimulatory signals in a rapid signal transduction cascade. IL-10 binds its receptor and activates two tyrosine kinases, Jak1 and Tyk2. Tyk2 acts as a constitutive reservoir for Src homology 2 domain-containing protein tyrosine phosphatase 1 (SHP-1) in resting T cells and then tyrosine phosphorylates SHP-1 on activation. SHP-1 rapidly binds to CD28 and ICOS costimulatory receptors and dephosphorylates them within minutes. As a consequence, the binding of phosphatidylinositol-3 kinase to either costimulatory receptor no longer occurs and downstream signaling is inhibited. Accordingly, spleen cells from SHP-1-deficient mice show increased proliferation with CD28 and ICOS stimulation in comparison to wild-type mice, which is 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 alter SHP-1 functions and abolish the suppressive effect of IL-10. In conclusion, the rapid inhibition of the CD28 or ICOS costimulatory pathways by SHP-1 represents the mechanism for direct T-cell suppression by IL-10 (47).

The role of Treg cells is not limited to suppression of Th2 cells. Peripheral tolerance utilizes multiple mechanisms to suppress allergic inflammation. Apparently, Treg cells contribute to the control of allergen-specific immune responses in five major ways: suppression of antigen-presenting cells that support the generation of effector Th2 and Th1 cells; suppression of Th2 and Th1 cells; suppression of allergen-specific IgE and induction of IgG4 and/or IgA; suppression of mast cells, basophils, and eosinophils; interaction with resident tissue cells and remodeling (35).

Regulation of Allergen-Specific IgE and IgG Subtype Responses During Allergen-SIT

Specific IgE in serum and on the surface of mast cells and basophils bound to FcεRI in allergic patients is a hallmark of atopic disease. Although peripheral T-cell tolerance is rapidly induced during SIT, there is no evidence for B-cell tolerance in the early course (32). Natural exposure to a relevant allergen is often associated with an increase in the IgE synthesis. Similarly, SIT frequently induces a transient increase in serum-specific IgE, however, followed by gradual decrease over months or years of treatment (48,49). In pollen-sensitive patients, allergen-SIT prevents elevation of the serum-specific IgE during the pollen season (50). However, the changes in IgE levels cannot explain the diminished responsiveness to specific allergen due to SIT, since the decrease in serum IgE is relatively late and does not correlate with clinical improvement from SIT.

Antibody responses induced during allergen-SIT are functionally heterogeneous, which may account for the conflicting data in relation to the protective effects of IgG (34,48,49). Subclasses of IgG antibodies, especially IgG4, are thought to capture the allergen before reaching the effector cell-bound IgE and thus prevent the activation of mast cells and basophils. However, the relationship between the efficacy of SIT and the induction of allergen-specific IgG subgroups remains a controversial issue, with serum concentrations of allergen-specific IgG correlating with clinical improvement in some studies, but not others (51,52). Allergen-specific IgG may be directed against the same epitopes as IgE, resulting in direct competition for allergen binding and a "blocking" effect. By contrast, induction of IgG specific for other epitopes may result in a failure of the IgG response to compete with IgE, even when IgG is present in molar excess. The concept of blocking antibodies is being reevaluated. Analysis of the IgG subtypes induced by SIT shows specific increases in IgG1 and, in particular, IgG4, with levels increasing 10- to 100-fold (53,54). There is cumulating evidence that SIT also influences the blocking activity on IgE-mediated responses by IgG4, and cellular assays are commonly used to investigate these changes (55).

A novel assay, which detects allergen-IgE binding using flow cytometry, has been used to detect "functional" SIT-induced changes in IgG antibody activity. Results suggest that

successful SIT is dependent on the blocking of the binding of allergen to IgE instead of the induction of a new production of IgG. The efficacy of SIT is not dependent on the induction of IgG.

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Mechanism of Allergen-Specific IgE and IgG Subtype Responses

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successful SIT is associated with an increase in IgG blocking activity that is not solely dependent on the quantity of IgG antibodies (56,57). It seems to be relevant rather to measure the blocking activity of allergen-specific IgG or IgG subsets, particularly IgG4 and also IgG1, instead of measuring their levels in sera. In this context, the role of anti-IgE treatment in the induction phase of allergen-SIT on safety and efficacy has been questioned. Anti-IgE mAb pretreatment enhances the safety of SIT for allergic rhinitis and may be an effective strategy to permit more rapid and higher doses of allergen immunotherapy (58). Its function on long-term efficacy is still under investigation.

