

Allergen Immunotherapy

Robert E. Esch, PhD and Jay Portnoy, MD

Address

Research and Development, Greer Laboratories,
PO Box 800, 639 Nuway Circle, Lenoir, NC 28645, USA.
E-mail: esch@greerlabs.com

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Allergen immunotherapy plays an important role in the treatment of allergic diseases and asthma. This article is a brief review of the current approaches, including patient and allergen selection, routes of administration, and use of standardized allergen vaccines. New approaches offering potentially useful strategies based on recent studies of T-cell epitopes, cytokines, and anti-IgE and DNA vaccines also are considered.

Introduction

Allergen immunotherapy for the treatment of allergic disease has been practiced since the beginning of the 20th century, based on the pioneering clinical work of Noon [1] and Freeman [2] with allergic rhinitis. Immunotherapy quickly became a major treatment modality, as there was no alternative to offer allergic patients. The acceptance of immunotherapy for the treatment of allergic disease varies among physicians. For example, it would be difficult to deny immunotherapy to patients with life-threatening insect venom hypersensitivity with demonstrable allergen-specific IgE, given the safety, efficacy, and cost of venom immunotherapy. For allergic rhinitis and asthma, immunotherapy traditionally has been an adjunct to environmental control and pharmacologic treatment. Environmental control is, in most cases, the first line of treatment for animal dander allergy and pharmacotherapy for allergic rhinitis and asthma. Immunotherapy generally is felt to be indicated for patients with evidence of a clinically relevant IgE-mediated disease and in whom environmental control and pharmacotherapy are insufficient. According to the National Institutes of Health Expert Panel Report 2, Guidelines for the Diagnosis and Management of Asthma [3], immunotherapy may be considered for asthma patients when 1) there is clear evidence of a relationship between symptoms and exposure to an unavoidable allergen to which the patient is sensitized, 2) symptoms are persistent, and 3) pharmacologic control of the asthma is difficult. Other considerations include the quality of allergen vaccines available for treatment, ability of patients to

comply, availability of facilities to treat untoward reactions, and the presence of underlying immunologic disease conditions and medications such as beta blockers [4]. A number of controlled trials showed that immunotherapy reduces the severity of allergic symptoms and medication requirements in children as well as adults [5•,6•,7,8••]. Today, even with the availability of a variety of pharmaceuticals to treat the symptoms of allergy, immunotherapy remains the only treatment that affects the natural course of the disease.

The 'Allergy March'

It is becoming clearer that the classic changes in allergen-specific IgG and IgE antibody levels during the course of immunotherapy are a manifestation of the process called "immune deviation," wherein the antibody shifts are regulated by changes in the activity of T_H1 and T_H2 cells [9]. In this model, allergen provocation in atopic subjects induces the production of IL-4, IL-13, and IL-5 by T_H2-type cells, causing B cells to switch to IgE synthesis. In addition, IL-5 promotes the differentiation, activation, and persistence of eosinophils in tissue sites. Immunotherapy reduces allergen-induced symptoms, production of inflammatory mediators, and nasal eosinophil and epithelial mast cell numbers. A rise in allergen-specific IgG ("blocking antibody") and a blunting of the seasonal rise in allergen-specific IgE titers also are hallmarks of immunotherapy. These characteristic changes may be due to the stimulation of T_H1 responses (*ie*, IL-2 and interferon- γ production), which promote IgG production by B cells, and/or to the induction of T_H2/T_H0 cell anergy, which diminishes T_H2 responses. These responses at the cellular and molecular levels serve as potential targets for new immunotherapy modalities.

The development of T_H1- and T_H2-type immune responses shortly after birth and during early childhood may determine the progression of allergic disease. Based on a prospective study following 216 children during the first 6 years of life, the incidences of allergic sensitization to inhalant allergens increased with age from 1.5% at the first year to 26% at 6 years [10•]. Marked increases in sensitization rates were observed mainly after the third year. A hypothesized reason for the global increase in allergic diseases is a failure in the natural conversion from the T_H2 to T_H1 phenotype due to a lack of antigenic stimulation in clean environments (the so-called hygiene hypothesis). An important question is whether immunotherapy can prevent this progressive disease or allergy march either by preventing sensitization to

allergens related to the asthma or by preventing the inflammation caused by allergen exposure. If it can, it may do so by providing a missing environmental exposure that previously drove the phenotypic conversion.

