

ALLERGY

Advances in allergen immunotherapy: Aiming for complete tolerance to allergens

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Allergen-specific immunotherapy (AIT) has been used for more than 100 years as a tolerance-inducing therapy for allergic diseases and represents a potentially curative method of treatment. AIT functions through multiple mechanisms, including regulating T and B cell responses, changing antibody isotypes, and decreasing mediator release and migration of eosinophils, basophils, and mast cells to affected tissues. Despite the relative success of AIT, attempts are being made to improve this therapy in order to overcome problems in standardization, efficacy, safety, long duration of treatment, and costs. These have led to the development of biotechnological products with successful clinical results.

Induction of immune tolerance has become a prime target for prevention and treatment for many diseases that involve dysregulation of the immune system, including asthma, allergy, autoimmunity, cancer, organ transplantation, chronic infections, and infertility (1–3). However, tolerance induction has achieved varying success in these different areas, with allergen immunotherapy (AIT) being the one of the most established. AIT is routinely applied in the clinic either subcutaneously (SCIT) or sublingually (SLIT) and is suitable for both children and adults for a variety of allergens, such as pollen, cat and dog, house dust mite, and venom (4). AIT affects symptoms of allergic inflammation but also has been documented to modify disease pathology long-term, resulting in less drug usage, decreased disease severity, a long-term curative effect after stopping the treatment, and prevention of additional allergen sensitization. These disease-modifying effects are critical because patient nonadherence to long-term medication regimens is a common challenge in AIT. Indeed, 55 to 82% of the patients on SLIT and 13 to 89% on SCIT have shown to be noncompliant (5, 6). Increasing safety while maintaining or even augmenting efficiency is the main goal of research in vaccine development and drives efforts of improving treatment schemes in AIT (4).

MECHANISMS OF ALLERGEN TOLERANCE IN ALLERGEN IMMUNOTHERAPY

Allergy is thought to result from dysregu-

lated type 2 immunity, which consists of GATA-3⁺ type 2 innate lymphoid cells (ILC2s), cytotoxic T cells (TC2), and T helper (T_H2) cells that produce interleukin-4 (IL-4), IL-5, and IL-13. These cytokines induce mast cell, basophil, and eosinophil activation as well as immunoglobulin E (IgE) antibody production, which in healthy individuals protect against helminths and venoms but when dysregulated contribute to allergic disease (7). Clinical tolerance to allergens can be defined as the establishment of long-term absence of allergic symptoms upon natural exposure, allergen chambers, or in vivo challenges. However, if there is no allergen exposure, this established tolerance may start to wane in some individuals (8). Immunologically, tolerance implies changes in memory-type allergen-specific T and B cell responses, regulation of allergen-specific IgE and IgG, as well as increases in mast cell and basophil activation thresholds, resulting in prevention of allergic symptoms (Fig. 1).

Regulatory T cells and allergen tolerance. Mouse immune tolerance models have been well studied, and adoptive transfer models have shown that regulatory T cells (T_{regs}) are critical for inducing and maintaining allergen tolerance in mice (3). Data in humans are less complete, but there are multiple lines of evidence suggesting a similar role of T_{regs} in allergen tolerance in humans. Direct investigation of affected tissue biopsies and skin late-phase responses from allergic individuals has shown a decrease in T_H2 cells and eosinophils and a parallel increase in T_{regs} and their cytokines in these tissues after AIT (9). Moreover, cell culture experiments have shown that tolerance-associated cytokines IL-10 and transforming growth factor- β (TGF- β) are

increasingly produced by antigen-specific T_{regs} after tolerance induction. Furthermore, direct analysis of ex vivo human peripheral blood cells during AIT and in healthy beekeepers upon allergen exposure demonstrates that allergen-specific T_{regs} increase and T_H2 cells decrease in these clinical allergen tolerance models (10).