The noninflammatory role for IgG4 may be because the IgG4 hinge region has unique structural features that result in a lower affinity for certain Fc_γ receptors and the ability to separate and repair leading to bispecific antibodies that are functionally monomeric (59). Furthermore, IgG4 does not fix complement and is capable of inhibiting immune-complex formation by other isotypes, giving this isotype anti-inflammatory characteristics.

By using well-defined recombinant allergen mixtures, all treated subjects developed very strong allergen-specific IgG4 and also increased IgG1 antibody responses. Some patients who are not initially sensitized to Phl p 5 develop strong specific IgG4, but not IgE antibody responses to Phl p 5 (53). This demonstrates that extract-based antibody measurements may provide wrong information, and studies on mechanisms of allergen-SIT should be performed with single allergens. Nevertheless, IgG4 antibodies can be viewed as a marker of introduced allergen dose, and they have the ability to modulate the immune response to allergen and the potential to influence the clinical response to allergen. In addition, the affinity of newly produced IgG4 and decreasing IgE to allergens has not been intensely studied and may have a very decisive role. Affinity maturation of specific antibodies in allergen immunotherapy and preseasonal versus postseasonal changes in their affinity remain to be elucidated (60).

For SLIT, a significant increase in specific IgG and IgG4 antibodies has not been consistently demonstrated. This has been investigated in a study related to the evaluation of serum IgG4 antibodies specific to grass pollen allergen components in the follow-up of allergic patients undergoing SCIT and SLIT. Preliminary data indicate that preseasonal high-dose SLIT without a build-up phase is safe and well-tolerated by allergic patients. Compared with IgG4 levels induced by SCIT, only a high-dose SLIT regimen results in an appreciable serum-specific IgG4 increase (61).

IL-10, levels of which are enhanced by SIT, appears to counterregulate antigen-specific IgE and IgG4 antibody synthesis (33). 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, it also regulates specific isotype formation and skews the specific response from an IgE- to an IgG4-dominated phenotype (Fig. 2). The healthy immune response to Der p 1 increases specific IgA and IgG4, small amounts of IgG1, and almost undetectable IgE antibodies in serum (34). House dust mite-SCIT does not significantly change specific IgE levels after 70 days of treatment; however, a significant increase in specific IgA, IgG1, and IgG4 is observed (34). The increase of specific IgA and IgG4 in serum coincides 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 (33,62). Most probably the decrease in IgE/IgG4 ratio during allergen-SIT is because of the skew of the predominant allergen-specific T-cell subset from Th2 cells to Treg cells. However, although Treg-cell generation happens within days, a significant decrease in IgE/IgG4 ratio occurs after years. The reason for the long-time gap between the change in T-cell subsets, but not IgE/IgG4 levels, is not easily explained by the half-life of antibodies. The role of bone marrow-residing IgE-producing plasma cells with very long life span remains to be investigated (63).

Mechanisms of Suppression of Effector Cells and Inflammatory Responses During Allergen-SIT

Peripheral T-cell tolerance to allergens, characterized by functional inactivation of the cell to antigen encounter, can overcome both acute and chronic events in allergic reactions (Fig. 2). Allergen-SCIT efficiently modulates the thresholds for mast cell and basophil activation and decreases IgE-mediated histamine release (64). In addition, IL-10 reduces proinflammatory cytokine release from mast cells (65). Furthermore, IL-10 downregulates eosinophil function and activity and suppresses IL-5 production by human resting Th0 and Th2 cells (66).

Moreover, although demonstrated in a model of myocarditis, IL-10 gene transfer significantly reduces mast cell density, local histamine concentration, mast cell growth, and prevents mast cell degranulation (67).

