

Review article

Mechanisms of allergen-specific immunotherapy

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Allergic diseases basically are immunologic disorders related to the activation of a distinct cytokine pattern in T cells, including increased secretion of certain allergic inflammatory cytokines, particularly IL-4, IL-5, and/or IL-13 (1–3). Whereas the symptoms of immediate and late-type allergic reactions can be ameliorated by various pharmacologic treatments, allergen-specific immunotherapy (SIT) represents the only curative approach for specific type I allergy (4–9). SIT is most efficient in allergy to insect venoms and allergic rhinitis (4–9). However, the mechanism by which SIT achieves clinical improvement remained unclear until recently. A rise in allergen-blocking IgG antibodies, particularly of the IgG4 class (10, 11), the generation of IgE-modulating CD8⁺ T cells, and a reduction in the number of mast cells and eosinophils and release of mediators (12–14) were found to be associated with successful SIT. Furthermore, SIT was found to be associated with a decrease in IL-4 and IL-5 production by CD4⁺ T cells, and, in some cases, with a shift toward increased IFN- γ production (9, 15–22). However, it appeared that the induction of an unresponsive or anergic state in peripheral T cells and the reactivation of the response by cytokines from the tissue microenvironment are basic intermediate key steps in the mechanism of SIT (15–17). Thus, conditions of the immunologic microenvironment and production of cytokines by tissue cells may finally determine whether SIT will be successful or unsuccessful. Therefore, for successful and safe

SIT, allergen variants should be created of which recognition sites for T cells remain intact, whereas binding sites for IgE antibodies are removed. Intact T-cell epitopes are required to enable the induction of specific T-cell tolerance or anergy against the antigen/allergen. Not only are the antibody or B-cell epitopes a prerequisite for elicitation of adverse reactions, but IgE antibodies also focus the allergen efficiently onto antigen-presenting B cells, which present it to T cells in a way that favors development of a Th2-dominated cytokine pattern.

A model especially suited for studies of human cellular and molecular mechanisms, regulating specific allergy and normal immunity provides the immune response to bee venom (BV) (15–19, 23–29). BV phospholipase A₂ (PLA) represents the major antigen and allergen of BV, and SIT with whole BV (BV-SIT) or short PLA peptides representing immunodominant T-cell epitopes (PLA-PIT) was applied successfully.

Allergen concentration and affinity of antigenic peptide to MHC-II and TcR molecules govern the generation of distinct T-cell cytokine profiles

By using a panel of PLA-specific human T-cell clones, it was demonstrated that the secretion of both absolute and relative amounts of cytokines and distinct cytokine patterns depend on the concentration of the antigen added to the cultures. Typically, a 10–50 times lower threshold amount of antigen was

required for the induction of IL-4 than for IFN- γ . Increasing antigen concentrations favored IFN- γ production by T cells, whereas IL-4 decreased at high antigen doses (25). The same was true for Th2 clones, but at much higher antigen concentrations. Accordingly, cytokine patterns do not necessarily represent stable phenotypes and can be modulated by the dose of antigen. Low antigen concentration and suboptimal antigenic peptide-binding capacity of MHC-II molecules generate weak T-cell activation, an IL-4-dominated Th2 cytokine pattern, and IgE antibodies. Thus, at the same antigen concentration, individuals with high affinity to immunogenic peptides display a higher density of MHC-II/antigen complexes on the APC surface and induce stronger T-cell activation, generating sufficient IFN- γ to suppress IgE (27). Therefore, the cytokine-regulating forces may be driven by an individual's HLA-class II type and the strength of epitope binding in an APC/peptide/T-cell interaction. The modulation of T-cell cytokine pattern by the dose of antigen represents a driving force in differential IgE and IgG antibody formation, resulting in either allergy or immunoprotection (26, 28, 29).

