

Specific immunotherapy in pollen allergy

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Pollen allergy affects at least 10% of the global population, and up to one-third of the affected individuals displaying hay fever symptoms will later develop allergic asthma. The efficacy of allergen-specific immunotherapy (SIT) in seasonal allergic rhinitis has been confirmed in many clinical studies using grass, ragweed, and birch pollen extracts. SIT prevents the onset of new sensitizations to different allergens, and reduces the development of asthma in patients with allergic rhinitis caused by inhaled allergens, including pollen. Several rational approaches are being developed to improve the efficacy of SIT by reducing immunoglobulin (Ig)E-mediated adverse reactions. The use of chemically modified, recombinant or genetically engineered allergens is being explored, in addition to the investigation of novel adjuvants and alternative routes of administration. The first clinical trials using this therapy provided encouraging results, suggesting that allergen extracts can be successfully substituted by molecule-based formulations. These new formulations, for use in the routine management of pollen allergies, should become available in the near future.

Keywords Allergen-specific immunotherapy, DNA vaccines, genetically engineered allergen vaccines, hypoallergens, peptide immunotherapy, pollen allergy, recombinant allergens

Introduction

Allergic and asthmatic diseases affect more than 25% of the population in industrialized countries [1,2]. Typically, affected individuals produce immunoglobulin (Ig)E antibodies specific to various environmental proteins termed allergens. Allergenic proteins originate from various sources, including pollen of grasses, weeds and trees, spores of molds, foods, mites, cockroaches, and dander from pets and other domestic animals. Clinical manifestations can be localized (eg, as seen in allergic rhinitis or hay fever, allergic asthma, food allergy, allergic skin inflammation, and ocular allergy) or systemic (as seen with anaphylaxis). Pollen allergy affects at least 10% of the global population, and up to one-third of the affected individuals having hay fever symptoms will later develop allergic asthma [1,2].

The etiology of allergic diseases is multifactorial. Genetic susceptibility, route of exposure, the dose of the allergen and, in some instances, the nature or structural characteristic of the allergen, have been shown to influence allergic immune response [1,2]. During sensitization, allergens

prime CD4⁺ T-helper (Th)2 cells, resulting in the production of Th2-cytokines such as IL-4 and IL-13. These cytokines promote class switching to the ϵ immunoglobulin heavy chain, culminating with the production of IgE antibodies by B-cells (Figure 1A). IgE antibodies then bind to the high-affinity receptor Fc ϵ R1, expressed in mast cells and basophils. Allergen-mediated cross-linking of the IgE-Fc ϵ R1 complexes causes mast cells and basophils to degranulate and release pharmacologically active mediators such as vasoactive amines (eg, histamine), lipid mediators (eg, prostaglandins and leukotrienes), chemokines, and other cytokines. These events are characteristic of the immediate phase of the allergic reaction [1,2].

Allergen-specific immunotherapy (SIT) involves the repeated administration of allergens to sensitized individuals [3]. SIT is a clinically effective allergen-specific form of treatment, capable of inducing active immunity to the allergen [3]. The decision to use SIT demands the careful assessment of a patient's condition and the role of allergic triggers. Typically, patients are started on a very low, subcutaneous dose of allergen. The dose is then increased in a series of weekly injections until a plateau or maintenance dose is achieved. The maintenance dose is then given at 4 to 6 weekly intervals for three to five years. Other injection regimes use several doses on each day, or a whole series of increasing doses in a single day (rush protocols).

