

Mechanisms of allergen-specific immunotherapy

Diverse mechanisms of immune tolerance to allergens

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Key Messages

- Allergen-specific immunotherapy (AIT) is an immune tolerance–inducing treatment, effective in reducing symptoms of allergic rhinitis and asthma as well as venom allergy and food allergy.
- Regulatory T cells (Tregs) exert their immunosuppressive function via interleukin 10 (IL-10) release, direct blocking of mast cell degranulation, and using mechanisms engaging cytotoxic T lymphocyte antigen 4 (CTLA-4) and programmed death 1 (PD-1).
- Regulatory B cells (Bregs) may contribute to induction of tolerance during AIT through IL-10–mediated suppression of effector T cell, inhibition of dendritic cell maturation, induction of Tregs, and production of anti-inflammatory IgG4 antibodies.
- Allergen-specific immunotherapy has been associated with a decrease in the number of Lin[−] CD127⁺ CRTH2⁺ group 2 innate lymphoid cells (ILC2).
- Several cellular and humoral markers such as basophil responsiveness, serum inhibitory activity for IgE-mediated allergen binding (IgE-FAB), appearance of IL-10–producing regulatory T and B cells, and IgE/IgG4 ratio show potential to be used as biomarkers of AIT efficacy, but further research is needed to interpret and confirm their association with the clinical response to AIT and how they relate to allergen tolerance after AIT cessation.

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ABSTRACT

Objective: The aim of this review is to provide an overview of the current knowledge on the mechanisms of allergen immunotherapy based on the recent publications and clinical trials.

Data sources: PubMed literature review.

Study selections: In this review, we focus on diverse mechanisms of AIT and provide an insight into alternative routes of administration. Additionally, we review and discuss the most recent studies investigating potential biomarkers and highlight their role in clinical settings.

Results: Successful allergen-specific immunotherapy (AIT) induces the reinstatement of tolerance toward allergens and represents a disease-modifying treatment. In the last decades, substantial progress in understanding the mechanisms of AIT has been achieved. Establishment of long-term clinical tolerance to allergens engages a complex network of interactions, modulating the functions of basophils, mast cells, allergen-specific regulatory T and B cells, and production of specific antibodies. The reduction of symptoms and clinical improvement is achieved by skewing the immune response away from allergic inflammation.

Conclusion: Although the complex nature of AIT mechanisms is becoming more clear, the need to discover reliable biomarkers to define patients likely to respond to the treatment is emerging.

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Introduction

Successful allergen-specific immunotherapy (AIT) induces establishment of long-term clinical tolerance against allergens, resulting in reduction of symptoms and a decrease in the need for pharmacotherapy, ultimately improving quality of life for patients suffering from immunoglobulin (Ig)E-mediated diseases.¹

In recent years, improved insight into the mechanisms of AIT has allowed a better understanding of the complexity of allergen-specific immune responses. Immune tolerance induced in successful AIT implies several cellular and molecular events responsible for inhibition of both early- and late-phase responses to allergen challenge.^{1,2}

Although the disease-modifying effect of AIT and its high efficacy have revolutionized the treatment of allergic diseases, some patients do not respond to the treatment. Therefore, development of laboratory tests and biomarkers to assess the effects of AIT on development of immune tolerance to allergens, and to allow early identification of responder patients, is needed. Many efforts have been undertaken to identify biomarkers with a potential to predict and monitor the clinical outcome for more effective AIT. In this review, we explore and discuss the most recent findings on the diverse mechanisms of immunotherapy and potential biomarkers for monitoring clinical and immunological response to AIT, focusing on various forms of AIT together.³

Advances in AIT Routes—Novel Approaches and Their Mechanisms

Thus far, subcutaneous allergen immunotherapy (SCIT) has been the gold standard administration route of AIT. Currently investigated novel AIT routes include oral (OIT), sublingual (SLIT), intralymphatic (ILIT), epicutaneous (EPIT), intradermal (IDIT), and local nasal (LNIT) administration. Oral immunotherapy involves the regular oral administration of small but increasing amounts of food allergens such as cow's milk, egg, peanut, and wheat.^{4–6} Although it induces desensitization, whether persistent tolerance can be achieved is not clear.⁴ Mild adverse events during OIT are frequent, for example, oral or throat itching and abdominal pain. Presently, OIT has not been standardized beyond studies ongoing with peanut.

