

Review article

Mechanisms of allergen specific immunotherapy – T-cell tolerance and more

Specific immune suppression and induction of tolerance are essential processes in the regulation and circumvention of immune defence. The balance between allergen-specific T-regulatory (Treg) cells and T helper 2 cells appears to be decisive in the development of allergic and healthy immune response against allergens. Treg cells consistently represent the dominant subset specific for common environmental allergens in healthy individuals. In contrast, there is a high frequency of allergen-specific T helper 2 cells in allergic individuals. A decrease in interleukin (IL)-4, IL-5 and IL-13 production by allergen-specific CD4⁺ T cells due to the induction of peripheral T cell tolerance is the most essential step in allergen-specific immunotherapy (SIT). Suppressed proliferative and cytokine responses against the major allergens are induced by multiple suppressor factors, such as cytokines like IL-10 and transforming growth factor (TGF)- β and cell surface molecules like cytotoxic T lymphocyte antigen-4, programmed death-1 and histamine receptor 2. There is considerable rationale for targeting T cells to increase efficacy of SIT. Such novel approaches include the use of modified allergens produced using recombinant DNA technology and adjuvants or additional drugs, which may increase the generation of allergen-specific peripheral tolerance. By the application of the recent knowledge in Treg cells and related mechanisms of peripheral tolerance, more rational and safer approaches are awaiting for the future of prevention and cure of allergic diseases.

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Allergen-specific immunotherapy (SIT) is highly effective in the treatment of IgE-mediated diseases such as rhinitis, conjunctivitis, asthma and venom hypersensitivity. It is the only treatment which leads to a life-long tolerance against previously disease-causing allergens due to restoration of the normal immunity. However, it is not an alternative but important part of the complex treatment including anti-histamines, anti-leukotrienes, β_2 adrenergic receptor antagonists and corticosteroids aiming at suppression of mediators and immune cells. Allergen-SIT is most efficiently used in allergy to insect venoms and allergic rhinitis, particularly seasonal pollinosis (1–4). Immunotherapy also improves asthma and inhibits seasonal increases in bronchial hyper-responsiveness (5). It has also been shown to prevent onset of new sensitiza-

tions (6) and reduce development of asthma in patients with rhinitis caused by inhalant allergens (7, 8).

The allergen specificity of immunotherapy is crucial in the understanding of its benefits and the underlying mechanisms, which are slowly being elucidated. In 1911, the original report of Noon (9) suggested that grass pollen extracts, used for immunotherapy of hay fever, induced a toxin, causing allergic symptoms. It was suggested that in response to injection of pollen extract, antitoxins develop and prevent the development of disease. Indeed, generation of neutralizing antibodies was demonstrated during SIT (10, 11). Later on, it has been acknowledged that activated T cells and their products play a major role in the pathogenesis of allergic diseases and allergen-specific T cells were considered the major target for SIT (Table 1) (3, 12–19). SIT was found to be associated with a decrease in IL-4 and IL-5 production by CD4⁺ Th2 cells, and a shift towards increased interferon- γ (IFN- γ) production by Th1 cells. A new light was shed when a further subtype of T cells, with immunosuppressive function and cytokine profiles distinct from either T helper (Th) 1 and Th2 cells, termed regulatory/suppressor or T regulatory (Treg) cells has been described (20–23). The evidence for their existence in humans has been demonstrated (14, 23–25).

Abbreviations: APC, antigen-presenting cells; CTLA-4, cytotoxic T-lymphocyte antigen-4; DC, dendritic cell; ECP, eosinophilic cationic protein; GTR, glucocorticoid-induced tumor-necrosis factor receptor family-related gene; HR, histamine receptor; LPR, late phase response; PD-1, programmed death-1; SIT, specific immunotherapy; SLIT, sublingual immunotherapy; TGF, transforming growth factor; Th, T helper; Tr1, type 1 Treg; Treg, T regulatory.

