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REVIEW ARTICLE

Review Series: Immunotherapy and Tolerance—Cutting Edge

Mechanisms of Allergen-Specific Immunotherapy and Novel Ways for Vaccine Development

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ABSTRACT

Allergen-specific immunotherapy (SIT) is the only available curative treatment of allergic diseases. Recent evidence provided a plausible explanation to its multiple mechanisms inducing both rapid desensitization and long-term allergen-specific immune tolerance, and suppression of allergic inflammation in the affected tissues. During SIT, peripheral tolerance is induced by the generation of allergen-specific regulatory T cells, which suppress proliferative and cytokine responses against the allergen of interest. Regulatory T cells are characterized by IL-10 and TGF-beta secretion and expression of important cell surface suppressive molecules such as cytotoxic T lymphocyte antigen-4 and programmed death-1 that directly or indirectly influence effector cells of allergic inflammation, such as mast cells, basophils and eosinophils. Regulatory T cells and particularly IL-10 also have an influence on B cells, suppressing IgE production and inducing the production of blocking type IgG4 antibodies. In addition, development of allergen-specific B regulatory cells that produce IL-10 and develop into IgG4 producing plasma cells represent essential players in peripheral tolerance. These findings together with the new biotechnological approaches create a platform for development of the advanced vaccines. Moreover, reliable biomarkers could be selected and validated with the intention to select the patients who will benefit most from this immune-modifying treatment. Thus, allergen-SIT could provide a complete cure for a larger number of allergic patients and novel preventive approaches need to be elaborated.

KEY WORDS

allergy vaccines, B regulatory cells, desensitization, immune tolerance, T regulatory cells

INTRODUCTION

Immune tolerance is the "Holy Grail" of many fields in which dysregulation of the immune system plays a central role including allergy, asthma, autoimmunity, organ transplantation and infertility. It is the prime target for prevention and treatment strategies of these disorders. Immune tolerance to allergens is characterized by establishment of a long-term clinical tolerance.[1-4] It is now becoming clear that allergic diseases are complex disorders and that there are several disease variants caused by distinctive cellular and molecular mechanisms. Although there are several clinically relevant phenotypes for allergic rhinitis, asthma, atopic dermatitis, chronic rhinosinusitis and urticaria, these phenotypes do not provide any insights into the mechanisms that underpin the diseases.[5,6] It is now thought that some clinical trials may have previously been unsuccessful because they were performed without attempting to classify patients into sub-groups defined by distinct pathophysiologies, namely 'endotypes'.[5-7] It is generally ac-

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Table 1 What is unknown in the mechanisms of allergen-SIT

- Molecular mechanisms of how Treg cells are generated *in vivo*
- Better adjuvants that specifically induce Treg cells
- *In vivo* life span of Treg cells induced by allergen-SIT
- If there are deleterious roles of Treg cells, such as immune tolerance to tumor antigens and chronic infectious agents?
- Role of resident tissue cells in immune tolerance
- Molecular mechanisms of spontaneous healing, remissions and exacerbations of allergic disease
- Local tissue events during SLIT and epicutaneous SIT
- Early molecular markers and predictors to decide to start, stop and success
- Is there differences in the mechanisms of high dose and low dose allergen-SIT?
- Mechanisms of long term maintenance of allergen tolerance
- Is there any role in defective barrier function in the successful response to allergen-SIT?

cepted that there is an endotype/phenotype of these diseases that responds best to allergen-SIT,[8] however there are no appropriate biomarkers for patient selection. The best suitable models to study the mechanisms underlying immune tolerance include the development of a healthy immune response during high dose of allergen exposure in beekeepers and cat owners as well as the development of tolerance to allergens induced by allergen-SIT.[9,10] The described mechanisms include changes in the profile of allergen-specific memory T and B cell responses, the synthesis of specific antibody isotypes that skew the immune response towards a non inflammatory pattern, as well as decreased activation, tissue migration and degranulation of effector cells including mast cells, basophils and eosinophils. The role of miRNAs in the immune system is being studied since the discovery of miRNAs in mammalian cells, and may represent biomarkers for SIT, because recent studies clearly demonstrate the presence of extracellular miRNAs in body fluids and propose the involvement of miRNAs in cell-cell communication.[11] In addition, defects in barrier function may play an important role in disease pathogenesis and better understanding of these mechanisms is required to develop better treatment modalities.[12] It seems that there is substantial need for research to elucidate the so far unknown mechanisms (Table 1).[13]