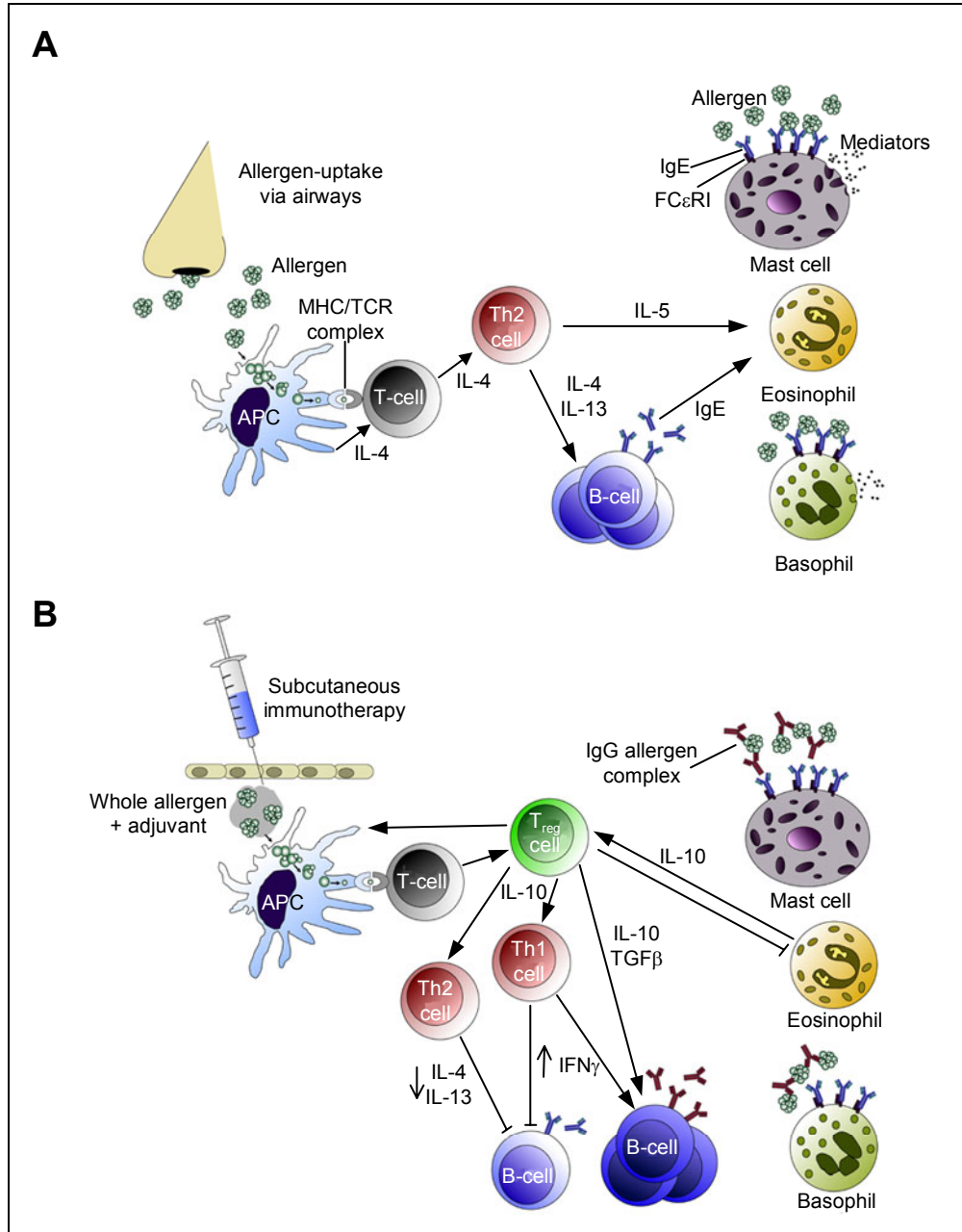
In this review, the immunological mechanisms underlying successful SIT are summarized and approaches under consideration to improve the safety and efficacy profiles of SIT, including allergen preparations, adjuvants, and routes of administration, are discussed. Clinical trials of SIT for pollen allergy using novel vaccines, which were carried out within the last three to four years, are specifically discussed.