Sublingual immunotherapy or oral allergy drop involves administering the allergens under the tongue on a daily basis. It is safe, effective, short, and does not require a build-up phase. Because it may exert a preventative effect on the development of asthma and the onset of new sensitizations, SLIT is indicated in grass pollen, ragweed, and house dust mite allergic rhinitis.⁷ Indirect evidence from Cochrane meta-analyses have demonstrated that both SLIT and SCIT are effective in patients with seasonal allergic rhinitis, but no clear evidence exists for their efficacy in perennial allergic rhinitis.

However, several limitations have been noted, including heterogeneity of patients with perennial allergy, geographic variation in allergen exposure, and differences in research standards between older and modern studies, which might have marked influence on the outcome of such a comparison.⁸

Direct intralymphatic injection of the antigen, ILIT, enhances the effectiveness of AIT by reducing the number of applications and duration of treatment. Furthermore, ILIT is characterized by fast alleviation of symptoms and good safety profile.⁹ When compared with SCIT, ILIT also enhances interleukin (IL)-1, interferon gamma (IFN- γ), IL-4, and IL-10 secretion.¹⁰ Recent clinical trials have investigated the effect of ILIT for pollen and cat dander allergy. Despite the induction of rapid tolerance, ILIT might provoke severe systemic/local reactions in hypersensitized patients.¹¹

Epicutaneous immunotherapy features allergen administration by using patches mounted on the skin. By targeting epidermal Langerhans cells, EPIT can reduce both local and systemic adverse effects.¹² Several clinical trials have used EPIT to deliver allergens of grass pollen, house dust mite, and food (cow's milk and peanut).^{13,14}

Epicutaneous immunotherapy has led to substantial and significant increases in allergen-specific T cell proliferation (CLA+ and CCR4+ T cell responses) in birch pollen–allergic patients with a positive atopy patch testing reaction, but no relevant changes of Bet v 1–specific IgE and IgG responses have been reported.¹³ Moreover, peanut EPIT increased peanut-specific IgG₄ levels and IgG₄/IgE ratios in patients with peanut allergy, along with trends toward reduced basophil activation and peanut-specific Th2 cytokines.¹⁴

Local nasal immunotherapy seems to be effective only on rhinitis symptoms and requires a particular technique of administration.¹⁵ Because of these technical difficulties, the use of LNIT is progressively decreasing.

Another AIT administration route is IDIT, which requires allergen injection into the dermis of the skin. It has been shown to be safe and leads to relief of symptoms after administration of only a few doses; however, worsening of respiratory allergic symptoms have been reported as a side effect.¹⁶

Besides alternative routes of administration, the use of structurally modified allergens is also being investigated.^{17,18}

Cellular and Molecular Mechanisms of AIT

Causes for the rising incidence of allergic disorders seem to be associated with increased urbanization. Reduced microbial exposure is associated with the development of allergy, which is mediated by dysregulated type 2 immunity and increased concentrations of allergen-specific IgE.¹⁹ The IgE-dependent degranulation of mast cells and basophils after allergen cross-linking on the cell surface causes a subsequent release of preformed mediators (ie, histamine) from the intracellular granules. Together with metabolites synthesized de novo from arachidonic acid (leukotrienes, platelet-activating factor, prostaglandins), all of these factors contribute to allergic inflammation and trigger the symptoms of asthma and allergic rhinitis on allergen exposure.²⁰ Therefore, the primary goal of AIT is to restore immune tolerance to allergens by inducing several mechanisms that inhibit both early- and late-phase allergic responses, such as a decrease in mast cell and basophil activity, induction of peripheral and local allergen-specific regulatory T cells (Tregs) and regulatory B cells (Bregs), and an increase in allergen-specific IgG₄ (Fig 1). Development of allergen tolerance engages cytokines—IL-10, transforming growth factor beta (TGF- β), and suppressor mechanisms involving histamine receptor 2, programmed death 1 (PD-1) and cytotoxic T lymphocyte antigen 4.²¹ Moreover, this effect is accompanied by reduction of histamine and tryptase in nasal fluid, and a decrease in number of eosinophils as well as Th2 cells and their cytokines.^{22,23} Various forms of AIT may involve different mechanisms.