Long-term SCIT is associated with reduction of the immediate and the late-phase reactions (LPRs) to allergen provocation in the nasal and bronchial mucosa or the skin. The mechanism of LPR is different from the mast cell-mediated immediate reaction and involves the recruitment, activation, and persistence of eosinophils, and activated T cells at the sites of allergen exposure (3). Successful SIT results not only in the increase of allergen concentration necessary to induce immediate or LPR in the target tissue but also in the decreased responses to nonspecific stimulation. Bronchial, nasal, and conjunctival hyper-reactivity to nonspecific stimuli, which seems to reflect underlying mucosal inflammation, decreases after SCIT and correlates with clinical improvement (68). During birch pollen SCIT, reduced plasma levels of eosinophil cationic protein (ECP), a marker of eosinophil activation, as well as chemotactic factors for eosinophils and neutrophils correlated with decreased bronchial hyperreactivity and clinical improvement (69). Inhibition by SCIT of the seasonal increase in eosinophil priming has also been demonstrated (70). In biopsies obtained during grass pollen SCIT, decreased eosinophil and mast cell infiltration in nasal and bronchial mucosa after SCIT correlates with the anti-inflammatory effect (71). Similar to these findings, SLIT achieved a significant clinical benefit in birch pollinosis, reduced the eosinophil infiltration in nasal mucosa, and significantly improved pulmonary function during the pollen seasons (72).

UNDERLYING MECHANISMS OF NOVEL AND EMERGING SIT VACCINES AND NOVEL ADJUVANTS FOR THE IMPROVEMENT OF INJECTION AND SUBLINGUAL SIT

Intensive studies to improve efficacy and safety of allergen-SIT are underway. A basic requirement for an allergen vaccine in achieving successful SIT without the risk of anaphylaxis is to express T-cell epitopes, which induce T-cell tolerance and lack antibody-binding sites that mediate IgE cross-linking (73). Conformation dependence of B-cell epitopes and linearity of T-cell epitopes may induce a different regulation of allergen-specific T-cell cytokine toward a nonallergic phenotype. Native allergens utilize IgE-facilitated antigen presentation by dendritic cells and B cells, which activates T cells to produce Th2-type cytokines and B cells to produce further IgE in a secondary response. In contrast, B-cell epitope deleted allergens, which do not bind IgE, do not initiate effector cell degranulation. They utilize phagocytotic or pinocytotic antigen uptake mechanisms in dendritic cells, macrophages, and B cells (74). T cells may be subsequently induced to generate a balanced Th0/Th1-type cytokine pattern in lower quantities as well as T-cell tolerance, which involves Treg cells. Accordingly, targeting T cells and bypassing IgE by modified allergens will enable the administration of higher doses of allergens, which is required to induce T-cell tolerance without the risk of anaphylaxis (74).

Therefore, immunotherapy using peptides (PIT) is an attractive approach for safe SIT. Induction of T-cell tolerance and increased IL-10 production has been demonstrated both in cat Fel d 1 and bee venom Api m 1 PITs (75). A potential barrier to PIT is the apparent complexity of the allergen-specific T-cell response in terms of epitope usage and dominant epitopes in humans and stability of peptides.

Genetically engineered recombinant hybrid molecules that span the whole T-cell repertoire, but do not bind IgE, have been developed to overcome these problems. Folding is complicated by the formation of intra- and intermolecular disulfide bond formation in cysteine-containing proteins, whereas any formed disulfide bond can fix the conformation and limits the freedom of further folding. The probability of a correct or native-like folding rapidly decreases due to the increasing probability of incorrect disulfide bond formation with increasing numbers of cysteines. A fusion protein consisting of the two major allergens of bee venom, Api m 1 and Api m 2, has been generated to investigate this concept. Destroyed conformational B-cell epitopes, but intact T-cell epitopes of the two allergens, characterize this protein. By providing decreased allergenicity with preserved T-cell tolerance inducing capacity, the Api m [1/2] fusion protein represents a novel vaccine

prototype for allergen fragments and full-length allergens reassembled mosaic vaccines (76). In order to not miss T cells, demonstrating two approaches is necessary: recombinant allergens with preserved dependence on

Another interesting approach is the use of allergen, Bet v 1, which has been completed in the suppression of seasonal allergic rhinitis by a mixture of five recombinant allergens. In symptomatic men, subjects developed a tolerance to SIT vaccines are given where they must be studied. In a study, an MHC class II immunogenicity was demonstrated. Intranasal activity and interaction with infections and tumours in the inguinal lymph nodes.