Mechanisms of SIT

The heterogeneity of allergen preparations, administration routes and vaccination protocols used in different studies hamper our understanding of specific molecular mechanisms following SIT. However, it is well accepted that immunotherapy modifies the responses of antigen-presenting cells (APCs), T-cells, B-cells and effector cells, which mediate the allergic response and, therefore, influence both immediate- and late-phase reactions to allergen exposure. Recently, the immunological mechanisms of action of SIT (see Figure 1) have been extensively reviewed [4••] and are summarized below.

- Modulation of APC function: clinical trials have illustrated that SIT increases the production of IL-10 by B-cells, monocytes and macrophages. This effect might lead to increased generation of IL-10-secreting T-regulatory 1 (Tr1)-like cells, which inhibit subsequent inflammatory responses.
- Modulation of T-cell responses: SIT increases the allergen-induced ratio of Th1 cytokines to Th2

Figure 1. The allergic reaction and immunological mechanisms associated with successful specific immunotherapy.



(A) In individuals susceptible to developing allergies, uptake of potentially allergenic proteins via the airways can result in the activation of specific T-cells in an IL-4-dominated environment. In the absence of regulatory mechanisms, allergen exposure can result in the generation of T-helper (Th)2 cells producing high levels of IL-4, IL-5 and IL-13, which favors class switching to IgE and clonal expansion of allergen-specific B-cells. IgE bound to cell-surface receptors of antigen-presenting cells (APCs) facilitates allergen uptake and presentation, indirectly increasing T-cell activation and IL-4 production. Upon allergen contact, immediate-phase reactions are triggered by allergen-dependent cross-linking of Fc ϵ RI-bound IgE antibodies on mast cells and basophils. This activates effector cell degranulation, resulting in release of vasoactive amines, leukotrienes, chemokines and cytokines. Late-phase reactions mediated by T-cells and eosinophils in the lungs of patients can result in persistent inflammation following inhalant allergen contact. TCR T-cell receptor.

(B) The cardinal feature of specific immunotherapy comprises immune deviation, influencing both immediate- and late-phase reactions of the allergic reaction. Adjuvant co-absorbed with the allergen is applied to activate tolerogenic APCs. Expression of IL-10 in combination with low levels of costimulatory molecules by these APCs favors the generation of allergen-specific regulatory T-cells (T_{regs}). Upon activation, T_{regs} produce IL-10, which helps to adjust the Th1/Th2 imbalance in an allergic individual in part by repressing the production of the Th2 cytokine IL-4. IL-10 decreases the number of tissue mast cells as well as the survival of eosinophils, suppresses mediator release of effector cells of the allergic reaction, and might itself augment the generation of pro-regulatory APCs. Furthermore, T_{regs} upregulate transforming growth factor (TGF) β and, in concert with IL-10, this event induces the production of IgA, IgG1 and IgG4 antibodies by allergen-specific B-cells. Such antibodies can compete with IgE for allergen-binding sites, have an anti-inflammatory effect as a result of the formation of immunological inactive complexes, inhibit IgE-mediated antigen presentation, and block mast cell and basophil degranulation.

cytokines via IL-10-dependent epitope-specific induction of T-cell anergy. Whole allergen or T-cell epitope-containing synthetic peptide SIT generates allergen-specific regulatory T-cells that can suppress the responses of effector T-cells. SIT increases the production of cytokines with regulatory activity (eg, IL-10 and transforming growth factor [TGF]β). SIT, using Th1-inducing adjuvants (eg, toll-like receptor [TLR] ligands), results in specific induction of Th1-cell responses.

- Modulation of antibody responses: increased levels of IL-10 and TGFβ produced by regulatory T-cells in SIT can potentially suppress IgE production and increase the production of the non-inflammatory isotypes IgG4 and IgA. Therapy-induced IgG reduces IgE-mediated degranulation of mast cells and basophils, inhibits IgE-facilitated allergen presentation to T-cells, and reduces the number of allergen-specific memory B-cells that have undergone class switching to IgE.