Mast Cells and Basophils

Mast cells and basophils play a significant role in inflammatory responses on allergen challenge. The high affinity receptor for IgE (Fc ϵ RI)-dependent activation of these cells results in degranulation and release of their preformed and de novo synthesized mediators and type 2 cytokines (such as IL-4, IL-13) that promote allergic inflammation and IgE production. Allergen immunotherapy has been demonstrated to cause the early desensitization of mast cells and basophils, which is reflected by lower responsiveness to allergens, regardless of the elevated allergen-specific IgE levels at the initial stage of immunotherapy.²⁴ Late effects of AIT are related to a decrease in tissue infiltration and mediator release of mast cells and basophils. The mechanisms that underlie the successful suppression of mast cell and basophil activities are not fully elucidated. Allergens are known to co-aggregate Fc ϵ RI with low-affinity IgG receptors (Fc γ RIIa and Fc γ RIIb). Basophil activation has been demonstrated to be inhibited by IgG antibodies produced during AIT in the mechanisms that involve both Fc γ RIIa and

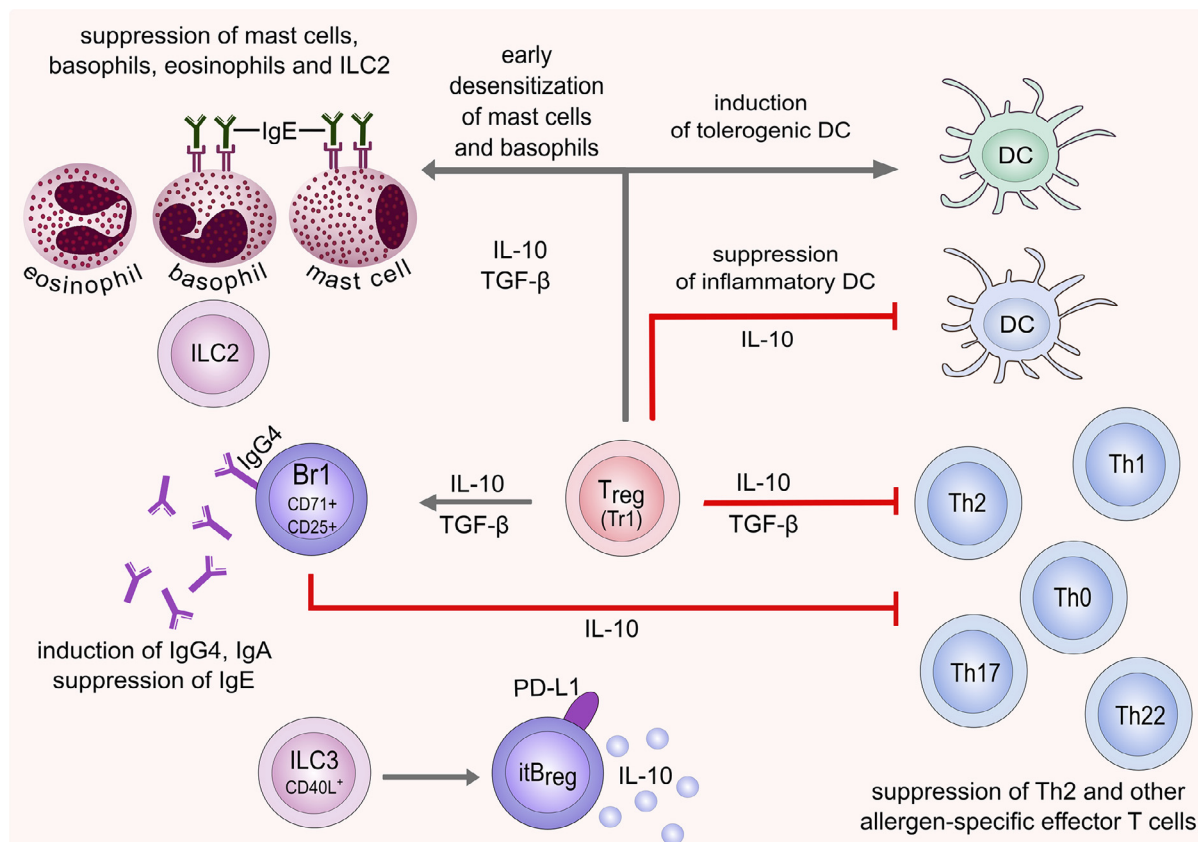


Figure 1. The mechanisms of immune tolerance to allergens during successful allergen-specific immunotherapy (AIT). One of the early effects of successful AIT is desensitization of mast cells and basophils. Induction of regulatory T cells (Tregs), which release interleukin 10 (IL-10) and transforming growth factor- β (TGF- β), results in suppression of the effector cells involved in allergic inflammation, such as mast cells, basophils, and eosinophils, as well as Th2 and other effector T cells. It also prevents seasonal increases in group 2 innate lymphoid cells (ILC2). Additionally, IL-10 and TGF- β induce immunoglobulin class-switch, promoting the production of IgG4 and IgA. By competing with IgE for allergen binding, IgG4 indirectly limit IgE-driven activation of mast cells and basophils. Inflammatory dendritic cells (DC) are suppressed by IL-10 released from Tregs. Immature transitional regulatory B cells (itBreg)-derived IL-10, which may be induced with the help of CD40L⁺ group 3 innate lymphoid cells (ILC3), also contributes to allergen tolerance. The long-term effect of AIT includes decreased IgE-to-IgG4 ratio and reduced number of mast cells and eosinophils in the tissue as well as the limited release of their mediators.

Fc γ RIIb.²⁵ The ability of various subclasses of IgG to inhibit IgE-mediated basophil degranulation have been recently investigated by MacGlashan et al.²⁶ IgG3 and to a lesser extent IgG2 were found to be the most effective in suppressing Fc ϵ RI activity on basophils.²⁶ The frequency of basophils expressing Fc γ RII in the peripheral blood was significantly increased after 1 year of AIT.²⁷ These data suggest that elevated expression of low-affinity IgG receptors may be a mechanism contributing to reduced basophil activation observed after AIT. Another mechanism that could explain early desensitization is the rapid up-regulation of histamine type 2 receptor, which has a suppressive effect on Fc ϵ RI-mediated activation and degranulation of basophils. This early allergen tolerance has been demonstrated in patients undergoing venom immunotherapy.²⁴ Although the effect of basophil desensitization during AIT has been documented in several studies, the underlying mechanisms need to be further investigated.