Table 1. Effects of allergen-specific immunotherapy on clinical and immunological parameters

Mechanism

Clinical parameter

- Long-term cure
- Decreased clinical symptoms and drug usage
- Decreased response to allergen challenge tests
- Decreased size and cellular influx in skin late phase response
- Decreased skin type I hypersensitivity response

Mast cells

- Reduction of tissue numbers
- Decrease in mediator release
- Decrease in proinflammatory cytokine production

Basophils

- Decrease in mediator release
- Decrease in proinflammatory cytokine production

Eosinophils

- Reduction of tissue numbers
- Decrease in mediator release

T cells

- Decreased allergen-induced proliferation
- Induction of Treg cells
- Increased secretion of IL-10 and TGF-beta
- Suppression of Th2 cells and cytokines
- Decreased T-cell numbers in late phase response

B cells

- Decreased specific IgE production
- Increased specific IgG4 production
- Increased specific IgA production
- Suppressed IgE-facilitated antigen presentation

Dendritic cells

- Suppressed IgE-facilitated antigen presentation

Skewing of allergen-specific effector T cells to Treg cells appears as a crucial event in the control of healthy immune response to allergens and successful allergen-SIT (26, 27). Thus, subsequent variants of the theory of 'yin-yang' balance (i.e. T suppressor/Th, Th1/Th2 cell, or Treg/Th2 cell) have been proposed. In addition, mediators of allergic inflammation that trigger cyclic adenosine monophosphate (cAMP)-associated G-protein-coupled receptors, such as histamine receptor (HR) 2 may contribute to peripheral tolerance mechanisms (28–30). Most studies available so far examined the mechanisms of subcutaneous immunotherapy. There is much less clear evidence on the immunological effects of immunotherapy by alternative, especially the sublingual route (31) (Fig. 1).

T regulatory cells in allergen-specific immunotherapy

T cells constitute a large population of cellular infiltrate in atopic/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 (32, 33). T cells are activated by aeroallergens, food antigens, autoantigens and bacterial superantigens in allergic inflammation. They are under the influence of

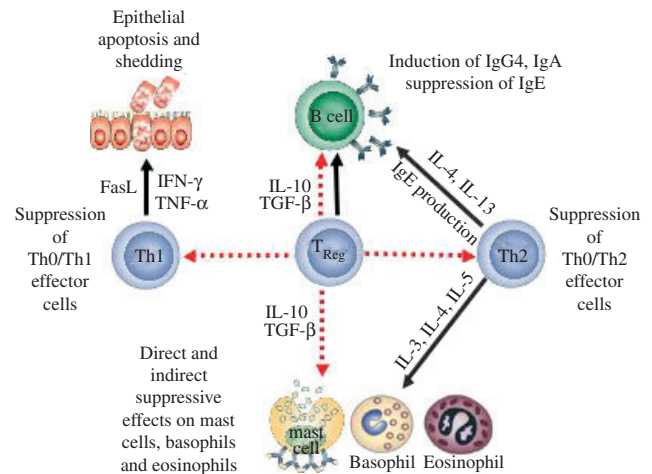


Figure 1. Immune deviation towards Treg-cell response is an essential step in SIT and natural allergen exposure of nonallergic individuals. Treg cells utilize multiple suppressor factors, which influence the final outcome of SIT. Treg cells suppress proliferation, tissue infiltration, pro-inflammatory 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 immunoglobulin (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.

skin, lung or nose-related chemokine network and they show organ-selective homing. A prolonged survival of the inflammatory cells in the tissues and consequent reactivation is observed in the subepithelial tissues (34–36). Finally, T cells display effector functions, which result in the induction of hyper IgE, eosinophil survival and mucus hyperproduction (35–37); and interact with bronchial epithelial cells and keratinocytes causing their activation and apoptosis (33). Peripheral T cell tolerance to allergens can overcome all of the above pathological events in allergic inflammation, because they all require T cell activation (Table 1).

The initial event responsible for the development of allergic diseases is the generation of allergen-specific CD4⁺ T helper cells. Under the influence of interleukin 4 (IL-4), naive T cells activated by antigen-presenting cells (APC) differentiate into Th2 cells (38–41). Once generated, effector Th2 cells produce IL-4, IL-5 and IL-13 and mediate several regulatory and effector functions. These cytokines induce the production of allergen-specific IgE by B cells, development and recruitment of eosinophils, production of mucus and contraction of smooth muscles (38–41). The degranulation of basophils and mast cells by IgE-mediated cross-linking of receptors is the key event in type I hypersensitivity, which may lead to chronic allergic inflammation. Distinct type 1 and type 2 subpopulations of T cells discriminated on the basis of cytokine secretion and function counter-regulate each other and play a role in distinct diseases. Importantly, Th1 cells also contribute

to chronicity and effector phase in allergic diseases, particularly by activation and apoptosis of resident tissue cells (42–47). IFN- γ , TNF- α and Fas pathways play essential roles in epithelial cell activation and apoptosis, which leads to spongiosis in atopic dermatitis and epithelial shedding in asthma (32, 33).