The concept of using immunotherapy as prophylaxis against further sensitization is best illustrated in the study of Des Roches *et al.* [8••], who showed that 10 of 22 asthmatic children younger than 6 years receiving immunotherapy to standardized mite vaccines for 3 years developed new sensitivities, while all of the children in the control pharmacotherapy treatment group did. Preliminary results of a European multicenter preventive allergy treatment study showed that in children aged 5 to 13 years with rhinoconjunctivitis receiving immunotherapy to birch and/or Timothy grass pollen vaccines, only 24% developed asthma symptoms. In contrast, 51% of the children in the control group receiving only pharmacotherapy developed asthma symptoms [11]. Thus, it appears likely that intervention with immunotherapy early in childhood can reduce both the risk of developing new sensitization to environmental allergens and the allergic inflammation caused by allergen exposure.

Patient Selection

As mentioned above, immunotherapy traditionally has been an adjunct to environmental control and pharmacologic treatment. Aggressive use of environmental control along with pharmacotherapy, for example, was shown to provide sufficient control of allergic asthma in children, so that immunotherapy did not offer an additional benefit [12•]. Such treatment is not achievable under real-world circumstances, however, because of the high rate of patient noncompliance. For that reason, it may be necessary to initiate immunotherapy in children with poorly controlled asthma who do not take their medications reliably or alter their environment adequately. Another potential benefit is the possibility of altering the natural history of the disease with early use of immunotherapy.

The indications for inhalant immunotherapy include 1) the presence of allergic rhinitis or allergic asthma, 2) documented IgE-mediated sensitivity to allergens associated with symptoms, 3) the inability to adequately avoid exposure to the relevant allergens, and 4) the availability of an allergenic extract for the antigen suspected of causing the symptoms. In addition, environmental control and pharmacotherapy should be recommended prior to initiation of immunotherapy. There is no age below which immunotherapy should not be provided; however, children under 2 years frequently have not had sufficient time and exposure to develop allergic sensitivities. In addition, since this form of treatment carries with it a risk of anaphylaxis, it is important that adequate communication be possible with the patient. For that reason, it is unwise to initiate immunotherapy in patients younger than 3 or 4 years. Since it also is desirable to prevent progression of the

allergy as described above, immunotherapy should be initiated as soon as it is feasible.

Antigen Selection

Most extracts used in immunotherapy are derived from pollens of trees, grasses, and weeds, as well as molds, animal dander, dust mites, and stinging insects of the order Hymenoptera. Since immunotherapy is specific for the antigen used, all relevant antigens need to be included in the treatment extract because any omission may decrease its effectiveness. It also is important that each constituent in the mixture is present at a high enough concentration for the immunotherapy to be effective. For that reason, when a large number of allergens are suspected to be relevant, a decision has to be made regarding the relative importance of each based on knowledge of the aerobiology of the region in which the patient lives.

The significance of indoor allergen exposure is more difficult to determine, since it is difficult to identify allergens for a particular patient. A history of a furry animal in the house, water damage with mold, or a history of insect infestation may be helpful in estimating a patient's exposure. Though it is possible to send a sample of house dust from the patient's home to a reference laboratory for allergen measurement to confirm exposure, reliance on allergen sensitivities alone to identify relevant antigens may be the only method available.

A number of factors must be considered when determining which antigens to include in an extract, including cross-reactivity, the need for an optimal dose of each constituent, and potential interactions among different extract types when mixed. Cross-reactivity is the tendency of different extract constituents to be recognized as the same or similar by the patient's immune system. When large numbers of allergen vaccines are prescribed for immunotherapy, the possibility increases for disproportionately high or low concentrations of some cross-reacting allergen groups to be included. Therefore, it usually is not desirable to include two cross-reacting antigens in the same mixture. There may be increased risk of adverse reactions toward cross-reactive allergens present in excess, and, conversely, deficiencies in other allergen components may reduce the effectiveness of treatment. Significant evidence now exists in the literature to support the use of only a limited number of representative allergens from a cross-allergenic group for allergy diagnosis and immunotherapy [13,14]. Some of the more important cross-reacting allergen groups are listed in Table 1. The application of this information could simplify formulation practices by significantly reducing the number of allergen products required for allergen immunotherapy. In general, the patterns of allergenic cross-reactivity among pollen and possibly fungal species follow their taxonomic relationships.