INDUCTION OF IMMUNE TOLERANCE TO ALLERGENS AS AN ESSENTIAL MECHANISM OF ALLERGEN-SIT

DEVELOPMENT OF DESENSITIZATION OF EFFECTOR CELLS

A number of mechanisms is involved in rendering mast cells and basophils unresponsive to allergens even if these cells are "sensitized" by specific IgE bound to their FcεRI receptor. After the first injections of allergen-SIT, a very early decrease in the susceptibility of basophils to degranulation and systemic anaphylaxis can be observed while all of the treated individuals have high quantities of specific IgE.[14] The rapid desensitization of skin mast cells seems to be more difficult to achieve.[15] The underlying molecular pathways seem similar to the rapid desensitization of the efector cells in anaphylactic reactions to drugs.[16] Histamine is one of the main mediators released upon FcεRI triggering of basophils and mast cells, and it exerts its functions through histamine receptors (HRs).[17,18] We recently demonstrated a rapid upregulation of H2R within the first 6 hours of the build-up phase of venom-SIT.[19] H2R strongly suppressed FcεRI-induced activation and mediator release of basophils, including histamine and sulfidoleukotrienes, as well as cytokine production *in vitro*. These data suggest that immunosilencing of FcεRI-activated basophils by a selective suppression mechanism mediated by H2R is highly relevant for the very early desensitization effect of venom-SIT.[19] The release of mediators from mast cells and basophils at low levels, below the "normal" threshold of systemic anaphylaxis is probably taking place during allergen-SIT.[20,21] Thus, successful hyposensitization is associated with the altered magnitude of mediator release from the effector cells.[20]

INDUCTION OF PERIPHERAL T CELL TOLERANCE TO ALLERGENS

Generation of allergen-specific Treg cells is central in the induction of allergen tolerance during allergen-SIT.[22-24] Peripheral tolerance is initiated by IL-10 and TGF-β, which are increasingly secreted by the allergen-specific Treg cells during the course of allergen-SIT.[22,23] Both IL-10-secreting (Tr1) and $FOXP3^+$ (Treg) subsets have been implicated, suggesting that there is an overlap between these subsets of Treg cells in humans.[25,26] The supporting evidence for the central role of Treg cells in the induction of allergen-specific tolerance was provided by the demonstration of the association of increased numbers of $FOXP3^+CD25^+CD3^+$ cells in the nasal mucosa after immunotherapy with clinical efficacy and the suppression of seasonal allergic inflammation.[27] $CD4^+CD25^+$ Treg cells from atopic donors are less effective in the inhibition of proliferation of $CD4^+CD25^-$ T cells, which indicates the failing mechanisms of peripheral allergen tolerance.[28] Studies that calculated the frequency of allergen-specific T cell subsets such as Th1, Th2 and Tr1 demonstrated a clonal shift towards Tr1 during allergen tolerance.[9,29] In addition, by using human MHC-class II tetramers to investigate allergen-specific T cells during induction of clinical allergen tolerance showed that this led to a switch in the frequencies of antigen-specific T-cells producing certain cytokines. There was a marked loss of IL-4-producing T-cells and an increase in the number of FOXP3+ and IL-10-producing antigen-specific $CD4^+$ T-

cells.[30] Along these lines, peptide immunotherapy in asthmatics results in decreased Th2 responses due to IL-10-dependent peripheral T cell tolerance. Injection of peptides of selected T cell epitopes from the major cat allergen Fel d 1 induced suppression of T cell proliferation after stimulation with other "linked" T cell epitopes from the same molecule.[31] Some models, such as suppression of germinal center reactions and intestinal lymphoid tissue, show that T cell suppression can take place both in the secondary lymphoid organs and in the affected tissues.[32]

Studies in human high dose allergen exposure models such as non-allergic bee keepers and cat owners showed that Treg cells specific for the major allergens of venom and cat saliva represent the major T cell subset in healthy individuals.[9,10] They utilize IL-10, TGF-β, cytotoxic T lymphocyte antigen 4, programmed death 1 and a number of other suppressive mechanisms.[9,29] The expression of FOXP3 correlates with the suppressive capacity of Treg cells.[33] Consistently, increased FOXP3 expression negatively correlates with IgE, eosinophilia and IFN-γ levels. Remarkably, the ratio of $FOXP3^+$ T cells to total $CD4^+$ T cells is significantly lower in asthmatics or atopic dermatitis patients compared to healthy individuals.[34]

