

EAACI/PTA Symposium Reviews

Mechanisms of specific immunotherapy of allergic diseases

Kowalski ML, Jutel M. Mechanisms of specific immunotherapy of allergic diseases.

Allergy 1998; 53: 485–492. © Munksgaard 1998.

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Accepted for publication 27 January 1998

The clinical effectiveness of specific immunotherapy (SIT) in the management of IgE-mediated diseases of the respiratory tract and venom allergy has been well established (1–3). However, the exact mechanisms of the treatment effect remain obscure. So far, the putative mechanisms have largely been deduced from observations of fluctuations in various immunologic parameters during allergen desensitization with reference to the clinical course of the diseases. Thus, elucidation of the mechanisms of SIT has followed closely the progress in understanding of the mechanisms of allergic disorders, reflecting currently available laboratory techniques.

In 1911, the original report of Noon (4) suggested that grass-pollen extracts, used for immunotherapy of hay fever, induced a toxin, causing allergic symptoms. In response to injection of pollen extract, a patient developed antitoxins that prevented the development of disease. Later authors considered that generation of neutralizing antibodies occurred during SIT (5, 6). With the progress in understanding the mechanisms of allergy, subsequent variants of the yin-yang hypothesis (e.g., T helper/T suppressor-cell balance or T helper 2-/T helper 1-cell subsets balance) have been proposed. Since most studies employed immunologic parameters in peripheral blood or, at best, in tissue fluids (e.g., in nasal or bronchial lavages), the relevance of these data for the situation in both the primary or secondary lymphatic and the effector tissue is often not clear. However, current technology,

including hybridization *in situ*, provides a much improved insight into local tissue responses.

Effect of SIT on specific responsiveness

Successful immunotherapy increases the patient's tolerance of natural exposure to a specific aero-allergen (7, 8). It is conceivable that the favorable clinical effects of SIT result from the reduction of the target organ or tissue responses to relevant allergens. In fact, SIT was shown to reduce specific cutaneous, bronchial, nasal, or ocular reactivity as assessed by skin and provocation tests with both seasonal and perennial inhalant allergens (1, 9–14). However, it is important to recognize that immunotherapy usually does not entirely abrogate the response to an allergen, but only increases the threshold dose. Thus, immunotherapy usually induces a shift in the tissue biologic reactivity, but not complete unresponsiveness to the allergen.

Long-term immunotherapy is associated with significant reduction of not only the early but also the late-phase reaction (LPR) induced by allergen in the nasal and bronchial mucosa and in the skin (9–12). SIT has also been shown to decrease the allergen-induced LPR more than the immediate-phase reaction in the skin (9). Since the LPR is associated with increased bronchial and nasal hyperresponsiveness and seems to mimic 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. Diminished end-organ responsiveness to specific stimulation may result from SIT intervention at several stages of the immune response, including allergen recognition, T-cell activation, immunoglobulin synthesis, or effector-cell reactivity.

Effect on serum immunoglobulins

As early as the 1930s, Cooke et al. suggested that immunotherapy is associated with the induction of blocking antibodies (5). Later, Lichtenstein et al. (6) assigned these blocking antibodies to 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, several studies demonstrated increases in specific IgG4 levels which correlated with clinical improvement (15). Along these lines, preincubation of specific antigen with IgG4 isolated from hyperimmune sera from desensitized patients results in a significant reduction of subsequent allergen-induced basophil histamine release (16). Furthermore, prolonged immunotherapy results in a shift from IgG1 to IgG4 synthesis (17).

However, in most studies, general correlation of specific IgG with the clinical benefit of immunotherapy is poor or cannot be demonstrated, especially for immunotherapy with aeroallergens (18–20). 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 (21–23). However, in some protected subjects, the increase in antivenom IgG cannot be demonstrated (24). Furthermore, very rapid (rush, ultrarush) immunotherapy protocols induce immediate clinical tolerance of large allergen doses, although the synthesis of blocking antibodies is not possible within several hours of treatment. Thus, the induction of blocking antibodies as the main reason for protection remains controversial. On the other hand, immunoglobulins synthesized in response to immunotherapy may modulate T-cell and B-cell activation via an intermediary of the anti-idiotypic or anti-isotypic regulation or modulation of antigen-antibody complexes. This assumption is supported by observations that administration of hyperimmune serum together with allergen can inhibit the development of adverse reactions to honeybee venom (25, 26). The induction of allergen-specific B cells secreting IgA and IgM antibodies was also demonstrated, suggesting that specific IgA and IgM antibodies may also be generated during immunotherapy (27).