Regulatory T Cells and Other T Cell Subsets

Induction and maintenance of peripheral tolerance to exogenous antigens, including allergens, requires fine balance between the functions, numbers, location, and time of action of antigen-specific Tregs and effector T cells.²⁸ Antigen recognition by Tregs and their further active suppression of other cells, including effector T cells, is required to fully establish cell-mediated tolerance.²⁹

Allergen-specific regulatory T cells and allergen-specific and non-specific effector T cells contain several distinct phenotypic compartments. Subsets of Tregs include thymic or natural Tregs (nTregs),

largely inclined to maintain tolerance to self-antigens. Inducible Treg subsets, such as IL-10-producing Tr1 cells, which develop from naïve conventional T cells on encountering non-self-antigens, presented by tolerance-inducing antigen-presenting cells in specialized niches in the periphery, such as gut and lungs.^{30,31} Subcutaneous allergen immunotherapy induced local expansion of Forkhead box P3 (FOXP3)⁺CD25⁺ regulatory T-cells in the nasal mucosa of treated patients.³² Moreover, SLIT, OIT, and EPIT induced epigenetic changes, including hypomethylation in the FoxP3 promoter region in Tregs, responsible for suppressive function and long-lasting production of Tregs.^{33,34} An interesting approach in milk-sensitized mice showed a potent induction of Treg cells when treated with SLIT combined with intraperitoneal injection of IL-2 complexes.³⁵ In humans, induction of IL-10-producing Tregs was reported after grass and birch pollen immunotherapy.^{36,37} Recently, aeroallergen-reactive Tregs, which were quantitatively and qualitatively intact, were detected in the peripheral blood of both healthy and allergic patients.³⁸ However, higher numbers of Th2 cells were found in allergic patients, suggesting that pathogenic Th2-cells at some point of their development escaped Treg-mediated suppression, probably because of targeting different proteins within the same allergens.³⁸ Accordingly, other authors identified, in patients allergic to tree pollen, food allergens, house dust mite (HDM), and cat allergens, the pathogenic subset of allergen-specific and nonspecific T cells, characterized by not only the high expression of prostaglandin D2 receptor (CRTH2), a known Th2-related surface marker, but also high expression of CD161 and CD49d and down-regulation of CD27, CCR7, CD7, and CD45RB.³⁹ This subset, called Th2A, is characterized also by high expression of *IL17RB* (gene encoding IL-25 receptor),