Although in early studies a switch from Th2 to Th1 type cytokines have been reported (19, 48), recent studies have demonstrated that peripheral T cell tolerance is crucial for a healthy immune response and successful treatment of allergic disorders (14, 18, 27, 49). The tolerant state of specific cells results from increased IL-10 secretion (14). The cellular origin of IL-10 was demonstrated as being the antigen-specific T cell population and activated CD4⁺CD25⁺ T cells as well as monocytes and B cells (14). Consistently, the increase of IL-10 both during SIT and natural allergen exposure has been demonstrated (14, 18, 27, 49). A recent study has been performed using IFN- γ , IL-4- and IL-10-secreting allergen-specific CD4⁺ T cells that resemble Th1, Th2 and Tr1-like cells, respectively. Healthy and allergic individuals exhibit all three subsets, but in different proportions. In healthy individuals Tr1 cells represent the dominant subset for common environmental allergens, whereas a high frequency of allergen-specific IL-4 secreting T cells (Th2-like) is found in allergic individuals (27). Hence, a change in the dominant subset may lead to either the development of allergy or recovery. Peripheral tolerance to allergens involved multiple suppressive factors such as IL-10, TGF- β , cytotoxic T lymphocyte antigen-4 (CTLA-4) and programmed death-1 (PD-1) (27). Accordingly, allergen-specific peripheral T-cell suppression mediated by IL-10 and TGF- β and other suppressive factors, and a deviation towards a Treg cell response was observed in normal immunity as a key event for the healthy immune response to mucosal antigens. The analysis of other IL-10 family cytokines such as IL-19, IL-20, IL-22, IL-24 and IL-26 demonstrated that suppressor capacity for allergen/antigen-stimulated T cells is only a function of IL-10 in this family (50).

Successfully treated patients develop specific T-cell unresponsiveness against the entire allergen as well as T cell epitope-containing peptides. These decreased proliferative responses do not arise from deletion as they are restored by the addition of IL-2 and IL-15. However, unlike in mucosal allergies no increases in TGF- β production during SIT were observed in venom allergy. Differences in the control mechanism which regulate immune responses to venoms and to aeroallergens might be due to different routes of natural allergen exposure as well as the induction of chronic events of allergic inflammation leading to tissue injury and remodeling in the latter case. Apparently, T cells which are becoming predominant during SIT and natural antigen exposure represent the so-called type 1 T regulatory (Tr1) cells in humans. CD4⁺ Treg cells that specialize in the suppression of immune response are pivotal in maintaining

peripheral tolerance (51–54). Treg cells are enriched within the CD4⁺CD25⁺ cells (55–58). Increases in numbers of CD25⁺ cells in the skin and nasal mucosa were also observed (49, 59). In humans, there is circumstantial evidence to suggest that Treg cells play a major role in the inhibition of allergic disorders. It has been reported that IL-10 levels in the bronchoalveolar-lavage fluid of asthmatic patients are lower than in healthy controls, and that T cells from children suffering from asthma also produce less IL-10 mRNA than T cells from control children (60, 61). Although some reports imply a role for TGF- β in the pathogenesis of asthma, particularly in remodeling of injured lung tissue in humans (62), a recent report indicated that the increased allergic inflammation observed after blocking of CTLA-4 is clearly associated with decreased TGF- β levels in the bronchoalveolar-lavage fluid of mice (63).

In the vast majority of the studies the cultures of PBMCs were examined. The question whether this events reflect the changes in the immune response in the mucosal tissues is of interest. T-cell responses after grass pollen immunotherapy have been examined in nasal mucosal and skin tissue. Increased IL-10 mRNA-expressing cells after SIT with grass pollen during the pollen season was demonstrated. However, unlike the findings in the periphery, IL-10 was not increased in nonatopic subjects exposed during the pollen season. Increased Th1 activity was demonstrated both in the skin and nasal mucosa (59, 64, 65). In addition, reduced accumulation of T cells in skin and nose after allergen challenge, but no decrease in T-cell numbers during pollen season were shown. Increases in IFN- γ observed after allergen challenge outside the pollen season correlated with the clinical improvement (66). During the summer pollen season increases of both IFN- γ and IL-5 with the ratio in favor of IFN- γ were observed (67). It seems, however, that the demonstration of the modulation of peripheral immune responses is pivotal for the effects of SIT. Local tissue responses do not necessarily reflect peripheral tolerance and are dependent upon several mechanisms like cell apoptosis, migration, homing, and survival signals, which are very much dependent upon natural allergen exposure and environmental factors (33).