Specific IgE in serum and on effector cells in tissues of allergic patients is a hallmark of atopic

disease. Natural exposure to a relevant allergen is often associated with an increase in the IgE synthesis. Desensitization frequently induces a transient increase in serum specific IgE, followed by gradual decrease over months or years of treatment (1, 28, 29). In pollen-sensitive patients, desensitization prevented elevation of the serum specific IgE titer during the pollen season (30, 31). 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, is relatively small, and is poorly correlated with clinical improvement after SIT (31). A certain re-evaluation of the previous findings might be necessary, since the radioallergosorbent test (RAST) for the whole allergen may not always indicate the development of IgE against particular major allergens. It has recently been reported that SIT with grass-pollen extract induced variable changes (both increases and decreases) in serum specific IgE levels against four purified (major) allergens of *Lolium perenne* (Lol p 1, 2, 3, and 5), changes which were poorly correlated with changes in serum specific IgE against total *L. perenne* pollen extract (32). Thus, it cannot be excluded that reduced target-cell sensitivity to natural or experimental allergen exposure may be caused, at least in part, by a decrease in formation of specific IgE against a major allergen, which cannot be assessed by conventional RAST.

Anti-inflammatory effect of SIT

The persistence of allergy in the respiratory tract can be best described in terms of chronic inflammation in the airway mucosa. The effect of immunotherapy on clinical and laboratory parameters of this inflammatory process has been intensively studied. Successful SIT has not only been shown to increase the quantity of allergen necessary to provoke a reaction in the target organ, but also to decrease responses to various nonspecific stimuli. Bronchial, nasal, and conjunctival hyper-reactivity to nonspecific stimuli, which seems to reflect underlying mucosal inflammation, decreases after SIT and is correlated with subjective improvement in the clinical course of the disease (33–35). On the cellular level, it has been shown that lower amounts of mediators of anaphylaxis (e.g., histamine or peptidoleukotrienes) are released during SIT (36–38). Birch-pollen immunotherapy has been shown to reduce plasma levels of eosinophil cationic protein (ECP), a marker of eosinophil activation, and chemotactic factors for eosinophils and neutrophils, in correlation with decreased bronchial hyperreactivity and clinical improvement (33, 39). Inhibition by SIT of the seasonal increase

in eosinophil priming has also been demonstrated (40). Another study showed that 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 (41). Furthermore, biopsies taken during grass-pollen immunotherapy have shown decreased eosinophil and mast-cell infiltration in nasal and bronchial mucosa after SIT, as compatible with the anti-inflammatory effect of this treatment (14, 42).

Effect on T cells

Activation of antigen-specific T cells and their clonal expansion is a prerequisite for development of allergic inflammation. It requires the delivery of coordinated signals through T-cell receptors (TCR) and adhesion molecules. The mechanisms of immunotherapy may also include the induction of unresponsiveness of allergen-specific CD4⁺ T cells. In experimental conditions, peripheral T-cell tolerance may result from several mechanisms, including clonal deletion or anergy, and TCR downregulation or suppression; theoretically, all these mechanisms may be present *in vivo*. It has been shown that human CD4⁺ T cells exposed to high concentrations of peptide (allergen) become unresponsive to restimulation: they fail to proliferate or provide B-cell help (43). At the same time, restimulated, anergic T cells fail to secrete interleukin (IL)-2, IL-4, or IL-5, although they may still generate interferon-gamma (IFN- γ). Accordingly, T cells from patients receiving SIT show decreased proliferative responses to relevant allergens *in vitro*, as well as decreased production of IL-2 (44).

Further understanding of the essential regulatory role of T cells in the IgE synthesis led researchers to postulate that atopy may involve an increased helper and/or decreased T-cell suppressor function, leading to overproduction of IgE. It has been reported that SIT induces a subpopulation of T cells that can suppress specific antigen-induced T-cell proliferation (45, 46). Thus, immunotherapy could act by shifting the balance from T helper toward T suppressor cells, switching off the production of specific IgE.

An important breakthrough in our understanding of the mechanisms of SIT was achieved by appreciation of the role of mononuclear cell-derived cytokines in regulation of allergic responses. One of the first studies focusing on the role of cytokines in SIT reported the effect of immunotherapy on the release of histamine releasing activity (which is a mixture of chemokines including MCP-1 and RANTES) from the peripheral blood mononuclear cells of desensitized patients (47).

While significantly enhanced release of HRA from mononuclear cells was found in atopic patients, SIT abrogated the generation of these cytokines, indicating the modulation of lymphocyte cytokine production to be an essential mechanism of SIT.