That indeed the strength of antigen binding by the MHC II-peptide-TcR complex governs T-cell activation and cytokine production is supported by recent studies on altered peptide ligands (APL) (26, 30). The effect of a particular amino-acid substitution on cytokine secretion may result from affinity changes of the peptide ligand to the MHC class II molecule. Exposure of T cells to APL induces a state of specific unresponsiveness or anergy, as defined by abrogated proliferation and cytokine synthesis on antigen rechallenge. Indeed, the cytokine changes resulting from a single amino-acid mutation in an antigenic PLA peptide increased the IgG4 production significantly and skewed the ratio of specific IgE:IgG4 antibodies toward normal immunity (30). Most remarkably, this demonstrates that physicochemical properties of immunologic reactions are fundamental in generation of distinct states of T-cell activation, cytokine patterns, and finally development of either disease or normal immunity (24, 27, 30). Such regulatory effects of allergen concentration on cytokine secretion may also reflect a physiologic mechanism in SIT in which repeatedly high allergen doses are injected over a longer period of time (24, 26, 28, 29).

The induction of specific anergy in peripheral T cells and reactivation of T cells are intermediate key steps in SIT

The immunologic mechanism of SIT was investigated in BV-SIT (15, 16) and further elucidated in PLA-PIT (19) with a mixture of three peptides representing the

immunodominant T-cell epitopes PLA⁴⁵⁻⁶², PLA⁸²⁻⁹², and PLA¹¹³⁻¹²⁴. In both BV-SIT and PLA-PIT, successfully treated patients developed specific T-cell unresponsiveness to the entire PLA allergen as well as the three T-cell-epitope-containing peptides. After 60 days of treatment, the specific proliferative T-cell response and secretion of the Th2 type cytokines IL-4, IL-5, and IL-13, as well as the Th1 cytokines IL-2 and IFN- γ , were suppressed. The PPD or TT control responses were not affected by these treatments, indicating that the suppressive effect of SIT and PIT was specific to the allergen.

The induction of an anergic state in Th2 cells is an active biochemical process, associated with increased levels of basal tyrosine kinase activity, cytokine production, and CD25 upregulation. It is related to alterations in the TcR-mediated signaling pathway. The anergized Th2 cells failed to respond to anti-CD3 stimulation with increased tyrosine phosphorylation of p56^{lck} and ZAP70 kinases. In addition, intracellular calcium flux, observed in untreated Th2 cells in response to anti-CD3 mAb, was absent in anergic Th2 cells (31).

The abrogated proliferative response was fully restored by antigen stimulation of anergic T cells in the presence of IL-2 or IL-15. The full capacity of IL-2 and IFN- γ secretion was also recovered by this cytokine treatment. In contrast, specific stimulation in the presence of IL-4 induced IL-4, IL-5, and IL-13, and therefore recovered a Th2 cytokine pattern typical of an allergic response. IL-2 and IL-15 basically display the same immunologic properties. However, while IL-2 is produced by activated T cells, most immunologically active cells, except T cells, secrete IL-15 (15, 17). Consequently, microenvironmental cytokines from the tissue recover and regulate T cells from SIT-induced anergy (15, 17). They can generate distinct Th0/Th1 cytokine patterns associated with successful therapy and normal immunity, or reactivate Th2 cells, supporting the persistence of the respective allergic response. Thus, successful SIT may be difficult to achieve in an established polyspecific allergy and atopy, and such treatment has to be applied at an early stage of the disease.

Decreased T-cell proliferative responses in SIT were demonstrated in allergy to ragweed, cat dander, and grass pollen (32-34). In mice, antigenic peptides of house-dust-mite and cat allergen were shown to induce anergy in T cells (35, 36), and recent studies with T-cell peptides of Fel d 1 clearly indicated peripheral tolerance induction in T cells by PIT of cat allergy (22, 37). In a recent study, T-cell epitope peptides of cat allergen were shown to initiate a T-cell-dependent late asthmatic reaction, without the requirement for an early IgE/mast-cell-dependent response, in sensitized asthmatic subjects (38).