In the clinic, both subcutaneous and sublingual versions of AIT have been shown to induce allergen-specific T_{regs} in humans. The immunological mechanisms of SLIT seem to be similar to SCIT; differences in most parameters are either modest or not observed. Immune tolerance induced by SLIT is associated with T_{regs}, IL-10 production, increases in sublingual Foxp3-expressing cells, and elevated allergen-specific IgG4, IgA, and serum that blocks IgE-facilitated allergen binding to B cells (11). Indeed, peripheral T cell tolerance to allergens is characterized mainly by generation of allergen-specific T_{regs} and a decrease in T_H2 and T_H1 cells (2, 12–14). However, inducing T_{regs} may not be enough: CD4⁺CD25⁺ T_{regs} from atopic donors have a reduced capability to suppress the proliferation of CD4⁺CD25⁺ T cells compared with those from healthy individuals (15, 16).

Regulatory B cells and allergen tolerance. In addition to T_{regs}, a regulatory subset of B cells that produce IL-10 (B_{regs}) have been reported, and lack of these cells leads to increased inflammation in mouse models of autoimmunity, transplantation, and chronic infection (17). Inducible IL-10-secreting B regulatory 1 (BR1) cells have also been characterized in humans. Indeed, in a study of allergic patients who received AIT, the frequency of IL-10⁺ phospholipase A2-specific B cells increased; this study demonstrated both the existence of suppressive B cells and that IgG4-expressing B cells develop from IL-10⁺ BR1 cells in human subjects (17). IL-10 is thought to be instrumental for this tolerizing function: Overexpression of IL-10 in B cells resulted in an immunoregulatory profile comprising up-regulation of suppressor of cytokine signaling 3, glycoprotein A repetitions, and predominant expression of CD25 and programmed cell death 1 ligand 1 (18). These cells showed a reduction in levels of many proinflammatory cytokines and augmented the production of anti-inflammatory IL-1 receptor antagonist and vascular endothelial growth factor. In addition, IL-10-overexpressing B cells secreted less IgE and showed a general ability to suppress T cells and dendritic cells (18).

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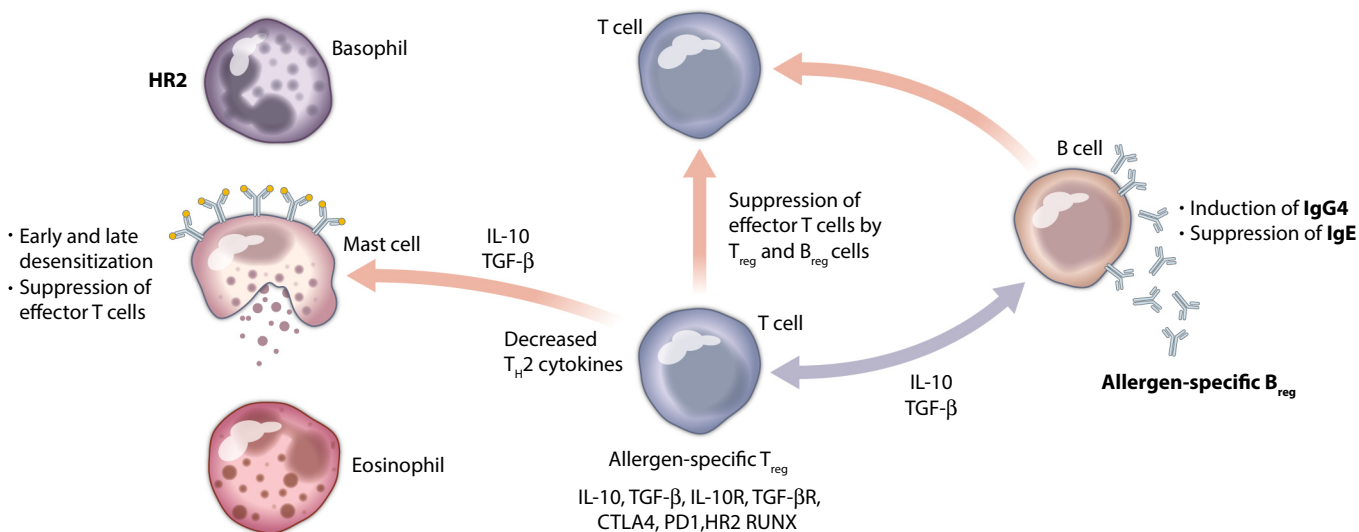