Investigation of Treg cells in allergic individuals provided further evidence for their role in peripheral allergen tolerance. It has been shown that mucosal tolerance induction against dietary antigens coincides with increased numbers of $CD4^+CD25^+$ Treg cells. Children who developed clinical tolerance to milk show suppressed peripheral blood mononuclear cell proliferation to bovine beta-lactoglobulin along with an increased frequency of circulating $CD4^+CD25^+$ Treg cells.[35] In allergic children, the numbers of Treg cells increase during the pollen season, which provides the mechanism by which other subsets of pollen allergen-specific T cells are controlled.[36] Notably, in both healthy and allergic individuals, all three major types of allergen-specific subsets of T cells, the Th1, Th2 and Tr1 cells, are found in different proportions. Thus, the shift in the balance between allergen specific Th2 and Treg cells is central to either development of allergen tolerance or allergic status or even the recovery from allergic disease.[9,29,37]

Breaking of peripheral T-cell tolerance to allergens can lead to the development of allergies, and a recent study showed some insight into these mechanisms. Human tonsils show very low levels of allergen-induced T-cell proliferation, thus representing a very suitable *in vivo* model to assess mechanisms of breaking allergen-specific T-cell tolerance.[38] Triggering of Toll-like receptor 4 or 8 combined with addition of proinflammatory cytokines IL-1beta or IL-6 breaks allergen-specific T-cell tolerance in human tonsils and peripheral blood through a mechanism dependent on the adaptor molecule myeloid differentiation primary response gene 88 (MyD88).[38] In particular, myeloid DCs and stimulations that activate these cells mediate the breaking of the tolerant state of allergen-specific $CD4^+$ T cells, whereas plasmacytoid DCs and stimulations that activate these cells did not have any tolerance-breaking effect. Tolerance-breaking conditions induced by different molecular mechanisms were associated with a mixed cytokine profile with a tendency toward increased levels of IL-13 and IL-17, which are Th2 and Th17 cytokines, respectively.

ALLERGEN-SPECIFIC IgE AND IgG RESPONSES

In contrast to the allergen-specific T cells, B cells do not show tolerance or unresponsiveness to allergens but are skewed from IgE-producing to IgG4-producing cells.[39] Allergen-SIT induces a transient increase in serum specific IgE followed by a gradual decrease usually visible after 3-6 months of treatment.[40] Measurements of the IgG subtype levels during SIT showed specific increases in the range of 10-100 fold in the concentrations of IgG1 and particularly of IgG4.[41] Specific IgG4 in serum shows a relatively early and rapid increase and continues to increase during the whole duration of SIT as it generally reflects the total allergen.

The suppressive cytokine IL-10 produced by Treg cells also affects the immunoglobulin synthesis through strong suppression of allergen-specific IgE, while it increases IgG4 production.[22,42] Thus, IL-10 regulates specific isotype formation towards a non-inflammatory phenotype - IgG4.[43] IgG4 antibodies are dynamic molecules that exchange Fab arms by swapping heavy-light chain pairs between IgG4 molecules with different specificities. This results in the production of bispecific antibodies with a substantially decreased capacity for cross-linking, because they are functionally monovalent.[44] The IgG4 hinge region has specialized structural features that result in a lower affinity for certain Fcγ receptors, and IgG4 does not fix complement and can inhibit immune-complex formation by other antibody isotypes.[45] In addition, IgG4 is a blocking antibody that prevents the activation and degranulation of effector cells by competing with allergen binding to the IgE on the Fcε receptors of mast cells and basophils.[43,46] The described shift in immunoglobulin isotype production cannot however, explain the therapeutic effect of SIT. In general, the decrease in serum IgE appears much later than clinical tolerance, which occurs relatively early during the course of SIT and does not correlate with the magnitude of clinical improvement after treatment. The production of IgE by bone marrow-residing plasma cells that show a very long life-span might the a plausible explanation for this discrepancy.[47]

In a recent study, human inducible IL-10-secreting B regulatory (Br1) cells and their immunoregulatory capacity has been investigated in highly purified IL-10-secreting allergen-specific human B cells.[43] Human Br1 cells produced high levels of IL-10 and po-