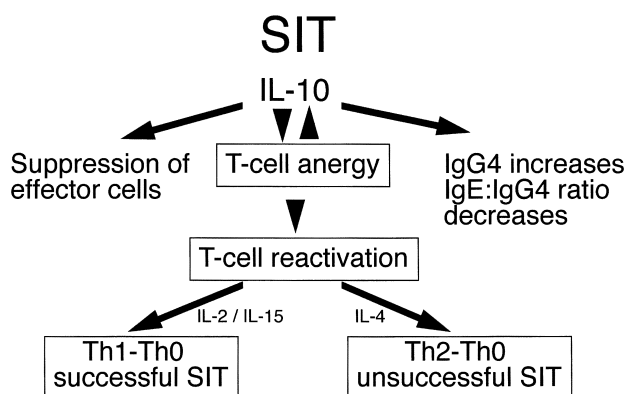


Figure 1. Immunologic mechanisms of SIT. Continuous treatment with high doses of allergen establishes specific anergy in peripheral T cells. This state is characterized by suppressed proliferative and T-cell-cytokine responses and simultaneous increase in IL-10 production. IL-10 suppresses specific T cells in an autocrine fashion. It also suppresses specific IgE and enhances IgG4 production. In consequence, activation, priming, and survival of allergic inflammatory effector cells are down-regulated. The anergic T cells can be reactivated by cytokines from the tissue microenvironment. In successful SIT, anergic T cells recover by the influence of microenvironmental IL-2 and/or IL-15 to produce Th0/Th1 cytokines. In an atopic individual, IL-4 may reconstitute a Th2 cytokine pattern and reactivate an allergic response.

T-cell anergy in SIT results from initial IL-10 production by specific T cells

The anergized cells showed suppressed PLA-specific T-cell proliferative and cytokine responses that could also be reconstituted by *ex vivo* neutralization of endogenous IL-10. This indicates that IL-10 is actively involved in development of anergy in specific T cells (Fig. 1). Whereas in both BV-SIT and PLA-PIT the antigen- and peptide-induced proliferative responses and Th1 and Th2 cytokine production decreased, the IL-10 production simultaneously increased and reached maximal levels after 4 weeks, when the specific anergy was fully established. The cellular origin of IL-10 was demonstrated by intracytoplasmic IL-10 staining in PBMC and co-expression of cellular surface markers (16). Intracellular IL-10 significantly increased after 7 days of SIT in the antigen-specific T-cell population and activated CD4⁺ T cells. After 4 weeks of SIT, intracytoplasmic IL-10 was also increased in monocytes and B cells, suggesting an autocrine action of T-cell-secreted IL-10 as a pivotal step in the induction phase of T-cell anergy and its maintenance by IL-10-producing APC and nonspecific bystander T cells (16). Interestingly, the same features of anergy were found in T cells of healthy beekeepers, who had been previously stung by high numbers of bees. Like allergic patients after BV-SIT, these naturally an-ergized individuals show increased numbers of IL-10-producing CD4⁺ CD25⁺ T cells and

monocytes. Neutralization of endogenous IL-10 in PBMC cultures from these individuals fully reconstituted the proliferative T-cell response and cytokine production (16).

IL-10 is a major regulatory cytokine of inflammatory responses and a general inhibitor of proliferative and cytokine responses in both Th1 and Th2 cells (39–46). IL-10 is released by Th1- and Th2-type lymphocytes, mononuclear phagocytes, and NK cells (40–43). *In vitro*, the inhibitory effect of IL-10 in T cells was observed exclusively in APC-dependent systems, but not in T cells stimulated by solid-phase-bound anti-CD3 (41, 44, 45, 47). This is because IL-10 blocks CD28-dependent costimulatory signaling pathways in T cells. IL-10 initiates peripheral T-cell anergy by blocking tyrosine phosphorylation of CD28 and subsequently the CD28 costimulatory signal. It appears that the CD28 is directly linked with the IL-10 receptor on T cells and coprecipitates with either mAb. In consequence, IL-10 inhibits the initial step of the CD28 costimulatory signaling pathway, the association of p85 phosphatidylinositol 3-kinase with CD28. This prevents binding of p110 phosphatidylinositol 3-kinase to CD28 and activation of the subsequent signaling cascade. Thus, inhibition of accessory molecule signaling may explain peptide-ligand-induced specific anergy in APC-free T cells (30, 31). In addition, IL-10 action at the level of cytokine gene transcription and inhibition of cytokine mRNA accumulation has been demonstrated (48, 49).