Benefits of SIT for pollen allergies

SIT has been widely used to treat allergic rhinitis caused by pollen sensitization [3]; the efficacy of SIT in seasonal allergic rhinitis has been confirmed in many clinical studies using grass, ragweed and birch pollen, and the beneficial effects of treatment were shown to last for at least six years after discontinuing therapy [5]. Furthermore, SIT has been shown to prevent the onset of new sensitizations to different allergens and reduce the chance of asthma development in

patients with allergic rhinitis caused by inhaled allergens, including pollen [6,7]. SIT has also demonstrated clinical efficacy in patients with severe seasonal rhinitis that is resistant to conventional drug therapy [3]. Table 1 provides a summary of recently reported clinical studies in pollen immunotherapy.

The demand for effective and rational approaches to deal with the underlying problem of allergy is increasing because of the awareness of the adverse effects of allergic rhinitis. In patients who have received treatment, SIT has been shown to improve the quality-of-life of treated individuals via the reduction of symptoms and medication usage [8].

Improving SIT vaccines

Despite its widespread effective use for treating allergic rhinitis and asthma, four major problems associated with SIT have been identified. (i) In its current form, SIT is performed with allergen extracts (containing mixtures of allergens, non- allergenic and/or toxic proteins, and other macromolecules) that are difficult to standardize. (ii) The induction of new IgE specificities has been reported for birch and grass pollen allergic individuals. In one study, 65% of patients treated with birch pollen extract rush immunotherapy developed low levels of IgE antibodies toward additional birch pollen allergens. (iii) Severe IgE-mediated side effects can occur during treatment because of the systemic administration of fully active allergens. (iv) Effective therapeutic doses cannot often be achieved because of nonstandardized extracts or side effects.

Table 1. Recent clinical studies of pollen immunotherapy.

Allergen preparation	Strategy	Efficacy	Reference
Allergen extracts	Children with grass pollen allergies were treated with grass pollen extracts in a double-blind, placebo-controlled immunotherapy study, patients were monitored for two pollen seasons	An improvement in the symptom-medication score as well as a tolerogenic effect toward the grass pollen allergens concerning cutaneous, conjunctival and bronchial allergen reactivity were observed in the actively treated group	[47]
	Grass allergen tablets (GRAZAX, ALK-Abelló) were tested in a double-blind, placebo-controlled study consisting of 634 patients from eight countries	Rhinoconjunctivitis as well as medication score improved significantly in the actively treated group; no severe side effects, neither systemic nor local, were observed	[48]
Allergoids	Six grass pollen extracts treated with formaldehyde were used to produce allergoids which were applied in a double-blind, placebo-controlled SIT study with grass pollen allergic individuals	A decrease of symptom and medication scores, an increase in allergen-specific IgG antibodies and allergen tolerance was reported in the SIT-treated but not in the placebo group	[49]
Recombinant allergens	A mixture of five recombinant grass pollen allergens was administered in a double-blind, placebo-controlled immunotherapy trial	Grass-pollen specific IgG antibodies were induced during therapy and treated patients reported improved symptom medication scores	[11••]
Allergen fragments	Fragments of Bet v 1a displaying reduced IgE binding capacity were administered subcutaneously in a double-blind, placebo-controlled multicenter study	Therapeutic IgG antibodies were induced against the natural wild-type allergen as well as against cross-reactive pollen allergens	[30••,50]
Allergen multimers	A Bet v 1a trimer with reduced <i>in vivo</i> allergenicity was administered subcutaneously in a double-blind, placebo-controlled multi-centre study	Bet v 1-specific IgG antibodies were induced during therapy; IL-5 and IL-13 expression were reduced in the trimer-treated patients compared with the control group	[30••,50]
Allergen immunostimulatory DNA sequence-conjugates	The major allergen of short ragweed Amb a 1 was conjugated to synthetic oligonucleotides containing immunostimulatory sequences from bacterial DNA; the conjugate was used in a placebo-controlled immunotherapy study	Short-course immunotherapy with modified Amb a 1 altered the cytokine profile in the nasal mucosa of treated patients, and an improved symptom score was reported in these patients after the second year of natural allergen exposure	[51]