Table 2 Characteristics of a good allergen-SIT vaccine

- Should induce long term allergen tolerance (curative)
- Should achieve clinical success in short time with few doses
- Should target individuals with allergy to identified allergens
- Biomarkers should be identified for patient selection and assessment on which population should be targeted, when to start and stop, and how to follow the patients
- To use multiple allergens at the same time should be possible
- Same approach could be useful for the preventive vaccines

tently suppressed antigen-specific $CD4^+$ T-cell proliferation. Interestingly, IgG4 was selectively confined to human IL-10^+ Br1 cells. Healthy beekeeper-derived B cells specific for the major bee venom allergen phospholipase A_2 showed increased expression of IL-10. Furthermore as a major contribution to mechanisms of allergen-SIT, the frequency of IL-10^+ PLA-specific B cells was significantly increased in patients receiving allergen-specific immunotherapy after 3 months demonstrating for the first time a functional B regulatory cell subset that may play a role in allergen tolerance in humans.[43]

SUPPRESSION OF EFFECTOR CELLS OF ALLERGIC INFLAMMATION

Allergen-SIT efficiently modulates IgE-mediated activation and histamine release from mast cells and basophils.[48] This process is regulated by Treg cells and their anti-inflammatory cytokines. Several molecular mechanisms have been proposed. Through the direct Treg cell-mast cell contact, Treg cells inhibit FcεRI-dependent mast cell degranulation.[49] IL-10 suppresses IL-5 production by human Th2 cells, reduces proinflammatory cytokine release from mast cells and downregulates eosinophil function and activity.[50] Mast cells are also involved in immune tolerance and exert suppressive functions in allergic inflammation. They are capable of downregulating allergic inflammation in UV-induced skin injury and venom-induced tissue damage models in which IL-10 plays key role.[51,52] Treg cells are potent suppressors in various models of eosinophilic inflammation including by schistosome infection-induced as well as asthma-like lung inflammation in mice.[53] Along these lines, decreased numbers of eosinophils, eosinophil chemoattractants, and their mediators in the nasal mucosa have been observed as a long term effect of SIT.[54]

NOVEL VACCINES FOR ALLERGEN-SIT

The future needs of allergen-SIT include increased efficacy and patient's adherence, reduced side effects and costs and treatment duration (usually 3-5 years). A good allergen-SIT vaccine should induce long term allergen tolerance; should achieve clinical success in short time with few doses; should target individuals with allergy to identified allergens; biomarkers should be identified for patient selection and assessment on which population should be targeted, when to start and stop, and how to follow the patients; the use of multiple allergens at the same time should be possible. The same approach could be useful for the preventive vaccines (Table 2).

Novel approaches to improve the efficacy and safety of vaccine-based allergen-SIT are outlined in Table 3. One promising approach includes bypassing IgE binding to avoid IgE-mediated side effects but targeting T cells to induce T cell tolerance,[55] which is based on our understanding of the conformation dependence of B cell epitopes and linearity of the amino acids sequence of T cell epitopes in the three dimentional structure of an allergen. Allergen-fragments, fusions, hybrids and chimeras have been used.[55-58] The prominent example of this approach is peptide immunotherapy that utilizes linear T cell epitope peptides.[59-61] In addition these modalities enable administration of higher doses of allergens (or their derivatives) without the risk of anaphylaxis.[55,62]

The second approach is the use of recombinant allergens or their cocktails, with the aim of partially mimicking an allergen extract. A study that tried to reconstruct the native grass pollen allergen extract using a mixture of five recombinant allergens was effective in reducing symptoms and the need for symptomatic medication in patients with grass pollen allergy.[41] All treated subjects developed high allergen-specific IgG1 and IgG4 antibody responses. A large number of clinical trials using recombinant allergens performed during the last decade showed significant clinical efficacy compared to the placebo group. Recombinant vaccines for grass pollen, birch pollen and house-dust mite represent the major focus in the future development. Vaccines for other allergens may not prove to be cost effective due to the large number of minor allergens.[63] Another attempt is to physically couple allergens to stimulators of the innate immune response. The large diversity of future approaches relies on infinite possibilities for combinations of multiple immune stimulators and methods for coupling.[64-66]