IL1RL1 (gene encoding ST2-a receptor for IL-33), and *CRLF2* gene encoding TSLP receptor), *IL13* and *IL5* genes, was present almost exclusively in allergic patients and was significantly diminished by OIT.³⁹ Similar subset of peanut-reactive T cells, expressing *IL17RB*, *IL4*, *IL13*, and *IL5*, was found in individuals allergic to peanut by means of single cell sequencing.⁴⁰ Also in long-term grass-pollen SCIT and SLIT, a clinical improvement during 2 years of treatment was associated with the decrease in numbers of allergen-specific CRTH2⁺CCR4⁺CD27⁺CD4⁺Th2 cells.⁴¹ Similarly, HDM-reactive IL-5⁺IL-13⁺CD27⁺CD161⁺CD4⁺ cells and ST2⁺CD45RO⁺CD4⁺ Th2 cells were significantly reduced in a HDM-allergic patients after 52 weeks of SLIT.⁴²

Interestingly, successful OIT was shown to induce expansion of a distinct cluster of allergen-specific T cells, sorted with major histocompatibility complex class II tetramers, carrying appropriate peptide. Gene expression profiling allowed phenotype clustering and identified an “anergic” CD28⁺CD38⁺IFN- γ IL4⁺IL13⁺IL10⁺allergen-specific CD4⁺T cell subset. The lack of CD28 costimulatory receptor and CD38, accompanied by diminished proliferating potential suggested unresponsiveness to T-cell receptor. The identified CD28⁺CD38⁺IFN- γ IL4⁺IL13⁺IL10⁺CD4⁺T cells were absent in the healthy controls and in allergic patients before OIT.⁴³ In terms of clonal expansion of allergen-specific T cells, OIT induces very diverse and polyclonal response, whereas similar clones constitute only a small fraction of the T cell responses.⁴⁴ Finally, other subsets of T cells with effector or regulatory capacity, such as CXCR5⁺T follicular helper cells or follicular regulatory T cells and their right balance, have been also recently linked to the maintenance of peripheral tolerance.⁴⁵

Regulatory B Cells

Although the involvement of B cells in the induction of allergen tolerance remains largely unexplored, an increasing body of evidence demonstrates their role in AIT. Their particular role is attributed to regulatory B cells (Bregs). The term *Bregs* is used regarding an immunosuppressive B cell subset that controls excessive inflammation mainly through the release of IL-10, which supports differentiation of regulatory T cells and results in the inhibition of proinflammatory responses and augments IgG4 production.⁴⁶ Human IL-10–producing Bregs (B_R1, named in analogy to inducible T_R1) are characterized by high expression of CD25 and CD71 and low expression of CD73 on the cell surface.⁴⁷ The prominent immunoregulatory profile of IL-10–producing Bregs characterized by up-regulation of CD25 (IL-2 receptor α chain), programmed death-ligand 1, suppressor of cytokine signalling 3, and glycoprotein A repetitions predominant has been demonstrated by Stanic et al.⁴⁸

The changes in the immune response induced on venom immunotherapy have shown that 3 to 4 months after the start of the treatment, the frequency of phospholipase A₂-specific IL-10–producing Bregs increased 2-fold to 5-fold, reaching a level comparable to that of healthy beekeepers during the season.^{20,47,49} Furthermore, a recent study on the role of B cells in immune tolerance to allergens demonstrated increased frequency of IL-10–producing B_R1 in both bee venom–allergic patients undergoing immunotherapy and nonallergic beekeepers exposed to high doses of bee venom. Interestingly, in both groups, striking similarities were found concerning PLA-specific IgG4-switched memory B cells, plasmablasts, and PLA-specific CCR5-expressing B cells.⁵⁰ Similarly, after peanut OIT, an increase in circulating allergen-specific B cells has been observed.⁵¹ Current evidence suggests a potential role of Bregs in initiation and maintenance of allergen tolerance induced by AIT.