Beside the SIT and PIT of BV allergy, evidence for induction of peripheral T-cell anergy was recently obtained in the SIT of wasp-venom allergy, grass-pollen asthma, conjunctivitis, and rhinitis (21, 33, 34, 50, 51). Furthermore, downregulated T-cell responses were reported in the PIT of cat allergy (22). Moreover, SIT-induced IL-10 increase was demonstrated also in wasp allergy, grass-pollen-allergic asthma, and the nasal immunotherapy of weed-induced allergic rhinitis (21, 50, 51). In mice, IL-10 administration before allergen treatment induced antigen-specific T-cell tolerance and established peripheral T-cell anergy (52). Recently, IL-10-derived regulatory CD4⁺ T cells, producing IL-10, but not IL-2 and IL-4, which suppressed the antigen-specific T-cell response, and prevented antigen-induced murine colitis, were identified in man and in mice (53).

Specific IgE and IgG4 antibody regulation by SIT and PIT

The serum levels of specific IgE and IgG4 antibodies delineate allergic and normal immunity to allergen. Whereas peripheral anergy was demonstrated in specific T cells, the capacity of B cells to produce specific IgE and IgG4 antibodies was not abolished.

In fact, specific serum levels of both isotypes increased during the early phase of treatment. However, the increase in specific IgG4 was more pronounced and the ratio of specific IgE to IgG4 decreased by 10-fold within a few weeks (15). Furthermore, the *in vitro* production of PLA-specific IgE and IgG4 antibodies by PBMC changed in parallel to the serum levels of specific isotypes. A similar change in specific isotype ratio was observed in the SIT of various allergies. Moreover, IL-10 that was induced and increasingly secreted during SIT appears to counterregulate antigen-specific IgE and IgG4 antibody synthesis. It is a potent suppressor of both total and PLA-specific IgE, while IgG4 formation is simultaneously increased (16, 17). Thus, IL-10 not only generates anergy in T cells but also regulates specific isotype formation and skews the specific response from an IgE- to an IgG4-dominated phenotype.

The effect of IL-10 on mast cells and eosinophils in allergic inflammation

Although the final decrease in IgE antibody levels and IgE-mediated skin sensitivity normally requires several years of treatment, most patients are protected against bee stings already at an early stage of BV-SIT. Increase of allergen-specific IgG4 antibodies, blocking IgE-binding to the allergen, may explain only the late phase of protection by SIT. However, in the early phase of SIT, a decrease in histamine and sulfidoleukotriene release from basophils may be of more relevance. This decreased basophilic mediator releasability (54) can be attributed to suppression of cytokines in anergic T cells. There is clear evidence

that effector cells of the allergic inflammation (mast cells, basophils, and eosinophils) require T-cell cytokines for priming, survival, and activity. In addition, IL-10 was shown to reduce TNF- α GM-CSF and IL-6 generation from mouse bone-marrow and rat peritoneal mast cells (55). Moreover, IL-10 downregulates eosinophil function and activity and suppresses IL-5 production by human resting Th0 and Th2 clones (45, 46, 56). It inhibits endogenous GM-CSF production and CD40 expression by activated eosinophils and enhances eosinophil cell death (57, 58) (Fig. 1).

Mechanisms of differential regulation of specific isotype responses by conformational allergen variants

Antigen presentation by different types of APC promotes development of CD4⁺ Th subsets with distinct cytokine patterns (59, 60). An intact three-dimensional structure and specific antigen recognition are pivotal in the development of distinct T-cell cytokine profiles by preferential usage of particular APC. Whereas specific B cells most efficiently present conformational intact antigen already at low concentrations, APC utilizing phagocytosis or pinocytosis for antigen uptake, such as monocytes, macrophages, and dendritic cells, internalize allergen molecules independently of their structural features (18, 23, 59–61) (Fig. 2). It has been shown that IgE bound to CD23 on B cells may be used to focus antigen to T cells (62–64). Both the high-affinity receptor for IgE (Fc ϵ RI) and the low-affinity Fc ϵ RII (CD23) may play a role in IgE-mediated antigen presentation (64–70). This mechanism operates selectively at very low doses of allergen, being focused and presented to CD4⁺