Aluminium hydroxide is an efficacious and safe adjuvant—stimulating through the Toll-like receptors (TLRs) patterns on micro-presenting cells. Overexpression of TLRs is effective in murine and human immunological CpG adjuvants have been used to a prolonged survival. The same Ambion adjuvant consecutive seasons of injection phase, the number of IL-4 and IL-5 a peptide antigen. IgG antibody titers and particles for allergens with histamine degranulation by adjuvants enhance in mice models.

On the basis of the translocation (MHC) targeting of the allergen consist of a cloned truncated Ii (involved in the trans-Golgi transport) proliferation and differentiation of individuals and proliferation of T cells in allergic mouse models and protection of mice and protection of the treatment of

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phils correlated with
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ated (70). In biopsies
infiltration in nasal
effect (71). Similar
rhinitis, reduced the
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underway. A basic
risk of anaphylaxis
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prototype for allergen-SIT (76). Another interesting approach is to cut the major allergens to fragments and fuse them in a different order without missing any T-cell epitopes in one reassembled mosaic allergen (77). In this study, two fragments of Api m 1, three fragments of Api m 2, and Api m 3 are reassembled in a different order with overlapping residues, in order to not miss any T-cell epitopes. Single injection of both vaccines, which only target T cells, demonstrates a preventive effect on IgE generation in mice. The advantage of these two approaches is that only one molecule has to be produced and purified instead of several recombinant allergens. T-cell epitopes are preserved and B-cell epitopes can be deleted or preserved depending on the type of the fusion molecule.

Another interesting approach is to use fragments and a trimer of major birch pollen allergen, Bet v 1, to treat birch pollen allergy. A double-blind, placebo-controlled study has been completed in three centers. It demonstrates increases in IgG1, IgG2, IgG4, and IgA and suppression of seasonal increases of IgE (78). In a different approach, the effectiveness of a mixture of five recombinant grass pollen allergens in reducing symptoms and the need for symptomatic medication in grass pollen-allergic patients was demonstrated. All treated subjects developed strong allergen-specific IgG1 and IgG4 antibody responses (53). Allergen-SIT vaccines are generally administered subcutaneously, intradermally, or sublingually, from where they must reach secondary lymphatic organs to induce an immune response. In a mouse study, an MHC class I-binding peptide from the lymphocytic choriomeningitis virus enhanced immunogenicity by as much as 106 times when compared with subcutaneous and intradermal vaccination. Intralymphatic administration induced CD8 T-cell responses with strong cytotoxic activity and interferon- γ (IFN- γ) production that conferred long-term protection against viral infections and tumors (79). The efficacy of allergen-SIT vaccines administered directly into inguinal lymph nodes of humans is currently being investigated.

Aluminium hydroxide is used as an adjuvant for allergen-SIT. These preparations are efficacious and have a good safety profile, but might be improved in efficacy. A new class of adjuvants—so-called immune response modifiers—act on antigen-presenting cells through the Toll-like receptors (TLRs). These recognize pathogen-associated molecular patterns on microorganisms, and depending on the type of TLR, different types of antigen-presenting cells can be targeted. TLR-triggering compounds that can control the overexpression of Th2 cytokines or skew the Th1:Th2 balance toward a Th1 profile are effective in murine models of allergy (73). Oligodeoxynucleotides, containing immunostimulatory CpG motifs that trigger TLR 9, linked to the allergen in ragweed allergy in humans have been utilized. Amb a 1-immunostimulatory DNA sequence conjugate SIT led to a prolonged shift from Th2 immunity toward Th1 immunity and appears to be safe (80). The same Amb a 1 CpG conjugate was effective for the treatment of allergic rhinitis for two consecutive seasons. Although early increases in Amb a 1-specific IgE occurred during the injection phase, a seasonal increase in Amb a 1-specific IgE did not occur. A reduction in the number of IL-4-positive basophils was reported (81). In another study, vaccination with a peptide antigen covalently coupled to highly repetitive virus-like particles induced high IgG antibody titers in humans. This suggests that allergens could be coupled to virus-like particles for allergen-SIT (82). As a different immunological approach, the fusion of allergens with human Fc γ is reported to inhibit allergen-induced basophil and mast cell degranulation by cross-linking Fc γ and Fc ϵ RI receptors (83). In addition, IL-10-inducing adjuvants enhance the efficacy of allergy vaccines in establishing allergen-specific tolerance in mice models (84).