Innate Lymphoid Cells

A relatively newly discovered group, the innate lymphoid cells (ILCs), have been found to exhibit functional diversity and—in

analogy to the main T helper (Th) lymphocytes—can be subdivided into 3 groups—ILC1, ILC2, and ILC3—based on the surface markers, transcription factors, and cytokines produced. In contrast to T and B lymphocytes, ILCs do not express rearranged antigen receptors (such as TCR and BCR), and they can be activated through natural cytotoxicity receptors or by cytokines. The ILC1s produce Th1-type cytokines and express T-bet. The ILC2s have been shown to express a cytokine profile similar to that of Th2 cells and GATA binding protein 3 (GATA3)⁺, whereas ILC3s release IL-17 and IL-22 and are retinoid-related orphan receptor gamma t (ROR γ t)⁺.⁵² A particular role in allergic diseases, due to resemblance to Th2, have been attributed to ILC2s. Rapid induction of this cell subset has been reported in the peripheral blood after exposure to cat allergen provocation.⁵³ Given the growing evidence for a role of ILCs in allergy, great interest exists to further explore their function in AIT. Recently, Fan et al.⁵⁴ have investigated the impact of AIT on ILC2. Allergen-specific immunotherapy caused a significant reduction in the frequency of ILC2 (Lin⁺CD127⁺CRTH2⁺) compared with those untreated. Interestingly, this difference was not observed between the AIT-receiving and the healthy control group.⁵⁴ This is consistent with a previous report showing that AIT suppresses peripheral increases in ILC2 on natural exposure to grass pollen.⁵⁵ Altogether, these observations contribute to better understanding of the role of ILCs in AIT, opening doors for potential clinical applications of ILCs as biomarkers.

Dendritic Cells

Dendritic cells (DCs), as specialized antigen-presenting cells, can either initiate and sustain allergic inflammation or support the induction of tolerance by regulating T-cell responses.³ Accordingly, DCs play a vital role during the course of AIT. A recent study by Tanaka et al.⁵⁶ has shown that oral CD103⁺CD11b⁺ classical DCs are involved in transport of sublingual antigens to submandibular lymph nodes and induction of antigen-specific Tregs. Induction of Tregs is an important aspect of allergen tolerance and has been demonstrated to be driven by tolerance-inducing DCs. Recently, retinoic acid–skewed DCs have been shown to possess a tolerogenic phenotype characterized by expression of IL-10, TGF- β , IL-27, and aldehyde dehydrogenase 1A2. Their ability to induce Tregs has been demonstrated in a mouse model of food allergy and showed potential as an alternative to classical immunotherapy.⁵⁷ Because DCs foster tolerance toward allergens and function as a link between innate and adaptive immune response, harnessing their properties may provide novel opportunities for targeted therapy.

IgE, IgG, and IgA Responses

Allergen-specific immunotherapy, both subcutaneous and sublingual, results in an early but transient increase in allergen-specific IgE in serum. This initial increase in IgE concentration is rarely accompanied by the occurrence of adverse effects, including an increase in allergic symptoms. Prolonged immunotherapy leads to a decrease of allergen-specific IgE levels that may foster long-term clinical tolerance.⁴¹

Induction of IgG4 that captures the allergen before it reaches IgE on the surface of mast cells or basophils seems to be an essential mechanism of immune tolerance to allergens, preventing activation of these cells. Whether IgG4 is the only subclass mediating this protective effect is unclear. A recent study by Freiburger et al.⁵⁸ has shown that IgG4 and—to a lesser extent—IgG2 were highly induced on ILIT in cat dander–allergic patients, but no production of IgG1 and IgG3 was seen. Because the induction of other IgG subclasses on AIT has not been studied thoroughly, one cannot exclude that the allergen tolerance-inducing effect of AIT is driven by cooperation of different subclasses.

Allergen-specific IgG competes with IgE for allergen binding. The importance of the IgG/IgE ratio has been highlighted recently by Orenge et al,⁵⁹ who reported that increasing the IgG/IgE ratio through direct administration of recombinant allergen-specific IgG antibodies is a well-tolerated and effective approach to reduce the symptoms of allergy.