Modulation of Th1/Th2 balance

It has become clear over the last 10 years that the capability of various subsets of CD4⁺ class II MHC-restricted T cells to recognize allergens and to generate cytokines is critical for regulation of IgE synthesis as well as the generation of inflammatory responses. The concept of T-helper cell heterogeneity with Th1 and Th2 subsets, which are characterized by a marked variation of their cytokine secretion patterns, has become a very common paradigm for description of the immunologic basis of allergic disorders, as well as the mechanism of SIT (48–53).

In atopic patients, the T-cell repertoire, both in peripheral blood and at the sites of allergic contact, contains allergen-specific T-cell clones of predominantly Th2 phenotype, which preferentially generate the so-called Th2-type cytokines (IL-4, IL-5, IL-10, and IL-13). Type 2 cytokines induce B cells to immunoglobulin isotype switching from IgG to IgE production and promote the development of allergic inflammation (54–57). On the other hand, Th1-type cells upregulate cell-mediated immune defense mechanisms and secrete cytokines with "antiallergic" properties (IL-2, IL-12, or IFN- γ). The numbers and activation of antigen-specific Th1-type cells seem to be decreased in atopic patients (49, 50, 56). Production of IgE antibodies is largely dependent on the ratio of IL-4 to IFN- γ cytokines; thus, enhanced allergen-specific IgE production in atopic disease may result from predominant or selective activation of allergen-specific Th2 cells. The occurrence of eosinophilia can be ascribed to the production of high levels of IL-5 by these cells. Allergen-specific Th2 cells also participate in the LPR and chronic inflammatory reactions. It may be hypothesized that if the activation of Th2 cells can be prevented, the negative effects, such as enhanced IgE synthesis or eosinophilic inflammation, may be abrogated.

For the last few years, a number of studies have focused on a possible modulation of T-cell cytokine production by SIT, providing some evidence of a beneficial effect of SIT on the Th1/Th2 balance. *In vitro* studies on specific allergen-driven peripheral blood T-cell lines have demonstrated the reduction in IL-4 secretion during both rush and conventional immunotherapy (38, 57–61). A decreased expression of mRNA for IL-4 in peripheral blood lymphocytes during SIT was also reported (62–64). The

production of other type 2 cytokines, such as IL-5 and IL-13, in allergen-driven peripheral blood T-cell lines after rapid (rush, ultrarush) bee-venom immunotherapy was also decreased (38, 60).

The effect of SIT on Th1 cytokine secretion seems to be less consistent. Although increased IFN- γ production by peripheral blood T cells was reported during venom immunotherapy (VIT) (38, 59, 60), Akdis et al. (60) observed suppressed secretion of both type 2 and type 1 cytokines (IL-2 and IFN- γ). However, this discrepancy could be explained by the differences in cell-culture conditions (e.g., the use of IL-4, an agent suppressing type 1 responses, in the former study. Similarly, during SIT with inhalant allergens, increased expression of IL-2 and IFN- γ mRNA, as well as the presence of intracellular IFN- γ in T cells, was reported (62, 65). However, other investigators could not confirm these results, and even decreased IFN- γ production after house-dust-mite immunotherapy was reported (63). Soderlund et al. (64) did not observe any effect of preseasonal immunotherapy with birch-pollen extract on IFN- γ mRNA expression in peripheral blood lymphocytes, although an inhibition of IL-4 mRNA expression could be clearly demonstrated. The discrepancy in SIT effect on T-cell subpopulations could be ascribed to differences in the previously mentioned cell-culture methodology or to differences between allergy to venoms and inhalant allergens. In contrast to mite and pollen allergy, Hymenoptera venom allergy – although IgE mediated – is not a typical atopic disease, since “true” atopic allergies such as hay fever, allergic bronchial asthma, or atopic dermatitis are not observed more frequently in patients with venom allergy than in the general population (66). Moreover, it has been demonstrated that cells specific to the major bee-venom allergen phospholipase A₂ (PLA), like Th0 cells, can secrete both IL-4 and IFN- γ (67, 68), whereas Der p 1- or Lol p-specific cells express a clear Th2 phenotype (49, 55). Since Th1 and Th2 cells probably stem from Th0 cells, PLA-specific cells may be particularly susceptible to signals affecting cytokine secretion. Accordingly, VIT seems to be significantly more efficient than allergen immunotherapy of inhalant allergies (2).