Various routes of vaccine administration are also investigated. It has been well-documented in the double-blind, placebo-controlled trials of sublingual immunotherapy (SLIT) that SLIT is clinical efficacious with a treatment benefit that might be slightly less than that achieved with subcutaneous SIT.[67] However the direct comparison of the two different ways of SIT are not possible at the moment due to very limited data from the head-to head studies and diversities in the clinical assessment methodology between studies. Sustained disease-modifying effects of SLIT have been established in large-scale randomized, double-blind, placebo-controlled trials in both adults as well as in children.[2,68] Although the magnitude of the change in most parameters is modest or

Table 3 Efforts for novel vaccine development for allergen-SIT

Type of the vaccine/approach	Description and mechanism
Bypassing IgE binding and targeting T cells	
Fusion of major allergens[56] and chimeric allergens[57]	Major allergens or their fragments are fused and expressed as a single recombinant protein. T cell reactivity is preserved, IgE binding is attenuated. Preventive effect on development of IgE is demonstrated in mice.
Hypoallergenic hybrid molecules[80]	Derived from Der p 1 and Der p 2, reduced IgE reactivity of hybrid proteins, induce higher T cell proliferation responses.
Fragments of major allergens[58]	NonIgE binding fragments of major allergen (Bet v 1). IgE binding is attenuated and T cell reactivity is preserved.
Peptide immunotherapy[59-61]	T cell epitope peptides (Fel d 1, Api m 1) that do not bind IgE and induce T cell tolerance have been used in cat and bee venom allergy.
Unrefolded native or recombinant allergens[62]	Major recombinant allergens (Api m 1, Bet v 1) are not refolded and lack the native conformation. IgE binding is abolished, T cell reactivity is protected.
Polymers of major allergens[58]	Major allergen (Bet v 1) is trimerized. Mast cell, basophil degranulation is attenuated, T cell reactivity is preserved in vitro.
Reconstitution of the natural extract with mixture of multiple recombinant allergens	
Mixture of several major recombinant allergens[41]	Phl p 1, Phl p 2, Phl p 5a, Phl p 5b, Phl p 6 were used as a mixture of five recombinant grass pollen allergens.
Allergens coupled to adjuvants that stimulate various aspects of innate immunity	
GpG oligonucleotide-conjugated allergens[64]	Toll-like receptor 9-triggering CpG oligonucleotide is fused to major ragweed allergen Amb a 1.
Allergens coupled to virus-like particles[65]	Highly repetitive virus capside-like recombinant particles coupled to house dust mite major allergen Der p 1.
Carbohydrate-based particles[81]	Carbohydrate-based particles-bound rPhl p 5b induced a stronger antibody and cytokine responses.
Hypoallergenic vaccine based on allergen-derived peptides fused to hepatitis B PreS[82]	Recombinant fusion proteins show reduced allergenic activity in basophil activation and no IgE reactivity.
Monophosphoryl lipid A (MPL) formulated with allergoid[66]	Th1-inducing adjuvant monophosphoryl lipid A (MPL) facilitates short-term SIT together with a grass pollen allergoid.
Novel routes of administration	
Intralymphatic vaccination[73]	Allergen-SIT vaccines administered directly into inguinal lymph nodes with the aim to deliver high amounts of allergens into secondary lymphatic organs.
Epicutaneous vaccination[72]	High numbers of antigen presenting cells (LCs), non-vascularized area, safe, needle-free, and potentially self-administrable.
Fusion of allergens with immune response modifiers	
Targeting FcγRII[74, 75]	Fusion of allergens with human Fcγ has been reported to inhibit allergen-induced basophil and mast cell degranulation by crosslinking Fcγ and FcεRI receptors.
Modular antigen translocation (MAT) vaccines[77]	The co-expression of major recombinant allergens together with transactivator of transcription (Tat) peptide and truncated invariant chain is able to target antigens to the MHC II molecules in the trans-golgi compartment.
Combination possibility with immune response modifiers	
Pre-treatment with anti-IgE mAb before SIT[78]	To reduce SIT induced side effects. To enable relatively rapid dose increase. To use relatively high doses.