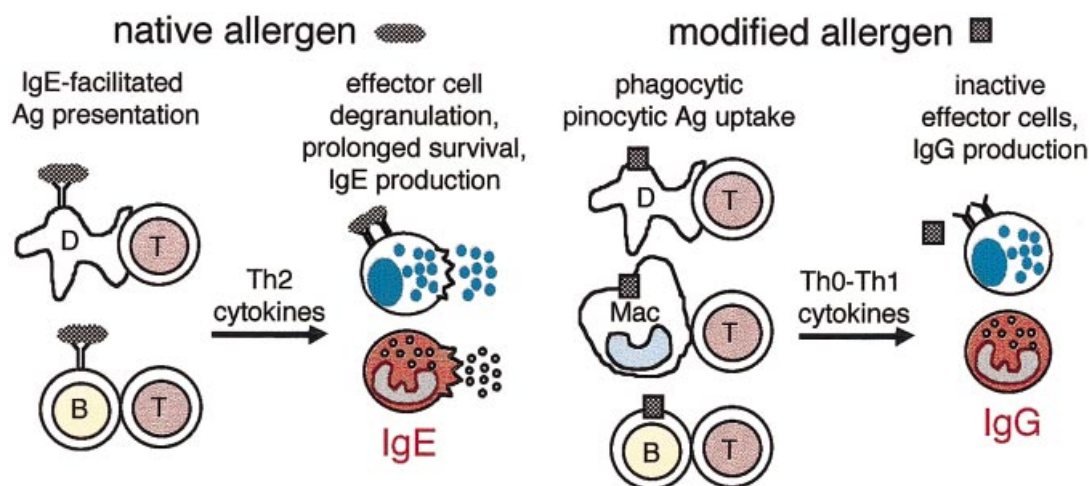


Figure 2. Difference in antigen presentation and immune response by native and modified allergens. Native allergens can degranulate mast cells and basophils and utilize an IgE-mediated antigen presentation, which leads to increased Th2 cytokine and IgE production. In contrast, modified allergens lacking IgE-binding sites utilize phagocytic or pinocytic antigen-uptake mechanisms by dendritic cells (D) and monocytes/macrophages (Mac), generating a balanced Th0/Th1-like cytokine pattern by T cells, and resulting in normalized isotype production by memory B cells. Ag: allergen.

T cells (68, 70). Consequently, blocking IgG antibodies, induced by SIT of birch-pollen allergy, inhibited the IgE-facilitated antigen presentation (70).

In addition, antigen capture by surface IgE and signal transduction were shown to be crucial in the elicitation of specific IgE responses (71). Indeed, PLA which expressed the correct tertiary structure, and which was recognized by IgE and IgG antibodies from bee-sting-allergic patients, induced high IL-4, IL-5, and IL-13 production in PBMC cultures. In contrast, nonrefolded recombinant (r) or native (n) PLA and chemically reduced and alkylated PLA induced higher IFN- γ and IL-2 production and proliferation (18). Differences in proliferation and cytokine patterns among correctly folded and nonrefolded PLA resulted from conformation-dependent involvement of different types of APC. Antigen-presenting B cells and monocytes recognized PLA in its natural conformation, stimulated Th2-type cytokines, and induced IgE antibodies. Nonrefolded or altered PLA was recognized, processed, and presented exclusively by CD14⁺ cells (monocytes), and it induced, mainly by increased IL-12 secretion, a Th1-dominated cytokine profile leading to IgG4 production (18). The increased activation of monocytes/macrophages was further substantiated by their enhanced IL-1 β secretion. The possibility that production of particular cytokine patterns and immunoglobulin isotypes was influenced by the enzymatic activity of PLA was excluded by using enzymatically inactive H34Q point-mutated refolded rPLA (18, 72).