In principle, these problems could be solved by the use of recombinant allergens (reviewed in references [9,10]). Cocktails of pure and standardized recombinant allergens can be formulated to substitute natural extracts, and the selected recombinant allergens can be modified to reduce the risk of IgE-mediated side effects. The section below describes new allergen preparations already clinically tested for their potential to substitute pollen extracts, as well as developments that are likely to result in new products for clinical application.

Allergen preparations

Recombinant allergens

The use of recombinant allergens that are structurally and immunologically equivalent to their natural counterparts offers important advantages over the use of natural extracts, especially because recombinant allergen preparations contain defined amounts of the active component and can be standardized. Two clinical trials were recently conducted that evaluated the efficacy of recombinant allergens [11•,12,13•].

A randomized, double-blinded, placebo-controlled study of a subcutaneously administered immunotherapy consisting of a mixture of five recombinant grass pollen allergens (Phl p1, Phl p2, Phl p5a, Phl p5b and Phl p6) was conducted in individuals with allergic rhinoconjunctivitis, with or without asthma. The recombinant vaccine proved to be an effective and safe treatment of allergic rhinitis. The clinical benefit was associated with modification of the specific immune response with synthesis of IgG4 and reduction of IgE antibodies, which is consistent with the induction of IL-10-producing regulatory T-cells [11•]. In another study, the efficacy and safety of subcutaneously administered immunotherapy with a standardized allergenic birch extract versus the major birch pollen allergen Bet v 1 (in natural and in recombinant forms) was analyzed in a multicenter, randomized, double-blinded, placebo-controlled trial [12,13•]. Results demonstrated that subcutaneous immunotherapy with the recombinant allergen was as effective and safe as natural birch pollen extract.

The results of these trials are highly encouraging, and suggest that the use of recombinant preparations in the routine management of allergic disease is promising.

Chemically modified allergens

Oligomerization produced by chemical modification of allergens with glutaraldehyde or paraformaldehyde (allergoids) appears either to destroy or to mask structural B-cell epitopes, resulting in significantly reduced IgE binding activity while maintaining immunogenicity. Commercially available allergoids are currently produced by chemical modification of allergen extracts with aldehydes, and are only available for a limited panel of allergens. They comprise bulky macromolecules with undefined structures and sequences.

Recombinant allergens could be a better choice of starting material for the production of allergoids than crude allergen extracts. Allergoids prepared from a recombinant

Phl p5b (rPhl p5b) grass pollen allergen have been shown to induce a lower T-cell proliferative response of Phl p5-specific T-cell clones (using PBMCs as APCs) when compared with purified rPhl p5b or untreated extract. Recombinant Phl p5b allergoid was efficiently presented by macrophages and dendritic cells, but not by B-cells, whereas rPhl p5b was presented by all APCs, including B-cells [14]. This is indirect evidence that allergoids bypass antibody-mediated allergen uptake and are targeted to APCs using phagocytic or pinocytic uptake mechanisms.

Targeting of allergens to so-called pattern recognition receptors has been proposed as a therapeutic strategy for immunomodulating immune responses to allergens. Two types of chemical modification have been described for targeting allergens: (i) treatment with maleic acid anhydride (maleylation), and (ii) conjugation with synthetic oligodeoxynucleotides (ODN) containing immunostimulatory CpG sequences from bacterial DNA. Maleylation results in alteration of the ϵ amino group of lysine residues contained in protein antigens and thereby converts them into ligands for scavenger receptors (SRs). SRs are members of the pattern recognition receptor family, are expressed by APCs and are involved in the recognition of foreign ligands during early phases of the immune response [15]. Delivery of protein antigens through SRs results in increased immunogenicity with a dominant Th1-type immune response [16]. Maleylation of whole Orchard grass pollen proteins has been reported to decrease IgE binding activity (tested by immunoblots) and to reduce skin reactivity [17]. Although there are no reports specifically addressing this topic, maleylation of recombinant allergens or allergen-derived nonanaphylactic peptides could be an attractive approach for producing and specifically targeting hypoallergens.