Generation and subsets of Treg cells

There are two major hypotheses concerning the generation of Treg cells. One of these suggests that Treg cells emerge from the thymus as a distinct subset of mature T cells with defined functions (52, 68). On the other hand, several studies have shown that Treg cells may differentiate from naive T cells in the periphery upon encountering antigens present at high concentrations (22, 54, 57). Numerous studies suggest that thymic differentiation accounts for Treg cells that are specific for self-peptides and are devoted to the control of autoimmune responses, whereas peripheral differentiation may be required for

environmental antigen-specific T cells for which an undesired immune response results in pathology.

Tr1 cells are defined by their ability to produce high levels of IL-10 and TGF- β (22, 69) and suppress naive and memory T helper type 1 or 2 responses. There is now clear evidence that IL-10- and/or TGF- β -producing Tr1 cells are in vivo generated in humans during the early course of allergen-SIT, suggesting that high and increasing doses of allergens induce Tr1 cells in humans (14, 18, 27, 70). Regulatory/suppressor Th3 cells which produce high levels of TGF- β , and variable amounts of IL-4 and IL-10 upon activation with appropriate antigen or anti-CD3 antibody are indicated in mucosal tolerance (20).

CD4⁺CD25⁺ Treg cells constitute 5–10% of peripheral CD4⁺ T cells and express the IL-2 receptor- α chain (CD25) (68). They can prevent the development of autoimmunity indicating that the normal immune system contains a population of professional Treg cells, involved in active mechanism of immune suppression (51–53, 68).

Subsets of Treg cells and their suppressor mechanisms are shown in Table 2. There are other Treg cells including CD8⁺ Treg cells, which may play a role in oral tolerance (55, 56), double negative (CD4[−] CD8[−]) TCR $\alpha\beta$ ⁺ Treg cells that mediate tolerance in several experimental autoimmune diseases (58) and $\gamma\delta$ Treg cells which can play a role in the inhibition of immune responses to tumors (71). In addition, a regulatory role for IL-10-secreting B cells and dendritic cells (DC), which have regulatory/suppressor properties has been recently suggested (72, 73). Some other cells may also show possible regulatory function. It has been demonstrated that natural killer cells, epithelial cells, macrophages and glial cells express suppressor cytokines such as IL-10 and TGF- β . Although their role has not been coined as professional regulatory cells, these cells may efficiently contribute to the generation and maintenance of a regulatory/suppressor type immune response (26).

CD4⁺ CD25⁺ T cells are the only lymphocyte subpopulation in both mice and humans that express CTLA-4 constitutively (53). The expression apparently correlates with the suppressor function of CTLA-4. Under some circumstances, the engagement of CTLA4 on the CD4⁺CD25⁺ T cells by antibody or by CD80/

CD86 might lead to inhibition of the TCR-derived signals that are required for the induction of suppressor activity. PD-1 is an immunoreceptor tyrosine-based inhibitory motif-containing receptor expressed upon T-cell activation. PD-1 : PD-ligand interactions inhibit IL-2 production even in the presence of costimulation and play a role in the suppressive function of Treg cells (27, 74). Exogenous IL-2 is able to overcome PD-ligand 1-mediated inhibition at all times, indicating that cells maintain IL-2 responsiveness. Glucocorticoid-induced tumor-necrosis factor receptor family-related gene (GITR) is expressed by CD4⁺CD25⁺ alloantigen-specific and naturally occurring circulating Treg cells (75, 76). Stimulation of CD25⁺CD4⁺ regulatory T cells through GITR breaks immunological self-tolerance (76). GITR is up-regulated in CD4⁺CD25[−] T cells after T-cell receptor stimulation and it also functions as a survival signal for activated cells (77). In addition, CD103 ($\alpha\text{E}\beta_7$ -integrin) and CD122 (β -chain of IL-2 receptor) are highly expressed on CD4⁺CD25⁺ Treg cells, which correlates with their suppressive activity (78, 79).