Potential Biomarkers for the Assessment of AIT Efficacy and Tolerance Acquisition

Even though AIT is highly effective, not all patients respond to treatment. Although our knowledge of the underlying mechanism of AIT is increasing, no surrogate biomarkers have thus far been identified as predictive of the clinical response to the treatment. Eventually, application of such knowledge could open the door to personalized medicine for allergic patients. Here, we discuss several biomarker domains that could assist in predicting clinical outcomes and in assessing AIT efficacy and the acquisition of tolerance.³

Basophil Responsiveness

Basophils store pro-inflammatory mediators, such as histamine and proteoglycans, in their cytoplasmic granules, which are released on activation by allergen cross-linking to surface-bound sIgE, causing a pro-allergic inflammatory response.⁶⁰ Quantification of basophil responsiveness has been reported as a potential biomarker to assess AIT efficacy. In both food and aeroallergen IT, a decrease in basophil responsiveness has been observed.⁶⁰ A recent study by Caruso et al⁶¹ suggested the use of the allergen sensitivity threshold (CD-Sens) by basophil activation tests to predict clinical efficacy of and monitor the immune responses to AIT. A significant decrease of allergic symptom severity in allergic rhinitis patients after 12 months of SLIT and increased tolerability of basophils to the pollen allergen *Parietaria* (CD-Sens) was observed, which was measured by expression of the basophil activation surface markers CD63 and CD203c on stimulated basophils. However, only CD203c appears to be correlated to clinical symptoms.⁶¹

Another study in patients with seasonal allergic rhinitis receiving SCIT or SLIT⁶² explored the potential of quantifying intracellular histamine-binding fluorochrome-labeled diamine oxidase, as a surrogate for histamine release, in whole-blood basophils together with measuring the surface expression of CD63, CD203c, and CD107a. Reduced basophil responsiveness and histamine release after both SCIT and SLIT was demonstrated and was correlated with lower allergic rhinitis symptom scores. Thus, these markers of basophil activation could serve as potential functional biomarkers of AIT efficacy and the attainment of tolerance, but this remains to be confirmed by large, controlled randomized trials.⁶² Several studies have failed to demonstrate a correlation between basophil activation and successful trials of AIT.⁶³ In the end, no consensus exists regarding using basophil activation as a biomarker for effective AIT.

Allergen-Specific IgE and Total IgE

Currently, elevated levels of allergen-specific IgE (sIgE) on allergen exposure is the only standard for confirming diagnosis and hence inclusion criteria for AIT. Several studies have reported a significant increase in sIgE levels during the first few months of AIT, followed by a progressive decrease after 6 to 12 months.⁶⁴ A long-term AIT study in patients allergic to house dust mite showed that sIgE levels further declined to baseline value after 2 to 3 years.⁶⁵ During the transient increase in sIgE levels, no clinical symptoms arose,³ and, similarly, the subsequent decrease was not associated with clinical improvement.⁶⁰ Results on changes in total IgE (tIgE) levels during AIT, however, are less consistent, which can mainly be explained by the time of sampling or the study duration.

Contradictory data exist regarding the efficacy of the sIgE/tIgE ratio in predicting the clinical response to AIT. Di Lorenzo et al⁶⁶ showed that, at least for pollen-allergic patients, calculating the sIgE/tIgE ratio could be promising in predicting responsiveness to AIT, whereas a subsequent randomized, controlled, open-labeled study could not replicate these results. A recent study further supported the suggestion from Di Lorenzo et al by showing a significant correlation between the sIgE/tIgE ratio and changes in visual analog and rhinitis symptom scores in mono-sensitized patients to house dust mite who underwent AIT.⁶⁷ Even though several studies have suggested the same correlations previously described, given the conflicting data, additional research must explore the reliability of using the sIgE/tIgE ratio in predicting clinical responses to AIT.