On the basis of protein transduction domain (PTD) technology, modular antigen translocation (MAT) molecules aimed to enhance antigen presentation through intracellular targeting of the MHC II presentation pathway have been engineered (85). MAT vaccines consist of a cloning cassette, which fuses transactivator of transcription (TAT) peptide to a truncated Ii (invariant chain), which is able to target antigens to the nascent MHC II molecules in the trans-Golgi compartment. MAT-conjugated allergens have the ability to stimulate T-cell proliferation and cytokine production in human PBMC cultures derived from allergic individuals and to elicit protective immune responses in mice. MAT vaccines induce a strong proliferation of PBMC at a low concentration and a Th2/Treg cell shift in the cytokine profile. In allergic mouse models, we showed that MAT vaccines are highly efficient in desensitizing mice and protecting them from anaphylactic shock. This technology is applicable not only for the treatment of allergies but also for the development of preventive vaccines (85).

ROLE OF HISTAMINE IN IMMUNE REGULATION

In addition to its dominant role in type I hypersensitivity reactions, histamine influences several immune/inflammatory and effector functions (86,87). Histamine can selectively recruit the major effector cells into tissue sites and affect their maturation, activation, polarization, and other functions leading to chronic inflammation (88). Histamine also regulates monocytes, dendritic cells, T cells, and B cells, as well as related antibody isotype responses. The diverse effects of histamine on immune regulation appear to be due to differential expression and regulation of four types of histamine receptors and their distinct intracellular signals (86,87). In addition, differences in affinities of these receptors for histamine are highly decisive for the biological effects of histamine and drugs that target histamine receptors.

Histamine possesses all the properties of a classic leukocyte chemoattractant, including agonist-induced actin polymerization, mobilization of intracellular calcium, alteration in cell shape, and upregulation of adhesion molecule expression. *In vivo*, allergen-specific wild-type but not H1R-deficient CD4⁺ T cells were recruited to the lungs of naive recipients following inhaled allergen challenge (88). Histamine inhibits neutrophil chemotaxis due to H2R triggering, which is mimicked by impromidine (H2R agonist) but not by betahistamine (H1R agonist) (89). Histamine contributes to the progression of allergic-inflammatory responses by enhancement of the secretion of proinflammatory cytokines such as IL-1 α , IL-1 β , IL-6, as well as chemokines such as RANTES or IL-8, in several cell types and local tissues (90-93). Histamine induces the CC chemokines, monocyte chemoattractant protein 1 and 3, RANTES, and eotaxin in explant cultures of human nasal mucosa via H1R, suggesting a prolonged inflammatory cycle in allergic rhinitis between the cells that release histamine and their enhanced migration to nasal mucosa (94).

Immunoregulatory effects of histamine on antigen-presenting cells such as dendritic cells and monocytes are known. In monocytes stimulated with TLR-triggering bacterial products, histamine inhibits the production of proinflammatory IL-1-like activity, tumor necrosis factor (TNF)- α , IL-12, and IL-18, but enhances IL-10 secretion, through H2R stimulation (90,95-97). Histamine also downregulates CD14 expression via H2R on human monocytes (98). The inhibitory effect of histamine via H2R appears through the regulation of ICAM-1 and B7.1 expression, leading to the reduction of innate immune responses stimulated by lipopolysaccharide (99). Histamine induces intracellular Ca²⁺ flux, actin polymerization, and chemotaxis in immature dendritic cells due to stimulation of H1R and H3R subtypes. Maturation of dendritic cells results in loss of these responses. In maturing dendritic cells, however, histamine dose dependently enhances intracellular cAMP levels and stimulates IL-10 secretion, while inhibiting production of IL-12 via H2R (100). Although human monocyte-derived dendritic cells express both H1 and H2Rs and can induce CD86 expression by histamine, human epidermal Langerhans cells express neither H1 nor H2Rs, mainly because of the effect of TGF- β (101).