no changes have been observed, the immunological mechanisms of SLIT seem to be similar to subcutaneous SIT. In the context of reduced treatment benefits, and modest changes in immunological markers, the further improvement of SLIT is to be expected. Multiple mechanisms of immune tolerance are induced by SLIT, and involve Treg cells, IL-10 production, increased sublingual FOXP3-expressing cells serum inhibitory activity for IgE-facilitated allergen binding to B cells due to elevated allergen-specific IgG4, IgA.[69,70] Allergen-specific FOXP3$^+$ Treg cells have been found in lingual and palatine tonsils in humans, and these cells may participate in oral tolerance and SLIT.[71]

Other routes of vaccine administration have been evaluated. The intralymphnode and epicutaneous applications of vaccine are currently tested. Both routes showed similar efficacy to subcutaneous injection im-

munotherapy in grass pollen allergy, but less applications and lower total doses of allergen were required using these routes.[72,73] T cell responses with strong cytotoxic activity and IFN-γ production that play a role in long-term protection against viral infections and tumors can be induced by intralymphatic vaccines.

Another promising strategy is the fusion of allergens to immune modifiers. FcγRIIb is an immune tyrosine based inhibitory motif containing receptor.[65] The coaggregation of FcεRI and FcγRIIb inhibits FcεRI signaling. The fusion of the inhibitory receptor FcγRIIb to allergens to downregulate downstream allergen-specific immune responses has been investigated. Another fusion protein of allergen and human Fcγ suppressed allergen-induced degranulation of basophils and mast cells by crosslinking Fcγ and FcεRI receptors.[74,75] In addition the major cat allergen Fel d 1 was cloned and expressed together with a human immune deficiency virus protein, TAT-derived membrane translocation domain, and a truncated peptide of the invariant chain (modular antigen translocation (MAT)-Fel d 1.[76] This MAT-Fel d 1 vaccine is much more efficiently internalized and potently presented to T cells by antigen-presenting cells. It induces potent T cell responses at doses that were approximately 100x lower than those of the native allergens. In a double-blind, placebo-controlled clinical trial, the MAT-Fel d 1 vaccine with alum adjuvant was administered in three increasing doses (1 μg, 3 μg, 10 μg) into inguinal, lymph nodes at 4 week intervals. It also showed a very good safety profile. Cats allergic patients who were treated with the MAT-Fel d 1 vaccines developed clinical tolerance to nasal challenge of cat dander extract in parallel with increased serum IgG4.[77]

Apart from the physical fusion in one agent, the conventional and novel methods of allergen-SIT may also be combined with immune-modifying biological therapies. For example the effectiveness of anti-IgE combined with allergen-SIT has been demonstrated in several studies.[78] One additional benefit was reduced side effects with a significant decrease in the risk of anaphylaxis caused by rush immunotherapy (a rapid dose increment approach to reach the maintenance dose as quickly as possible) and improved rescue medication scores (so, decreasing the need for a rescue medication to suppress the symptoms: for example anti-histamines for allergic rhinitis) of SIT with a good safety profile.[78,79] These add-on strategies with biologicals or biosimilars will also expand the treatment scope of allergen-SIT by including of the high-risk patients such as food allergy etc. Recent developments provided hope for better treatments capable of providing a complete cure for allergic disease. The findings of the recent studies utilizing the model of antigen tolerance provided by allergen SIT might also provide insights into the further therapeutic approaches in other immune-mediated disease, which are related to dysregulation of the immune system.

CONCLUSIONS AND FUTURE PERSPECTIVES

Today the major challenges for allergen-SIT include reducing the number of nonresponders and side effects, providing a long term recovery or even a complete cure, and reduce the costs as well as the burden for the patients related to the multiple visits and the long (3-5 years) duration of the therapy. Thus, there is a strong rationale for the development of new vaccines including potent biological immune response modifiers as well as new routes of administration. Prevention strategies for allergic diseases also form a very exciting horizon. However, the major challenges include the requirement for very early intervention, safety problems for a pediatric usage, and missing early biomarkers of who will develop allergy and to which particular allergen.

The advances in immunology and bioengineering are being applied to biologicals to improve their clinical efficacy and feasibility of production by optimizing their design and clinical efficacy. Novel diagnostic biomarkers defining the various endotypes will help to select the best responders and provide them with an optimized patient-specific treatment. It is generally thought that the combination of immune response modifiers with allergen-SIT might provide a way for efficient immunomodulation of allergic diseases. All of these approaches could provide a complete and persistent, life-long cure of allergic disease to a large number of allergic subjects.

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