These findings demonstrate the decisive role of specific antigen recognition by different APC, depending on structural features and the existence of conformational B-cell epitopes (Fig. 2). Various approaches to avoid IgE binding to allergen and corresponding antigen presentation have been applied. Chemically modified allergen variants, with low IgE-

binding properties, are already in clinical practice (73–85). However, genetic engineering of allergens clearly represents the future (86–99) (Table 1).

Conclusions

Induction of specific anergy in peripheral T cells by IL-10 and subsequent reactivation of distinct cytokine patterns by cytokines from the tissue microenvironment are key events in the immunologic mechanisms of SIT. These mechanisms have implications which may reach beyond specific allergy treatment, and induction of specific anergy may be of importance also in autoimmunity and transplantation. It can foster tumor growth, parasite survival, and AIDS development. The immunologic key steps of SIT and PIT are depicted in Fig. 1. Both SIT and PIT generate IL-10, which in an autocrine way of action induces specific anergy in peripheral T cells. Both types of treatment decrease the antigen-specific IgE:IgG4 ratio in peripheral blood. The reactivation and modulation of distinct cytokine patterns in anergic T cells suggest a pivotal role of microenvironmental cytokines in the development of SIT. T-cell-secreted cytokines are essential for the priming, survival, and activity of inflammatory effector cells. Therefore, specific T-cell reactivity is directly involved in the pathogenesis of allergic inflammation. IL-10 not only induces anergy in T cells but also inhibits the activation of inflammatory reactions by mast cells and eosinophils. Finally, the generation of new Th2-type cells secreting allergic inflammatory cytokine patterns can be suppressed by administration of high allergen doses inducing IFN- γ -dominated cytokine patterns.

Various attempts have been made to increase the success of SIT and decrease its risk of side-effects. The availability of recombinant allergen technology will permit excellent standardization of allergen preparations for clinical use. The aim of allergen modification is to decrease the allergenicity while retaining its immunogenicity (100). This could be achieved by destroying conformational B-cell epitopes and simultaneously preserving linear T-cell epitopes in allergens. Fig. 2 demonstrates the different ways of antigen presentation and immune response by native and modified allergens. Native allergens utilize IgE-facilitated antigen presentation, leading to increases in production of Th2 cytokines. High amounts of IL-4 and IL-13 produced by classical allergens induce higher IgE production, whereas high IL-5 production leads to eosinophil activation and increased eosinophil life span. Because of severe side-effects, the high doses required for successful SIT may not be reached with native allergen extracts. For example, they cannot be used in anaphylactogenic food and in latex allergy. In contrast,

Table 1. Strategies to modify allergens in past and for future SIT

Chemical modification of allergens

- Mineral oil precipitation (73)
- Alum precipitation (74)
- Urea denaturation (75)
- Polyethylene glycol precipitation (76, 77)
- Poly-D-glutamic acid:D-lysine conjugation (78)
- Formaldehyde/glutaraldehyde conjugations (79–83)
- Anhydride conjugation of allergens (84)
- Potassium cyanate treatment (85)

Modification of B-cell epitopes

- Site-directed mutagenesis of amino acids in B-cell epitopes (86–91)
- Deletion of amino acids in B-cell epitopes (88, 89)

Modification of allergen conformation

- Unfolded recombinant allergens (18)
- Reduction and alkylation of cysteine residues (18)
- Site-directed mutagenesis of the cysteine residues (89, 92, 93)

Hypoallergenic allergen fragments (94–97)

Hypoallergenic allergen oligomers (96–99)

T-cell-epitope peptides and altered peptide ligands (19, 22, 30, 37)

modified allergens lacking IgE-binding sites or effector cell degranulation do not employ IgE-mediated antigen presentation (100). They utilize phagocytic or pinocytic antigen-uptake mechanisms, which induce a balanced Th0/Th1-like cytokine pattern by T cells, resulting in

lower IgE and higher IgG production by memory B cells. Bypassing IgE and targeting T cells by modified allergens enables administration of higher doses to induce Th2-type T-cell tolerance without risk of anaphylaxis.

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