The balance between production and death is important in the control of cell numbers within physiological ranges. Cell accumulation in the tissues may be a consequence of either increased cell production or decreased cell death. Because apoptosis of cells is a powerful mechanism for deleting the cells, it raises the interesting possibility that unequal apoptosis of effector cells may lead to preferential deletion of one subset over another (102). The effect of histamine on the life span of immune-competent cells has not been investigated so far. Soga and colleagues show that histamine affects monocyte life span via the H2R. These findings provide additional evidence supporting the immunomodulatory effect of histamine on monocytes. Histamine prevents monocytic apoptosis in a dose- and time-dependent manner and this effect is mediated by the H2R and cAMP pathway. (103). This phenomenon results in part from the histamine-induced endogenous production of IL-10. These results may indicate that the H2R signals prolong the life span of monocytes and infiltration to the site of inflammation in modalities such as chronic allergic disorders, including asthma and atopic dermatitis. In addition, studies suggest that histamine may play an important role in the modulation of the cytokine network in the lung via H2R, H3R, and H4R that are expressed in distinct cells and cell subsets (104,105). Apparently, due to the same signal transduction patterns, β_2 -adrenergic receptors may function similarly to H2R in humans (106).

Endogenous histamine is actively synthesized during cytokine-induced dendritic cell differentiation (107). Histamine actively participates in functions and activity of dendritic cell

histamine influences can selectively recruit, polarization, and regulates monocytes, responses. The diverse potential expression and molecular signals (86,87). In highly decisive for the

coattractant, including tum, alteration in cell gen-specific wild-type recipients following motaxis due to H2R by betahistamine (H1R inflammatory responses by IL-1 β , IL-6, as well local tissues (90-93). and 3, RANTES, and gesting a prolonged histamine and their

such as dendritic cells bacterial products, tumor necrosis factor stimulation (90,95-97). monocytes (98). The of ICAM-1 and B7.1 stimulated by lipopolymerization, and and H3R subtypes. during dendritic cells, and stimulates IL-10 with human monocyte-CD86 expression by Rs, mainly because of

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precursors as well as their immature and mature forms. Dendritic cells express all four histamine receptors (108-110). They mature from monocytic and lymphoid precursors and acquire dendritic cell 1 and dendritic cell 2 phenotypes, which in turn facilitates the development of Th1 and Th2 cells, respectively. In the differentiation process of monocyte-derived dendritic cells, H1R and H3R act as positive stimulants that increase antigen-presentation capacity and Th1-priming activity. In contrast, H2R acts as a suppressive molecule for antigen-presentation capacity, enhances IL-10 production, and induces IL-10-producing T cells or Th2 cells (96,100,111).

Differential patterns of histamine receptor expression on Th1 and Th2 cells determine reciprocal T-cell responses following histamine stimulation (112). 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- and Th2-type responses are negatively regulated by H2R, due to activation of different biochemical intracellular signals (112). In mice, deletion of H1R results in suppression of IFN- γ and dominant secretion of Th2 cytokines (IL-4 and IL-13). H2R-deleted mice show upregulation of both Th1 and Th2 cytokines. In addition, IL-3 stimulation significantly increases H1R expression on Th1 but not on Th2 cells. Furthermore, histamine stimulation induces IL-10 secretion through H2R (113). Increased IL-10 production in both dendritic cells and T cells may account for an important regulatory mechanism in the control of inflammatory functions by histamine (Fig. 3). In conclusion, histamine and four different histamine receptors constitute a multifaceted system with distinct functions of receptor types due to their differential