Fig. 1. Mechanisms of AIT. Allergen-specific T_{reg} cells generate and suppress other effector T_H1 and T_H2 cells and directly or indirectly suppress mast cells and basophils; early and late desensitization of mast cells and basophils occurs. Suppression of allergen-specific IgE and induction of IgG4 by T cells and induction of allergen-specific IL-10⁺ B_{reg} cells lead to the suppression of effector T cells and dendritic cells.

Regulation of mast cells, basophils, and eosinophils by AIT.

In mouse models, early desensitization takes place starting from the first injection of AIT and is linked to decreased mast cell and basophil degranulation (Fig. 2) (19). This effect is similar to rapid desensitization for hypersensitivity reactions to drugs. One of the main soluble factors released from mast cells and basophils after allergen challenge is histamine, which mediates its effects through histamine receptors (HRs). Immunosilencing of high-affinity IgE receptor (FcεRI)-activated basophils by means of selective suppression mediated by H2R might be highly relevant for the very early desensitization effect of AIT (20). This suppression of eosinophils, mast cells, and basophils continues during the course of AIT, suggesting that it could possibly decrease risk of anaphylaxis as well. Changes in these innate immune cells also have the potential to be useful as biomarkers for AIT. Decreased basophil sensitivity to IgE-mediated degranulation has been suggested to represent changes in blood basophils and tissue mast cells. Early improvement in basophil sensitivity predicts symptom relief with grass pollen immunotherapy (21). Furthermore, basophil expression of diamine oxidase shows an increase after AIT and has been suggested as a biomarker of allergen immunotherapy response (22).

Innate lymphoid cells and AIT. Innate lymphoid cells are a recently introduced cell subset that may enhance inflammation in many diseases. Type 2 innate lymphoid

cells in particular have been implicated in asthma and upper respiratory inflammation (23). Although there is still much to be learned about the contribution of these cells to allergic disease, recent data that seasonal increases in peripheral innate lymphoid type 2 cells are inhibited by subcutaneous grass pollen extract AIT support a role for these cells in disease pathology (24).

Modulation of allergen-specific IgE and IgG responses during AIT. Allergen-specific IgE increases in pollen-allergic individuals during the pollen season, and AIT prevents this increase. Indeed, after AIT there is a transient increase in serum allergen-specific IgE followed by a gradual decrease over months or years of treatment. However, this decrease in serum IgE is relatively late and does not correlate with clinical improvement after AIT.

Instead, allergen-specific IgG antibodies, especially IgG4, may contribute more directly to AIT. These antibodies are thought to capture the allergen before it reaches the effector cell-bound IgE and thus prevent the activation of mast cells and basophils. IgG4 antibodies can be viewed as a marker of introduced allergen dose, but they also have the ability to modulate the immune response to allergen. Allergen-specific IgG may be directed against the same epitopes as IgE, resulting in direct competition for allergen binding and a “blocking” effect (25). There is accumulating evidence that AIT influences the blocking activity of IgG4 on IgE-mediated responses. However, the re-

lationship between the efficacy of AIT and the induction of allergen-specific IgG subgroups remains a controversial issue.

There are several features of IgG4 that may contribute to its noninflammatory role. The two arms of IgG4 have the ability to separate and repair by means of dynamic Fab arm exchange, which leads to the production of bispecific antibodies that are functionally monomeric (26). Furthermore, IgG4 does not fix complement but is capable of inhibiting immune-complex formation by other isotypes, giving this isotype anti-inflammatory characteristics (27). It is highly possible that the decrease in the IgE/IgG4 ratio during AIT is influenced by the skewing of the T cell population from allergen-specific T_H2 to T_{reg}s as well as by increases in B_{reg}s number. IL-10, which is produced by both T_{reg}s and B_{reg}s, is a potent suppressor of both total and allergen-specific IgE while it simultaneously increases IgG4 production (13, 28).