In addition to targeting the allergen to TLRs, conjugation to CpG-containing ODNs also masks allergenic determinants and thus interferes with cross-linking of Fc IgE receptors on mast cells and basophils [18]. A study showed that treatment of ragweed allergic patients with an Amb a 1 (a ragweed allergen)-CpG ODN conjugate markedly increased allergen-specific Th1 responses in the peripheral blood. These responses were characterized by increased production of IFN γ and CXC chemokine ligands 9 and 10, and decreased production of IL-5 and CC chemokine ligands 17 and 22 [19•,20•]. Although the Amb a 1-CpG-ODN conjugate was produced using purified natural Amb a 1, recombinant-based CpG-ODN conjugates would be the obvious development of this approach for SIT.

Peptide-based immunotherapy

Short peptide sequences containing relevant T-cell epitopes of allergens are able to immunomodulate T-cells, but have a reduced ability to crosslink IgE and activate effector cells [4•]. These properties are attractive for strategies targeting T-cell responses in the treatment of allergy and asthma. Clinical trials of peptide-based immunotherapies have been performed for bee venom and cat allergy [4•]. These studies showed that successful outcomes are associated with induction of specific T-cell anergy and with a decrease in

allergen-specific IgE:IgG4 ratio, initiated by the autocrine action of IL-10 and/or TGF β . Although peptide immunotherapy has not been implemented in pollen allergies, a single immunodominant T-cell epitope has been identified in the allergen Art v 1, the major allergen in mugwort pollen [21•].

Genetically engineered allergens

Genetic engineering involves the targeted modification of genes in order to alter function or properties in a predictable manner. Accordingly, allergens have been modified to display reduced IgE-binding epitopes (hypoallergens), while preserving structural motifs necessary for T-cell recognition (T-cell epitopes) and for induction of IgG antibodies reactive with the natural allergen (blocking antibodies). Presently, cDNA sequences of approximately 388 allergen genes have been elucidated and can be found in the allergen databank [22]. From this library, 73 cDNAs encode pollen allergens, making templates for producing genetically engineered vaccines for important pollen allergies easily available.

Until now, approaches utilized for modification of pollen allergen genes have included changing specific base pairs (mutated gene), introducing a new piece of DNA (chimeric or hybrid gene, head-to-tail fusions), and deletions (truncated gene or fragments).

Linhart *et al* engineered a hybrid molecule in which the four major grass pollen allergens, Phl p1, Phl p2, Phl p5 and Phl p6, were fused into a single cDNA [23]. Immunization of rabbits and mice with the hybrid induced stronger and earlier IgG antibody responses than equimolar mixtures of the individual components. Vaccination-induced IgG antibodies were shown to block immediate allergic reactions in rat basophil degranulation assays. It will be interesting to compare the efficacy of this hybrid with the recombinant vaccine cited earlier in the article where the same components were administered as a mixture.

Hypoallergenic site-directed mutants of the major birch pollen allergen Bet v 1 and its homolog in apple have been produced, the latter mediating reduced allergenicity in a double-blinded, placebo-controlled, food-challenge study [24•,25,26]. In another approach, Vrtala *et al* disrupted the 3-D structure of Bet v 1 by expressing it in *Escherichia coli* as two fragments, corresponding to 1-74 and 75-160 of the cDNA [27,28]. The fragments exhibited random coil conformation and almost no allergenicity. Together, the fragments harbored all relevant T-cell epitopes. Skin reactivity and histamine release were greatly reduced when compared with the native intact Bet v 1 allergen. Furthermore, immunization of mice and rabbits with Bet v 1 fragments induced IgG antibodies that inhibited binding of IgE from allergic patients to wild-type Bet v 1. Oligomeric forms of Bet v 1 have also been constructed. Two or three copies of the full-length Bet v 1 molecule were linked by short spacers, expressed in *E. coli* and purified to homogeneity. *In vitro* studies using patient sera showed comparable IgE binding for the rBet v 1 monomer, and the engineered dimer and trimer. The trimer mediated reduced basophil histamine release compared with the monomer.