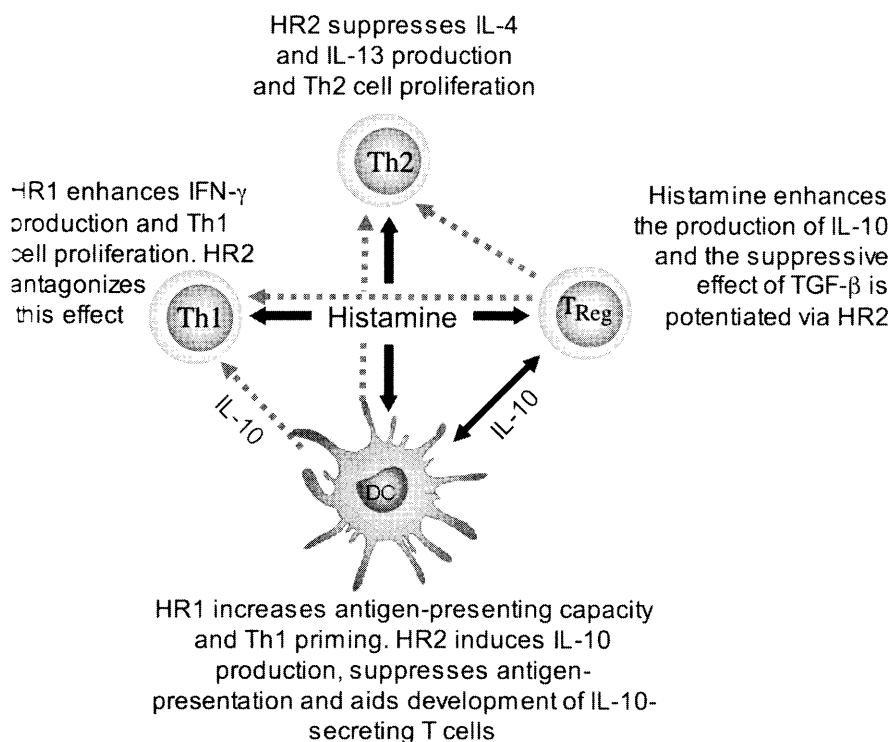


Figure 3 Histamine regulates the inflammatory functions of antigen-presenting cells and T cells in lymphatic organs and subepithelial tissues. The controlled release of histamine from effector cells of allergy induces IL-10 in DC and suppresses both Th1 and Th2 responses through the HR2. Furthermore, IL-10 affects the maturation of DC to IL-10-producing DC, which may further contribute to Treg cell generation. DC expresses all known histamine receptors. HR1 and HR3 induce proinflammatory activity and increased APC capacity, whereas HR2 plays a suppressive role. Th1 cells show predominant expression of HR1, whereas Th2 cells show a higher expression of HR2. HR1 induces increased proliferation and IFN- γ production in Th1 cells. HR2 acts as a negative regulator of proliferation, suppresses IL-4 and IL-13 production in Th2 cells. HR2 negatively regulates both Th1 and Th2 responses, induces IL-10 production, and potentiates the suppressive effect of TGF- β (solid line: activation, dotted line: suppression). *Abbreviations:* DC, dendritic cells; HR2, histamine receptor 2; IL, interleukin; IFN- γ , interferon- γ ; TGF- β , transforming growth factor β .

expression, which changes according to the stage of cell differentiation and influences of the microenvironment. These mechanisms are open to be used in the search for new adjuvants for SLIT.

CONCLUSION

There is growing evidence supporting the role for Treg cells and/or immunosuppressive cytokine, IL-10, and TGF- β as a mechanism by which SCIT, SLIT, and healthy immune response to allergens is characterized by suppression of Th2 responses, ensuring a well-balanced immune response and a switch from IgE to IgG4 antibody production. More studies are required to clarify mechanisms of action of SLIT. Nevertheless, the currently known mechanisms such as generation of Treg cells can be better used for the improvement of current treatment modalities using recombinant allergens or peptide therapy. The elaborations of more efficacious methods, including rapid protocols and targeting histamine receptors, also hold a promise for further development.

SALIENT POINTS

- Generation of Treg cells and peripheral T-cell tolerance is an essential event in allergen tolerance.
- Regulation of allergen-specific IgE and IgG subtype responses occurs during allergen-SIT.
- Immunosuppressive cytokines, IL-10, and TGF- β play a role in the mechanisms by which SCIT and SLIT respond to allergens. This is characterized by suppression of Th2 responses and ensuring a well-balanced immune response and a switch from IgE to IgG4 antibody production.
- Novel adjuvants are required to improve efficacy of SCIT and SLIT.
- Understanding the role of histamine in immune regulation opens a window for future therapies.
- Known mechanisms such as generation of Treg cells can be used to improve current treatment modalities using recombinant allergens or peptide immunotherapy.
- The elaborations of more efficacious methods, including rapid protocols, hold promise for future development.

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INTRODUCTION

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