Contribution of the microenvironment of vaccine delivery to mechanisms of AIT in SLIT. The site of antigen delivery also contributes to tolerance induction. The complex interplay of innate and adaptive immune responses during SLIT, which takes advantage of the tolerogenic environment of tonsils and oral mucosa, has been explored in several clinical studies. These studies have reported tolerogenic mechanisms similar to those observed for SCIT (29). After SLIT, immune changes occur in oral mucosa and periphery leading to

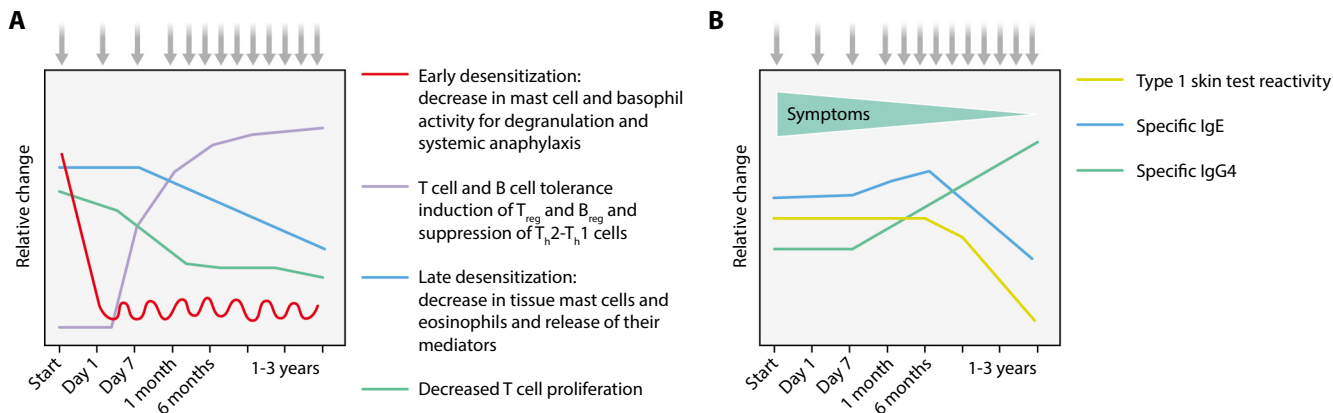


Fig. 2. Immunologic changes during the course of AIT. Starting with the first injection, decreases in mast cell and basophil activity, degranulation, and decreased tendency for systemic anaphylaxis take place within the first hours. This is followed by generation of allergen-specific T cell tolerance with the development of specific T_{reg} and B_{reg} cells and suppression of allergen-specific T_H1 and T_H2 cells. Specific IgE shows an early increase and relatively late decrease. Specific IgG4 continuously increases as long as the treatment continues. After several months, the allergen-specific IgE/IgG4 ratio decreases. In parallel to clinical improvement, decreases in tissue mast cells and eosinophils and release of their mediators and decreased skin late-phase response take place. A decrease in type I skin test reactivity is also observed relatively late in the course. It has to be noted that there is variation between donors and protocols.

decreased T_H2 responses and a shift to IL-10 and TGF- β production by T_{regs} . Tolerance-inducing dendritic cells have been found in tonsils and oral mucosa (14, 29, 30). In addition, SLIT also promotes the synthesis of allergen-specific IgG4 and IgA antibodies that block allergen-IgE complex formation and binding to inflammatory cells, thus encouraging an anti-inflammatory environment (14, 29). However, despite these similarities, the magnitude and intensity of the observed immunological changes in SLIT have not been as prominent as SCIT, and future studies are needed to differentiate the relative (perhaps antigen-specific) advantages for the different delivery methods.