Allergen products frequently contain active enzymes that are responsible for reducing the allergenic activity of

Table I. Cross-reacting pollen allergen groups*

Pollen type	Cross-reacting group	Representative genera
Grass	Pooideae	<i>Poa</i> (bluegrass), <i>Bromus</i> (brome), <i>Dactylis</i> (orchard), <i>Festuca</i> (fescue), <i>Lolium</i> (perennial rye), <i>Agrostis</i> (redtop), <i>Anthoxanthum</i> (vernal), <i>Avena</i> (cultivated oat), <i>Holcus</i> (velvet), <i>Phalaris</i> (canary), <i>Phleum</i> (Timothy), <i>Agropyron</i> (quack), <i>Elymus</i> (wild rye), <i>Secale</i> (cultivated rye), <i>Triticum</i> (cultivated wheat)
	Chloridoideae	<i>Cynodon</i> (Bermuda), <i>Bouteloua</i> (grama), <i>Distichlis</i> (salt)
	Panicoideae	<i>Paspalum</i> (Bahia), <i>Sorghum</i> (Johnson), <i>Panicum</i> (Para), <i>Zea</i> (corn)
Tree	Aceraceae	<i>Acer</i> (maples and box elder)
	Betulaceae	<i>Alnus</i> (alder), <i>Betula</i> (birches), <i>Corylus</i> (hazelnut)
	Cupressaceae	<i>Cupressus</i> (cypress), <i>Juniperus</i> (junipers and cedars), <i>Taxodium</i> (bald-cypress)
	Fabaceae	<i>Acacia</i> (mimosa), <i>Robinia</i> (locust), <i>Prosopis</i> (mesquite)
	Fagaceae	<i>Quercus</i> (oaks), <i>Fagus</i> (beech)
	Juglandaceae	<i>Carya</i> (hickory and pecan), <i>Juglans</i> (walnut)
	Moraceae	<i>Morus</i> (mulberry), <i>Broussonetia</i> (paper mulberry)
	Oleaceae	<i>Olea</i> (olive), <i>Fraxinus</i> (ash), <i>Ligustrum</i> (privet)
	Pinaceae	<i>Pinus</i> (pines)
	Platanaceae	<i>Platanus</i> (sycamore)
	Salicaceae	<i>Populus</i> (cottonwood and poplars), <i>Salix</i> (willows)
	Ulmaceae	<i>Ulmus</i> (elms)
	Weed	Chenopodiaceae
Asteraceae: Artemisia		<i>Artemisia</i> (mugworts, wormwood, and sages)
Asteraceae: Ambrosia		<i>Ambrosia</i> (ragweeds), <i>Xanthium</i> (cocklebur), <i>Iva</i> (poverty weed and marsh elders)
Amaranthaceae		<i>Amaranthus</i> (careless weed and pigweeds), <i>Acnida</i> (Western water hemp)
Plantaginaceae		<i>Plantago</i> (plantain)
Polygonaceae		<i>Rumex</i> (dock and sorrel)

**Eupatorium* (fennel), *Hymenoclea* (burrobush), *Urtica* (nettle), *Cocos* (Queen palm), *Tamarix* (salt cedar), *Myrica* (bayberry), and *Casuarina* (Australian pine) were excluded from the table because of their high specificity, their limited diversity, or lack of information regarding their cross-allergenicity.

therapeutic mixtures. Though the therapeutic efficacy of allergen products is not necessarily related to their allergenic activity, these changes make it difficult for manufacturers to ensure product quality and stability. Products containing high concentrations of proteolytic enzymes were implicated in reducing the allergenic potency of therapeutic mixtures [15]. *Aspergillus*, for example, was shown to reduce the IgE-binding activity of Timothy grass when the two were mixed, though the activity of ragweed was not affected. Preparations derived from insects and fungi tend to have the greatest enzymatic activities, and pollen and animal dander allergens are the most susceptible to their effects [16]. Based on this information, it is desirable for fungus- and insect-derived extracts to be separated from those derived from pollen and animal dander.

Although children have a higher incidence of suspected food allergy, there are no well-controlled studies that support the use of allergen immunotherapy for food hypersensitivity. Data on the effectiveness of allergen immunotherapy in the management of skin and mucous membrane diseases, such as atopic dermatitis, urticaria, and *Candida* vulvovaginitis, are conflicting [17–19].

Routes of Administration

Immunotherapy can be administered by subcutaneous, nasal, sublingual, or oral routes. Subcutaneous immunotherapy has been used for 90 years, and the effectiveness, safety, and specificity of this mode of immunotherapy have been well established since 1965 [4,5•,6•,7,8••,20]. Subcutaneous immunotherapy entails the risk of systemic anaphylactic reactions [21,22]; therefore, injections should be given only by physicians who are experienced in the administration of allergen vaccines and the emergency care of anaphylaxis or by trained health care professionals under the guidance of an allergy specialist. Risk factors for systemic reactions during immunotherapy include sensitivity of the patients, asthma, type of allergens used, and the rate of dosage increases during the build-up phase. Pre-medication with oral corticosteroids and the combination of an H₁ and an H₂ antagonist may be used to reduce systemic reactions during immunotherapy, especially for rush or cluster regimens [23].