SIT affects serum antibody isotypes

Specific IgE in serum and on effector cells in tissues of 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 (12). 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 (80–82). In pollen-sensitive patients, desensitization prevents elevation of the serum specific IgE titer during the pollen season (83, 84). However, the changes in IgE levels can hardly explain the diminished responsiveness to specific allergen due to SIT, since the decrease in serum IgE is late, relatively small, and is poorly correlated with clinical improvement after SIT.

The induction of blocking antibodies by SIT was suggested as early as in the 1930s by Cooke et al. (10). Lichtenstein et al. (11) assigned these blocking antibodies to immunoglobulin G (IgG). Research focused on the subclasses of IgG antibodies, especially IgG4, believed to capture the allergen before reaching the effector cell-bound IgE, and thus to prevent the activation of mast cells and basophils. In fact, a substantial number of studies demonstrated increases in specific IgG4 levels together with clinical improvement (85, 86). In the case of venom allergy, the rise of antivenom IgG correlates, at least at the onset of desensitization, with protection achieved by the treatment (87, 88). The concept of blocking antibodies has recently been reevaluated. Blocking antibodies seem not only to inhibit allergen induced release of inflammatory mediators from basophils and mast cells, but also inhibit IgE-facilitated allergen pres-

Table 2. Subsets of T regulatory cells and their suppressive mechanisms

T regulatory cells	Suppressor mechanism
Tr1	IL-10, TGF- β , CTLA-4, and PD-1
Th3	TGF- β and IL-10
CD4 ⁺ CD25 ⁺ Treg	Membrane TGF- β , CTLA-4, PD-1, GITR, and IL-10
CD8 ⁺ CD25 ⁺ CD28 [−] Treg	Same as CD4 ⁺ CD25 ⁺
Oa-1 dependent-CD8 ⁺	Oa-1-specific TCR
CD4 [−] CD8 [−] Treg	Induction of apoptosis
TCR $\gamma\delta$ Treg	IL-10 and TGF- β

entation to T cells as well as prevent allergen-induced boost of memory IgE production during high allergen exposure in pollen season. It has been demonstrated that grass pollen immunotherapy induced allergen specific, IL-10-dependent 'protective' IgG4 responses (89). The data established an absolute association between IgG4-dependent blocking of IgE binding to B cells in patients who underwent immunotherapy and a trend towards a correlation with clinical efficacy. It seems to be relevant rather to measure the blocking activity of allergen-specific IgG than the crude levels in sera. This can explain the lack of correlation between antibody concentration and degree of clinical improvement. However, 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 a study using well defined recombinant allergen mixtures all treated subjects developed strong allergen specific IgG1 and IgG4 antibody responses (90). 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 (91), but recent studies do not support this view and are consistent with induction of a tolerant immune response (90).

Interleukin-10 that is induced and increasingly secreted by SIT, appears to counter-regulate antigen-specific IgE and IgG4 antibody synthesis (Table 3) (14). IL-10 is a potent suppressor of both total and allergen-specific IgE, while it simultaneously increases IgG4 production (14, 92). 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. The healthy immune response to Der p1 demonstrated increased specific IgA and IgG4, small amounts of IgG1 and almost undetectable IgE antibodies in serum (18). House dust mite-SIT did not significantly change specific IgE levels after 70 days of treatment; however, a significant increase in specific IgA, IgG1 and IgG4 was observed (18). 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 (14, 93).

Suppression of effector cells and inflammatory responses during SIT

Long-term SIT is associated with significant reduction of not only the immediate response to allergen provocation, but also the late phase reaction (LPR) in the nasal and bronchial mucosa or in the skin. 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. The immunopathologic changes in the mucosal tissues of subjects chronically exposed to inhalant allergens resemble those seen during the late phase. Since LPR is associated with increased bronchial and nasal hyper-responsiveness and mimics the pathologic condition of chronic allergic inflammation, it has been postulated that the effect of SIT on the LPR is relevant to its clinical efficacy (94).