VACCINE DEVELOPMENTS FOR ALLERGEN IMMUNOTHERAPY

Despite its relative success, AIT faces several challenges, including worldwide standardization, limitations in efficacy, sometimes severe side effects, low patient adherence, and high costs due to the long duration (3 to 5 years) of treatment. A variety of approaches are being pursued to improve the standardization, efficacy, and safety of AIT (31–33). Moreover, studies on prophylactic AIT are also being performed (Table 1) (34).

Targeting T cells while bypassing IgE in AIT. One key approach to improve efficacy of AIT is to simultaneously target T cells to induce T cell tolerance and bypass IgE binding to avoid IgE-mediated side effects (35). The goal of this approach is to administer higher doses of allergens to induce

T cell tolerance while blocking IgE binding in order to lower the risk of anaphylaxis (35). To accomplish this, researchers are developing allergoids by altering the linearity of the amino acids sequence of T cell epitopes of an allergen by using a mixture of T cell epitope peptides, allergen fragments, fusions, recombinant allergen hybrids, and chimeras (36). There are currently many allergoid preparations containing structurally modified allergens in the market with decreased IgE-binding activity. The prototype of this approach—peptide immunotherapy that uses linear T cell epitope peptides—is showing fruitful results in the treatment of cat and birch pollen allergies with few doses (37, 38).

Mixtures of recombinant allergens to reconstitute a native-like combination.

Another approach has been to combine recombinant allergens for AIT with the aim of partially reconstituting an allergen extract and eliciting a broader tolerizing response. As an example, the mixture of five recombinant *Phleum pratense* grass pollen allergens showed effectiveness in reducing symptoms and the need for symptomatic medication in people with grass pollen allergy (39). All treated subjects developed strong allergen-specific IgG1 and IgG4 antibody responses.

Physical coupling of allergens to innate immune response-stimulating substances. This area is very open to further developments owing to a vast array of possibilities for combinations of multiple immune stimulators and different methods

of coupling. CpG oligonucleotide-conjugated allergens (40), allergens coupled to virus-like particles (41), carbohydrate-based particles (42), and the use of monophosphoryl lipid A formulated with allergoid (43) are representatives of this group.

Different routes of administration.

Different routes of vaccine administration have been proposed to improve efficacy and safety. Meta-analysis of double-blind, placebo-controlled trials has shown that SLIT is safe and clinically efficacious, with a treatment benefit approximately half of that achieved with subcutaneous SCIT (44). Disease-modifying effects of SLIT have been confirmed in large-scale randomized, double-blind, placebo-controlled trials as well as in children. In addition, intralymphatic vaccination and epicutaneous vaccination are more recently pursued routes. Both routes showed similar efficacy to SCIT in grass pollen allergy but require fewer applications and lower total doses of allergen (45). Notably, intralymphatic administration induced either T cell tolerance or strong T_H1 responses, depending on the type of the vaccine used (46, 47).

Fusion of allergens with immune response modifiers. Recently, the major cat allergen Fel d 1 was cloned and expressed in a way that enhanced its cellular entry and antigen presentation. Human immune deficiency virus TAT-derived membrane translocation domain was used for enhanced entry to cells, and a truncated peptide of the invariant chain was used to

Table 1. Developing vaccine approaches for allergen-SIT.