This reduced anaphylactic potential was verified *in vivo* by skin testing of birch pollen allergic patients. When compared to the Bet v 1 monomer, the Bet v 1 trimer induced similar proliferation and cytokine production upon stimulation of Bet v 1-specific T-cell clones. *In vivo* studies in animal models illustrated the ability of the Bet v 1 trimer to induce IgG-blocking antibodies, which inhibited binding of human IgEs to rBet v 1 [29].

Both Bet v 1 fragments and the Bet v 1 trimer have been used to vaccinate birch pollen allergic patients in a double-blind, placebo-controlled study. Active treatment induced protective IgG antibodies that inhibited allergen-induced release of inflammatory mediators. The researchers also observed a reduction of cutaneous sensitivity and an improvement of symptoms in actively treated patients. In addition, increases of allergen-specific IgE induced by seasonal birch pollen exposure were significantly reduced in vaccinated patients. This clinical trial was the first using modified recombinant allergen preparations [30••].

Allergen-gene vaccines

In a mouse model, DNA vaccines comprising genes encoding pollen allergens were shown to efficiently inhibit IgE expression, experimental airway inflammation and airway hyperresponsiveness by the induction of Th1-biased immune reactions (reviewed in reference [31]).

Two major concerns must be addressed before the clinical application of DNA vaccines for allergies can be considered: (i) the anaphylactic side effects observed as the result of cross-linking pre-existing IgE molecules by the translated native gene product, and (ii) the weak immunogenicity of parenterally injected conventional DNA vaccines in humans. The former concern has been addressed by coding for hypoallergenic derivatives, such as fragmented allergen genes [32], or hypoallergens developed by mutation or gene shuffling (reviewed in reference [33]). A routine method has been established to produce hypoallergenic vaccines with any given allergy gene of interest by forced ubiquitination [34••]. Replicase-based DNA vaccines have displayed immunogenicity at 10,000-fold lower concentrations than conventional DNA vaccines, thus elegantly addressing the immunogenicity problem. Even at such low doses, this vaccine type has displayed highly effective protection from allergic reactions in a mouse model [35••]. Taken together, these latest vaccine generations fulfill all necessary requirements for an allergy DNA vaccine for clinical trials. They can be produced with high purity, are allergen-specific, are safe (with respect to anaphylactic side effects), do not trigger new 'therapy-induced' IgE antibodies, and are expected to be applied at nanogram doses in humans.

Adjuvants

Although aluminum hydroxide is widely utilized to increase allergic sensitization in mouse models of allergy, this adjuvant is also traditionally used in subcutaneous SIT. Supporting its use, a study showed that aluminum hydroxide downregulates human Th2 cell responses to allergens *in vitro* [36]. Nevertheless, despite its proven efficacy and safety profiles, aluminum hydroxide might not be the ideal adjuvant for SIT.

Other adjuvants have been evaluated for their use in SIT. Lipopolysaccharide (LPS) is a ubiquitous molecule and a number of important immunoregulatory processes have co-evolved with it. With respect to allergy and asthma, there is still the question of whether LPS is beneficial or disease promoting. Th1-inducing capacity and the partial improvement of SIT efficacy (without harmful side effects) have been demonstrated in animal studies and clinical trials of LPS or monophosphoryl lipid A adjuvants co-administered with allergens [37,38]. In a food allergy model, the potential of heat-killed *Listeria monocytogenes*, an intracellular bacterium that triggers strong signals to the innate immune system (including Th1 danger signals), as a Th1 adjuvant was demonstrated [39].