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 hyperreactivity to nonspecific stimuli, which seems to reflect underlying mucosal inflammation, decreases after SIT and correlates with clinical improvement (95, 96). During birch pollen SIT, 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 (95, 97). Inhibition by SIT of the seasonal increase in eosinophil priming has also been demonstrated (98). In biopsies taken during grass pollen SIT decreased eosinophil and mast cell infiltration in nasal and bronchial mucosa after SIT correlated with the anti-inflammatory effect. In addition, plasma concentrations and *in vitro* production of endothelin-1 (a bronchoconstrictor and proinflammatory peptide) were significantly decreased in asthmatic children after 2 years of immunotherapy with mite extract (99, 100).

The cardinal difference between true atopic diseases like allergic rhinitis, asthma or atopic dermatitis and venom allergy is the lack of many chronic events of allergic inflammation leading to tissue injury and remodeling in anaphlactoid monoallergies (33). Despite, the fact that definite decrease in IgE antibody levels and IgE-mediated skin sensitivity normally requires several years of SIT, most patients are protected against bee stings already at an early stage of BV-SIT. An important observation starting on from the first injection is an early decrease in mast cell and basophil activity for degranulation and

Table 3. The mechanisms of action by IL-10 and TGF- β

IL-10	TGF- β
Suppresses allergen-specific IgE	Suppresses allergen-specific IgE
Induces allergen-specific IgG4	Induces allergen-specific IgA
Blocks B7/CD28 co-stimulatory pathway on T cells	Suppresses allergen-specific Th1 and Th2 cells
Suppresses allergen-specific Th1 and Th2 cells	
Inhibits DC maturation, leading to reduced MHC class II and co-stimulatory ligand expression	Downregulates Fc ϵ R1 expression on Langerhans cells
Upregulates Fox P 3 (indirectly)	Upregulates Fox P 3 (directly and indirectly)
Reduces release of pro-inflammatory cytokines by mast cells	Associated with CTLA-4 expression on T cells

systemic anaphylaxis. The mechanism of this desensitization effect is yet unknown. It has been shown that mediators of anaphylaxis (histamine and leukotrienes) are released during SIT without inducing a systemic anaphylactic response. Particularly, ultrarush protocols induce significantly increased release of these mediators to circulation. Their piecemeal release may affect the threshold of activation of mast cells and basophils. Although there are fluctuations and risk 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 generation of allergen-specific Treg cells and decreased specific IgE. This is particularly because they require T cell cytokines for priming, survival and activity, which are not efficiently provided by suppressed Th2 cells and activated Treg cells (101, 102). Peripheral T cell tolerance to allergens, which is characterized by functional inactivation of the cell to antigen encounter can overcome both acute and chronic events in allergic reactions. SIT efficiently modulates the thresholds for mast cell and basophil activation and decreases immunoglobulin E-mediated histamine release (103, 104). In addition, IL-10 was shown to reduce proinflammatory cytokine release from mast cells (105). Furthermore, IL-10 down regulates eosinophil function and activity and suppresses IL-5 production by human resting Th0 and Th2 cells (106). Moreover, IL-10 inhibits endogenous GM-CSF production and CD40 expression by activated eosinophils and enhances eosinophil cell death (107).

Mechanisms of sublingual immunotherapy

The immunological mechanisms of sublingual swallow immunotherapy are less established. In Cochrane analysis (108), the authors concluded on an increase in IgG4, but no stable effect on IgE levels in adults. In addition, the induction of allergen-specific IgA has been reported (109). There is conflicting data concerning lymphoproliferative responses (110, 111). So far, the evidence on the changes in Th1/Th2/Treg activity induced by sublingual immunotherapy (SLIT) need to be confirmed. The effects on T-cell reactivity and cytokine secretion show strong variation in many studies. One preliminary study showed reduced T-cell proliferation and peripheral IL-10 production in allergic patients successfully treated with house dust mite SLIT (110). Decreased ECP and serum IL-13 after 6 month of SLIT has also been demonstrated (112). In addition, nasal tryptase secretion after nasal allergen challenge test decreased (113). During 2 years of SLIT in children with grass pollen allergens, in spite of a positive effect on rescue medication, no significant effects on *in vitro* T-cell immune responses or immunoglobulins were observed (111). However, due to its well-established safety profile, with > 500 million doses administered to humans, SLIT is currently considered as an alternative to subcutaneous SIT.