Type of the vaccine/approach	Description and mechanism	Target cells and activity
Targeting T cells, bypassing IgE in AIT		
Fusion of major allergens and chimeric allergens	Major allergens or their fragments are expressed as a single recombinant protein	Antigen-presenting cells and T cells. IgE binding is attenuated, T cell reactivity is preserved.
Hypoallergenic hybrid molecules	Cloning several allergens together or mutating IgE binding epitopes or modifying natural conformational structure	Antigen-presenting cells and T cells. Reduced IgE reactivity of hybrid proteins, with higher T cell proliferation responses.
Fragments of major allergens	Major allergen divided into non-IgE-binding fragments	Antigen-presenting cells and T cells. IgE binding is attenuated and T cell reactivity is preserved.
Peptide immunotherapy	Non-IgE-binding T cell epitope peptides for cat, mite, pollen, and bee venom allergy	Antigen-presenting cells and T cells. Induction of direct T cell tolerance.
Unfolded native or recombinant allergens	Major recombinant allergens (Api m 1, Bet v 1) are not refolded to the native conformation.	Antigen-presenting cells and T cells. Reduced IgE binding, while T cell reactivity is preserved.
Polymers of major allergens	Major allergen is polymerized	Antigen-presenting cells and T cells. IgE binding and basophil degranulation and skin test reactivity is attenuated, and T cell reactivity is preserved.
Mixtures of recombinant allergens to reconstitute a native-like combination		
Mixture of several major recombinant allergens	A mixture of five recombinant grass pollen allergens (Phl p 1, Phl p 2, Phl p 5a, Phl p 5b, Phl p 6) was developed.	Antigen-presenting cells and T and B cells, surface IgE bearing mast cells, basophils
Physical coupling of allergens to innate immune response–stimulating substances		
GpG oligonucleotide-conjugated allergens	Major allergen (Amb a 1) is bound to a Toll-like receptor 9-triggering CpG oligonucleotide.	B cell and plasmacytoid dendritic cell activation and antigen presentation to T cells
Allergens coupled to virus-like particles	Der p 1 coupled to highly repetitive virus capsid-like recombinant particles	Predominantly B cells and other antigen-presenting cells, T cells. Rapid induction of high IgG antibody titers was observed in healthy human volunteers.
Carbohydrate-based particles	Carbohydrate-based particles-bound rPhl p 5b induced a stronger antibody and cytokine responses.	General immune response and use of carbohydrate-binding receptors on antigen-presenting cells
Hypoallergenic vaccine based on allergen-derived peptides fused to hepatitis B PreS	Recombinant fusion proteins show reduced allergenic activity in basophil activation and no IgE reactivity.	Predominantly B cells and other antigen-presenting cells
Monophosphoryl lipid A (MPL) formulated with allergoid	Pollen allergoid formulated with the T _H 1-inducing adjuvant monophosphoryl lipid A (MPL) facilitates short-term SIT.	TLR-4 expressing antigen-presenting cells such as myeloid dendritic cell activation and antigen presentation to T cells
Different routes of administration		
Sublingual immunotherapy	Efficient, safe, and widely used with native allergen extracts	Oral mucosal dendritic cells and general tonsillar immune response
Intralymphatic vaccination	Allergen-SIT vaccines administered directly into a lymph node. The aim is to deliver high amounts of allergens into secondary lymphatic organs.	Lymph node immune response. Predominantly B cells and T cells and other lymph node antigen-presenting cells
Epicutaneous vaccination	Allergen extracts applied to skin nonvascularized area, safe, needle-free, and potentially self-administrable.	Skin langerhans cells and regional lymph node T and B cells
Fusion of allergens with immune response modifiers		
Targeting FcγRII	Fusion of allergens with human Fcγ	Suppression of antigen-specific B cells, IgE-loaded basophils and mast cells
Modular antigen translocation (MAT) vaccines	The fusion of transactivator of transcription (TAT) peptide to both truncated invariant chain and allergens is able to target antigens to the nascent MHC II molecules in the trans-golgi compartment.	Potentiated antigen presentation to T cells
Combined usage of AIT vaccines with immune response modifiers		
Treatment with anti-IgE mAb before and during AIT	To reduce AIT-induced side effects. To enable relatively rapid dose increase To use relatively high doses	Decreased mast cell and basophil degranulation, decreased IgE-facilitated antigen presentation

increase antigen presentation (47, 48). This modular antigen translocation (MAT)–Fel d 1 vaccine is efficiently internalized and potently presented to T cells through antigen-presenting cells, which stimulated T cell responses in 100-times-lower doses as

compared with native allergens. In a double-blind, placebo-controlled clinical trial, the MAT–Fel d 1 vaccine was administered in three increasing doses into inguinal lymph nodes at 4-week intervals. In addition to a good safety profile, three doses of MAT–Fel

d 1 intra-lymph node vaccination induced clinical tolerance to nasal challenge of cat allergen extract in parallel with T cell tolerance and increased serum IgG4 in correlation with IL-10 (47, 48).