Allergen-Specific IgG4

During the first months of AIT, the serum level of allergen-specific IgG4 (sIgG4) has been shown to increase.^{62,68} The serum level of allergen-specific IgG4 can function as a blocking antibody by competing with the allergen binding of sIgE, which prevents the subsequent activation and degranulation of mast cells and basophils, as well as allergen presentation to T cells.^{3,60} This antagonistic activity can be quantified using an immune-solid-phase allergy chip,⁶⁹ and, additionally, a flow cytometry-based assay (IgE-FAB) has been developed to quantify IgE-facilitated allergen presentation and T cell activation.⁷⁰

Several studies have reported an increase of 10- to 100-fold in sIgG4 concentrations during AIT,⁷¹ and others have observed a correlation between sIgG4 and clinical outcomes. In fact, the induction of sIgG4 during AIT appears to be related to tolerance development.⁶⁸ However, sIgG4 is not commonly acknowledged as a biomarker to predict clinical responses to AIT.⁶⁸ The sIgG4 levels decrease back to pretreatment values when AIT is stopped,³ which occurs despite continued improvement in symptoms, whereas the inhibitory activity of serum for IgE-FAB has been shown to remain elevated posttreatment.⁷²

Altogether, sIgG4 alone may not serve as a reliable biomarker to predict clinical reactivity or outcomes to AIT.⁶⁰ Exploring the efficacy of IgE-FAB further, together with other IgE-blocking activities, as a potential biomarker of AIT efficacy, could be interesting. A recent review by Shamji et al³ elaborately discusses the potential of IgE-blocking activities as a biomarker of AIT efficacy.

IL-10–Producing Regulatory T Cells

The appearance of IL-10–producing Treg cells during and after AIT may reflect the development of tolerance. Several studies reported the suppression of effector cell function and induction of blocking antibodies, such as IgG4, by Treg cells during and after AIT.⁷³ Additionally, correlations were reported between the number of Treg cells and improvement of symptoms during AIT,⁷⁴ and between the local accumulation of Treg cells in the nasal mucosa after AIT and clinical efficacy.³² The regulatory cytokine IL-10 plays a critical role in the induction of peripheral tolerance, because effects of AIT were abrogated after blocking IL-10 signaling.⁷⁵ Therefore, IL-10–producing Treg cells could serve as a potential biomarker to assess AIT efficacy and the acquisition of tolerance. The Treg cells, however, are not the only IL-10–producing regulatory cells; regulatory B cells also have been reported to play a role in the suppression of allergic inflammation by secreting IL-10. Whether the AIT-induced changes in long-term memory reside in the T or B cell compartment remains unknown.

Dendritic Cells

By comparative transcriptomic and proteomic analyses, molecular markers associated with 4 monocyte-derived DC subclasses, termed DC1, DC2, DC17, and DCreg, have been identified.⁷⁶ These subclasses

stimulate the differentiation of Th1, Th2, Th17, or Treg cells, respectively. Using these markers, a recent study⁷⁶ demonstrated an inverse correlation between the expression of DCreG and DC2 markers in grass pollen–allergic patients after 4 months of AIT, reflected by the up-regulation of DCreG markers and the down-regulation of DC2 markers. Based on this, an optimal combination of 5 molecular markers predominantly expressed in DCs, such as CD141 and C1Q, or shared with natural killer cells, T cells, or both, such as FcγRIIIA, RIPK4, and GATA3, could be defined to discriminate responders from nonresponders after only 2 months of AIT.⁷⁶ Also, these results suggest that reorientation of the immune response from a pro-inflammatory Th2 to a regulatory profile is critical to successful AIT.^{3,76}

Several other studies have confirmed the impact of AIT on blood DCs.⁷⁷ Therefore, changes in molecular markers associated with DC2 and DCreG cells, reflecting the pro-allergic/regulatory response balance in peripheral blood, could be used as a potential biomarker of AIT efficacy and the acquisition of tolerance.

Conclusion

Although significant strides in understanding the mechanisms of AIT have been made, the cellular and molecular changes underlying the development of allergen tolerance are still under investigation.⁷⁸ Knowledge of AIT is progressively expanding in parallel with the increase of advanced laboratory and diagnostic techniques. The emergence of novel genome-editing tools may be used to target immune responses and promote tolerance. Establishing safe protocols for such interventions is one of the major challenges in the field of AIT. Another critical question relates to the importance of finding a surrogate marker of those likely to respond to AIT. Although several promising biomarkers of successful AIT have been identified, so far no generally accepted and validated indicator of AIT efficacy and acquisition of tolerance exists.

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