Role of histamine receptor 2 in peripheral tolerance

As a small molecular weight monoamine that binds to four different G-protein-coupled receptors, histamine has recently been demonstrated to regulate several essential events in the immune response (29, 30). Histamine receptor 2 is coupled to adenylate cyclase and studies in different species and several human cells demonstrated that inhibition of characteristic features of the cells by primarily cAMP formation dominates in HR2-dependent effects of histamine (114). Histamine released from mast cells and basophils by high allergen doses during SIT interferes with the peripheral tolerance induced during SIT in several pathways. Histamine enhances Th1-type responses by triggering the HR1, whereas both Th1 and Th2-type responses are negatively regulated by HR2 (Fig. 2). Human CD4⁺Th1 cells predominantly express HR1 and CD4⁺Th2 cells HR2, which results in their differential regulation by histamine (28). Histamine induces the production of IL-10 by DC (115). In addition, histamine induces IL-10 production by Th2 cells (116),

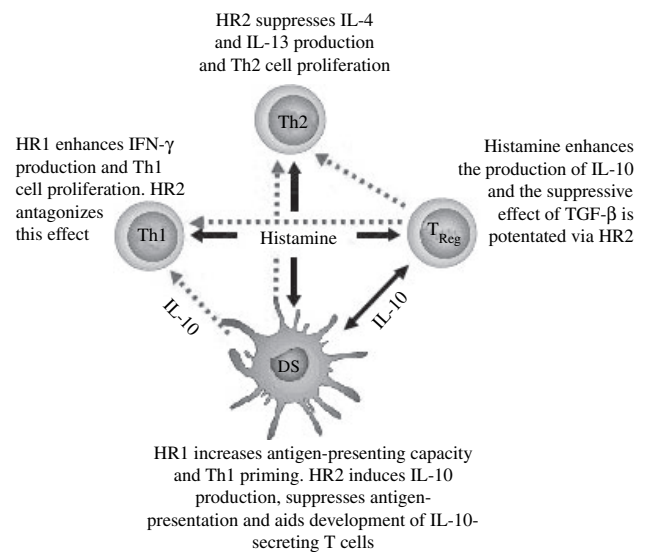


Figure 2. Histamine regulates the inflammatory functions of antigen-presenting cells and T cells in lymphatic organs and subepithelial tissues. The controlled release of histamine from effector cells of allergy induces IL-10 in dendritic cells (DC) and suppresses both Th1 and Th2 responses through the histamine receptor 2 (HR2). Furthermore, IL-10 affects the maturation of DC to IL-10-producing DC, which may further contribute to Treg cell generation. DC express 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, 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).

and enhances the suppressive activity of TGF- β on T cells (117). 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 (28). Apparently, these recent findings suggest that HR2 may represent an essential receptor that participates in peripheral tolerance or active suppression of inflammatory/immune responses. Histamine also regulates antibody isotypes including IgE (28). High amount of allergen-specific IgE is induced in HR1-deleted mice. In contrast, deletion of HR2 leads to a significantly less amounts of allergen-specific IgE production, probably due to direct effect on B cells and indirect effect via T cells.

The long-term protection from honeybee stings by terfenadine premedication during rush immunotherapy with honeybee venom in a double-blind, placebocontrolled trial was analysed (118). After an average of 3 years, 41 patients were re-exposed to honeybee stings. Surprisingly, none of 20 patients who had been given HR1-antihistamine premedication, but six 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 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 ultrarush immunotherapy, which may lead to a dominant expression and function of tolerance-inducing HR2 (119). Administration of antihistamines decreases the HR1/HR2 expression ratio, which may enhance the suppressive effect of histamine on T cells. Further studies are required to substantiate these promising findings supporting the use of antihistamine pretreatment in all venom SIT patients.

Novel developments and future prospects for allergen SIT

Recombinant DNA technology has enabled the cloning of many allergens thus facilitating investigations aimed at improving efficacy and safety of immunotherapy. Novel developments in allergen-SIT are summarized in Table 4. The effectiveness of a mixture of five recombinant grass pollen allergens in reducing symptoms and need for symptomatic medication in grass pollen allergic patients was demonstrated (90). In addition, all treated subjects developed strong allergen specific IgG1 and IgG4 anti-

body responses. Some patients, who initially were not sensitized to allergen group 5 (Phl p 5), developed a strong IgG antibody responses to that allergen.