Virus-like particles and strong adjuvants

have also been used to modulate the responses of allergen-specific T cells. A single dose of Fel d 1-linked virus-like particles prevented type I hypersensitivity in mice (49, 50). The coaggregation of FcεRI and FcγRII receptors and the fusion of allergens to human Fcγ has been reported to inhibit allergen-induced basophil and mast cell degranulation by cross-linking Fcγ and FcεRI receptors (51). In addition, fusion proteins consisting of nonallergenic peptides from the four major timothy grass pollen allergens and the PreS protein from hepatitis B virus as a carrier were expressed in *Escherichia coli*. This vaccine reduced T cell proliferation and proinflammatory cytokine release and increased blocking IgG activity (52).

Combined use of AIT vaccines with immune response modifiers. Another high potential approach to improve AIT is the combination of AIT with biological immune response modifiers. Anti-IgE combined with AIT has been evaluated in several studies and has resulted in a decrease in the risk of anaphylaxis caused by rush immunotherapy, with a quicker dose increment to reach the maintenance dose and improved rescue medication scores with a good safety profile (25, 53). Several other combinations are possible, including anti-IL-4 or anti-IL-13 as well as their receptor antagonists. These approaches represent many of the avenues being pursued to improve the efficacy and safety of AIT, some of which are expected to be used in the clinical setting in the coming years.

ENDOTYPES OF ALLERGIC DISEASES, BIOMARKERS, AND ALLERGEN IMMUNOTHERAPY

Allergic diseases are quite heterogeneous, and pathologies are driven by different molecular mechanisms. Phenotypes of allergic diseases describe clinical and morphologic characteristics and individual responses to treatment; however, they are not directly linked to disease mechanisms. Endotypes of allergy and asthma have been introduced recently that link a subgroup of an individual disease to the mechanism contributing to pathology, such as metabolic, inflammatory, immunological, and remodeling pathways (54, 55). For example, the type 2 endotype—which includes innate lymphoid cells, T_H2 cells, tissue eosinophilia, and IgE production—is clearly defined and linked to mechanisms of disease development, response to treatment, and disease outcomes in allergic disease (54, 55). Emerging en-

dotype-driven strategies, particularly the development of biologics that target a single molecular pathway, are being pursued in order to find a solution for individualized clinical problems on disease outcomes. It is now a major question whether there exists an AIT-responsive endotype of allergic diseases and asthma. However, the description of an endotype relies on biomarkers, which can be a key molecule associated with or directly contributing to a particular disease endotype. Unfortunately, there are so far no established biomarkers that are capable of predicting AIT-responder patient selection and successful clinical response.

CONCLUSION

AIT offers an efficient treatment of allergic diseases with a possibility of cure. There is strong evidence for clinical safety and efficacy of AIT and rapidly improving understanding of mechanisms of action. There are also intensive efforts to improve AIT vaccines by making them more convenient, efficient, and safer. One of the major bottlenecks in improving AIT vaccines has been the use of not fully universally standardized allergen extracts, which are not possible to perfect because of fundamental problems of natural allergen extracts. In addition, long-term AIT protocols face substantial challenges with patient adherence. The general use of disease endotypes for allergies and asthma and correct selection of the responder patient population with defined biomarkers remain essential unmet needs in the clinical settings. Yet, research is progressing to address these bottlenecks, and hopefully what we learn from the relative success of AIT can be leveraged for the treatment of other diseases of immune dysregulation.

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