The studies performed on T cells derived from peripheral blood and the measurement of immune response in the peripheral blood compartment have several limitations; e.g., immunotherapy-induced decrease in Th2-type lymphocytes in the peripheral blood may result from migration of a cell subpopulation to the site of allergen injection or to the lymphoid tissue. Therefore, it was important to assess the cytokine profile at the site of inflammation in the target organs. Studies performed in

effector tissues, using *in situ* hybridization of biopsies to detect the presence of mRNA for a number of cytokines, generally confirmed observations from peripheral blood. SIT induced a decrease in mRNA expression for type 2 cytokines (IL-4 and IL-5) and simultaneous increase in type 1 cytokine expression (IFN- γ and IL-12) in skin biopsies during the allergen-induced LPR (70, 71). A limited reduction in IL-4 mRNA expression and an increase in IFN- γ mRNA expression during the LPR in nasal biopsies were also reported (71). Taken together, these studies suggest that SIT may modulate the T-cell response to allergen by shifting the balance from Th2 to Th1 response, resulting in both suppression of IgE production and inhibition of allergic inflammation. However, this concept is still hypothetical as shown by the fact that decreased IL-4/IFN- γ ratios induced by SIT do not correlate closely with decreased levels of allergen-specific IgE (59).

The nature of the SIT-induced switch from the Th2 to Th1 cytokine profile production by CD4+ cells is not clear, and several potential mechanisms have been postulated. Immunotherapy may either induce Th2 cell unresponsiveness (“anergy”), or accelerate apoptosis of Th2 cells or even prompt a conversion of Th2 to Th1 cells, resulting in the predominance of Th1 cells (72, 73). Alternatively, immunotherapy may specifically enhance the generation and selective expansion of antigen-specific Th1 cells, thereby inducing “immune deviation”.

Antigen-presenting cells and other targets of SIT

The modulatory effect of immunotherapy on the cytokine profile of T cells may also result from its direct effect on antigen-presenting cells (74). It has been shown that cytokine secretion profiles are affected by high cumulative doses of antigen, as administered during allergen immunotherapy. High doses of allergen facilitate antigen presentation by macrophages/monocytes, instead of B cells, as it takes place by low antigen doses, seeming to favor Th1 cytokine production (58, 75). Carballido et al. (68) also demonstrated in PLA-specific clones that an increase in antigen concentration correlated with a significant increase of IFN- γ secretion, which might result from the increased panel of stimulated T-cell clones for which a threshold for IFN- γ production was reached.

The model of cytokine regulation becomes even more complicated with recent studies demonstrating that not only CD4+ T cells, but also CD8+ T cells, may generate two profiles of cytokines. The subpopulation called Tc1 secrete type 1 cytokines, and Tc2 secrete type 2 cytokines (76). An increased

production of IL-4 by CD8+ T cells in atopic asthma has been recently demonstrated, bringing into question the possible effect of SIT on this source of cytokines (77). Along these lines, immunotherapy with house-dust-mite extract has been shown to induce antigen-specific CD8+ T cells in children with atopic bronchial asthma (78). Involvement of other cellular sources of cytokines cannot be ruled out. For example, the increase in allergen-induced IL-12 mRNA expression in a cutaneous biopsy specimen during SIT was localized mostly to tissue macrophages (CD68+ cells) (70). These data suggest that several cell types may be involved in the immune response to immunotherapy, serving as a source of protective cytokines.

Mechanisms of immediate desensitization by very rapid immunotherapy protocols

Increasing evidence of the safety and efficacy of rapid specific immunotherapy protocols (29, 79), especially in Hymenoptera venom allergy, when the maintenance dose of venom extract is reached within 3–5 days (rush protocol) or even 3–6 h (ultrarush protocol), has prompted studies on the mechanisms involved in immediate desensitization. The rapid appearance of clinical tolerance of extremely large quantities of allergen, as observed during ultrarush venom immunotherapy, calls for investigation of the mechanisms preventing systemic reactions, including anaphylactic shock. Such studies are also valuable for the understanding of the mechanisms of anaphylactic reactions in general. As discussed above, most studies on the mechanisms of SIT have focused on the immunologic mechanisms involved in the persistent tolerance obtained after prolonged SIT such as changes in antibody formation and, more recently, in changes of the cytokine secretion profile of T cells. However, changes in these immunologic parameters cannot explain the appearance of clinical tolerance of allergens achieved within hours of treatment. In fact, no significant changes in IgE synthesis were observed during induction of ultrarush VIT (29). There are only conflicting anecdotal reports on the involvement of regulatory lymphocytes in this process (80, 81). Alternatively, the inflammatory effector cells seem to be other targets for rapid SIT. A plausible explanation of the mechanism of ultrarush VIT could be downregulation of the triggering mechanisms responsible for mast-cell/basophil activation or, alternatively, subclinical depletion of mediators from mast cells or basophils. The fact that at the beginning of ultrarush VIT the protection is short-lived also implicates desensitization of the effector cells, which are believed to be primarily responsible for acute