Recently, two studies demonstrated genetical engineering of several recombinant allergens in one protein, which comprises the whole amino acid sequences of major allergens (120, 121). The major allergens of honey bee venom, *Apis mellifera*, phospholipase A₂ (Api m 1), hyaluronidase (Api m 2) and melittin (Api m 3) fragments with overlapping amino acids were assembled in a different order in the Api m (1/2/3) chimeric protein. This vaccine preserved the entire T-cell epitopes, whereas B-cell epitopes of all three allergens were abrogated. In the Api m 1/2 hybrid, both Api m 1 and Api m 2 allergens were engineered end to end, which lead to a B cell epitope abrogated phenotype (120). Again T-cell epitopes were preserved. In both candidate vaccines, IgE cross-linking leading to mast cell and basophil mediator release was profoundly reduced. Supporting these findings, the Api m 1/2 and the Api m (1/2/3) induced 100–1000 times less type-1 skin test reactivity in bee venom allergic patients. Treatment of mice with both novel vaccines led to a significant reduction of specific IgE development towards native allergen representing a protective vaccine effect *in vivo* (120, 121). Another interesting approach was the use of birch pollen major allergen Bet v 1 trimers in a clinical study, which demonstrated that Bet v 1-specific IgG1, IgG2, and IgG4 were significantly increased and a significant reduction of Bet v 1-reactive IL-5- and IL-13-producing cells was observed (122).

Immunotherapy using peptides (PIT) is an attractive approach for investigation of peripheral T-cell tolerance in humans. Short allergen peptides, either native sequences or altered peptide ligands with amino acids substitutions do not contain epitopes for IgE cross-linking to induce anaphylaxis. There is considerable rationale for targeting T cells with synthetic peptides based on such T-cell epitopes. To date, clinical trials of peptide immunotherapy have been performed in two allergies and evidence for peripheral T-cell tolerance to whole allergens has been demonstrated (15, 123–126). Single amino acid alteration in T-cell epitopes can modify specific T-cell activation and cytokine production (127). Rodent studies suggest that, under highly controlled experimental conditions, allergic diseases can be inhibited by altered peptide ligand administration. Whether this is due to Th2 to Th1 immune deviation or the induction of Treg cells remains to be elucidated (127, 128). Although PIT is theoretically attractive as a means to avoid IgE-mediated early phase reactions, it is important to note that serum IgE in allergic individuals may sometimes bind to relatively short linear epitopes of protein allergens (129). A potential barrier to peptide immunotherapy of allergy is the apparent complexity of the allergen-specific T-cell response in terms of epitope usage and dominant epitopes in humans (130–132). It is not however clear, if by-passing the IgE-dependent responses using peptides

Table 4. Novel developments for future allergen-SIT

Development	Reference
Reconstitution of an extract by five recombinant allergens	90
Hybrid and chimeric reconstitutions of several major allergens in one protein as a T cell directed vaccine, which bypasses IgE-mediated effects	120, 121
Trimer and fragments of one major allergen	122
Peptide immunotherapy	15, 123–126

results in neglecting of some important mechanism of SIT, such as mast cell and basophil desensitization and effects of histamine via HR2.

Conclusion

Peripheral T-cell tolerance is the key immunological mechanism in healthy immune response to self- and noninfectious nonself-antigens. This phenomenon is clinically well documented in allergy, autoimmunity, transplantation, tumor, and infection. Changes in the fine balance between allergen-specific Treg and Th2 and/or Th1 cells is very crucial in the development and also treatment of allergic diseases. In addition to the treatment of established allergy, it is essential to consider prophylactic approaches before initial sensitization has taken place. Preventive vaccines that induce Treg responses could be developed, and allergen-specific Treg cells, which will become predominant may in turn dampen both the Th1 and Th2 cells and their cytokines, ensuring a well-balanced immune response. Treg

cell populations have proven possible, but difficult to grow, expand and clone *in vitro*. Further studies are needed to demonstrate in the clinic, whether *in vivo* generation or adoptive transfer of Treg cells and/or their related suppressive cytokines may change the course of allergy and asthma. Small-molecular weight compounds that may generate Treg cells or increase their suppressive properties is an important target not only for the use in allergy and asthma, but also for transplantation and autoimmunity. In this context, by the application of the recent knowledge in peripheral tolerance mechanisms, more rational and safer approaches are awaiting for the treatment, prevention and cure of allergic diseases.

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