Another approach evaluated has been the use of an allergen peptide co-adsorbed with maleylated poly-L-lysine on alum. This adjuvant combination was shown to target SRs and to elicit a Th1-dominant response in mice [40]. Co-adsorption could also find applications in the formulation of multi-vaccines for immunotherapy of patients allergic to several allergens.

Other substances, such as synthetic imidazoquinolines [41], microbicides [42] and antimycotics, have been reported to display Th2-suppressive properties [43] and may be added to the list of future adjuvants for supporting SIT. In addition, recombinant DNA technology represents an interesting mode of combining allergen with adjuvant, permitting the possibility of developing highly defined and standardized vaccines for SIT. A recombinant fusion protein comprising Bet v 1 and a bacterial cell surface (S-layer) protein has been shown to induce Th1 responses in mice and *in vitro* in cultured human cells [44•].

Routes of administration

The route of administration of allergens in SIT plays an important role in the subsequent development of immunological responses. Therefore, alternative routes to subcutaneous application have been the focus of recent interest. Oral and sublingual administration has gained special attention as a mode of allergen delivery. A meta-analysis of the double-blinded, placebo-controlled trials conducted over the past decade showed that sublingual SIT is associated with a favorable safety profile and is clinically efficacious, although the treatment benefit is about half of that achieved with subcutaneous injection [45•]. Similarly to subcutaneous administration, sublingual SIT has been shown to reduce the development of asthma in children with allergic rhinitis [45•]. The immunological mechanisms of sublingual SIT appear to be similar to those of injected SIT. A recent review on this topic showed that most studies using sublingual SIT reported increased amounts of serum IgG4 and IgA. However, decreases in the ratio of IgE to IgG4 have not been consistently observed [46•].

Future studies using larger sublingual doses of allergen might achieve similar clinical efficacy as subcutaneous SIT. However, such large doses may compromise the superior safety profile of sublingual delivery. Development of special adjuvant preparations and/or engineered allergens to meet

the specific features of the oral mucosa might be needed to improve the therapeutic profile of sublingual SIT.

Future directions and conclusions

Over the past few years, the field of allergology has witnessed tremendous progress in allergen characterization and recombinant production, as well as in our understanding of the molecular mechanisms of allergic diseases and SIT. The modulation of APC function and T- and B-cell responses has been studied and described in detail. It has become clear that successful SIT is associated with increases in allergen-specific antibodies (IgG1, IgG4 and IgA), reduction of allergen-specific T-cell proliferation paralleled with changes of cytokine secretion patterns which results from an increased ratio of Th1/Th2 cell responses, and induction of regulatory T-cells. Two cytokines, IL-10 and TGF β , appear to be key players in the immunological mechanisms underlying SIT. Both regulatory T-cell function and changes in serum antibody patterns are associated with expression of IL-10 and TGF β . A number of strategies can be envisioned for these pathways.

Clinical trials have shown that certain novel allergen preparations (synthetic peptides comprising T-cell epitopes, recombinant allergens, genetically engineered hypoallergens) and adjuvants (conjugates of allergen and immunostimulatory DNA sequences) are safe and efficacious. In addition, these new vaccines can be standardized to meet the highest pharmaceutical standards. Despite these new developments, much effort is still needed to turn SIT into a high-compliance form of treatment. The major perceived obstacles relate to a lack of rapid clinical effect, such as the requirement for a high number of injections under strict medical control over a long period of time, and IgE-mediated side effects. Promising approaches to improve SIT include the development of oral vaccines, more efficient adjuvants, and hypoallergenic preparations. Eventually, population studies to identify susceptible individuals could provide the basis for prophylactic strategies to prevent allergic diseases.

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