allergic symptoms. However, this hypothesis has not been confirmed *in vivo*; we found that skin mast cells had exactly the same reactivity to specific (bee venom) and nonspecific (codeine phosphate) activation before and after induction (3.5 h) of ultrarush bee-venom immunotherapy, indicating that "desensitization" of tissue mast cells is not responsible for the allergen-induced state of areactivity (38).

Since circulating blood basophils are also a potential source of mediators in acute systemic allergic reactions, and the administered allergen may reach the circulation more easily than it can reach tissues distant from the injection site, the effect of VIT on blood basophils has also been investigated (38, 82–84). Significant decreases of basophil histamine release were demonstrated after the maintenance dose was administered for several months, and a decrease of total histamine in peripheral blood leukocytes was observed just after completion of ultrarush or rush VIT, possibly reflecting a certain depletion of circulating basophils (82, 83, 85). However, *in vitro* histamine release from blood basophils in response to allergen stimulation was not significantly affected by rush or ultrarush VIT if the results were expressed as percent release of total histamine (85). On the other hand, rapid desensitization to venom allergens was associated with significant reduction in absolute amounts of released histamine, as well as LTC₄ formation in response to specific antigens. These data indicate that although rush immunotherapy does not render effector cells nonresponsive, it may, at least to a certain degree, affect the magnitude of mediator release. However, the rapid induction of tolerance of allergens by ultrarush VIT is unlikely to be due to a change in the responsiveness of basophils, and its mechanism remains a mystery. It is also unclear to what extent the mechanisms of VIT may be extrapolated to immunotherapy with inhalant allergens. Due to rapid application of high allergen doses during rush protocols, as compared to slow dose increase by conventional SIT, some differences in VIT influence on T-cell reactivity were also observed. Although rapid immunotherapy, like conventional immunotherapy, is associated with a shift from Th2- to Th1-type cytokine production by peripheral blood lymphocytes (61, 74), the modulation of T-cell cytokines during conventional VIT is much slower to develop (59, 61). Moreover, in contrast to ultrarush VIT inducing rapid T-cell areactivity, conventional VIT involves a transient increase in T-cell proliferation in response to allergen during the incremental phase of allergen administration followed by a state of specific hyporesponsiveness (61). The implications of these

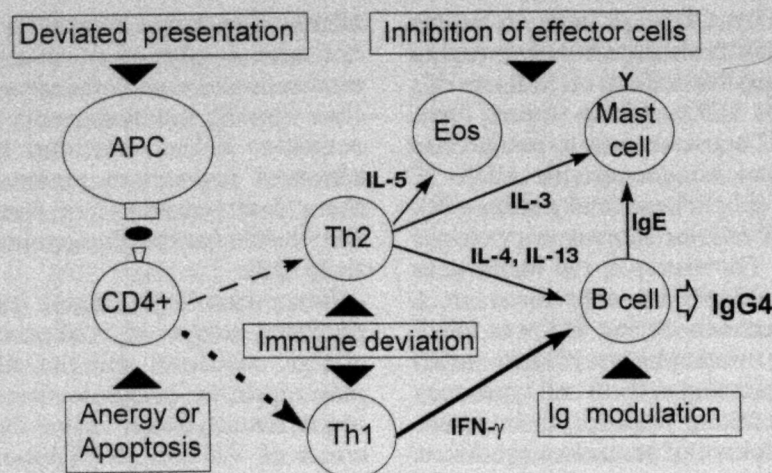


Fig. 1. Potential mechanisms of modulation of IgE-dependent allergic response by specific immunotherapy. APC: antigen-presenting cell; EOS: eosinophil.

observations in terms of clinical efficacy call for further investigation.

Although the mechanisms of SIT have not been completely elucidated, several mechanisms operating simultaneously or sequentially during immunotherapy have been documented (Fig. 1). At the early phase of SIT, desensitization of the effector cells contributes to decreased responsiveness to specific allergens. Simultaneous modification of antigen-presenting cells and CD4+ lymphocyte functions result in modulation of immunoglobulin production and further inhibition of effector-cell recruitment and activation. Over longer periods of time, all these mechanisms, acting in concert, lead to depression of allergic inflammation and clinical improvement in desensitized patients.

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