Local nasal, sublingual, and oral routes for immunotherapy also may be effective in reducing symptoms of pollen- and mite-induced rhinitis in children as well as adults [24–32]. Allergens administered orally may be destroyed by gastric digestion and therefore must be administered at

Table 2. Approved, pending, and candidate-standardized allergen extracts

Currently available standardized extracts approved by the FDA	Cat hair, cat pelt, <i>Dermatophagoides farinae</i> , <i>Dermatophagoides pteronyssinus</i> , short ragweed, Hymenoptera venoms (honey bee, yellow hornet, white-faced hornet, yellow jacket, paper wasp), and grass pollens (Bermuda grass, red top, Kentucky bluegrass, meadow fescue, sweet vernal grass)
License applications pending approval by FDA	Cockroaches (American, German, oriental), latex extract
Future candidates for standardization*	Johnson grass, bahia grass, giant ragweed, lamb's quarter, plantain, Russian thistle, mugwort, pigweed, oak, box elder/maple, elm, mountain cedar, ash, birch, <i>Alternaria</i> , <i>Aspergillus</i> , <i>Penicillium</i> , fire ant, dog, peanut, egg, milk, shrimp

*Based on a priority list submitted by the Standardization Committee of the American Academy of Allergy, Asthma, and Immunology [35].
FDA—Food and Drug Administration.

doses at least 100 to 1000 times greater than those required by the subcutaneous route to induce serologic and clinical effects. However, oral immunotherapy with grass pollen extract in enteric-coated capsules has been used effectively as booster therapy after a short course of conventional subcutaneous immunotherapy for priming [28]. In a more recent study, microencapsulated short ragweed pollen extract administered at doses only slightly higher than those used in high-dose subcutaneous immunotherapy reduced symptom-medication scores and blunted the seasonal rise in specific IgE antibodies [30]. In that trial, doses of short ragweed pollen vaccine were administered daily with incremental increases until a maintenance dose of 16 to 24 µg Amb a 1 units was reached. An immunologic and clinical effect was achieved in about 7 weeks after starting immunotherapy.

The risk of inducing systemic anaphylactic reactions and low patient compliance during subcutaneous immunotherapy has facilitated the use of alternative routes. The use of the sublingual and intranasal routes has been gaining popularity in Europe mainly because of their perceived safety and ease of administration. In the sublingual/swallow method, conventional allergen vaccines in drops are placed under the tongue and held for 2 minutes before being swallowed. The safety of sublingual immunotherapy was established in a study of 472 adults and 218 children with rhinoconjunctivitis and mild to moderate asthma receiving sublingual immunotherapy with a variety of inhalant allergen vaccines [33]. The treatment duration ranged from 4 months to 2 years. Gastrointestinal side effects were significantly more frequent as compared with the placebo group, but no serious systemic reactions were recorded in either the adult or pediatric patients. In trials with sublingual immunotherapy, even when a significant clinical benefit is shown, cytokine changes characteristic of immune deviation are not observed and the characteristic changes in allergen-specific IgG and IgE levels are not consistently detected. Thus, it is possible that the immunologic mechanisms responsible for the clinical effect may be different from those of subcutaneous immunotherapy. Standards for optimal treatment doses, regimens, and duration for the sublingual or oral routes needs to be established.

Use of Standardized Allergen Vaccines

Whenever they are available, standardized allergen vaccines are used in immunotherapy. The majority of allergen vaccines used in clinical practice are still unstandardized, however. The potency of unstandardized extracts are designated in terms of protein nitrogen units or extraction weight per volume. Their stability is based on formulation (eg, glycerinated vs aqueous) and not by validated studies. In contrast, standardized allergen vaccines are labeled with US standards of potency, and their shelf lives are based on the demonstration that they maintain their potency until the product's expiration date [34]. The assignment of potency units may differ among products, and the clinician should consult each product's package insert for information regarding dosage and administration. Current standardized allergen products approved the US Food and Drug Administration are listed in Table 2. The Allergen Standardization Committee of the American Academy of Allergy, Asthma, and Immunology also compiled a list of vaccines that are candidates for standardization on the basis of a survey of academy members to determine which products deserve the highest priority [35].

Based on current evidence for optimal effective doses for allergen immunotherapy, the total number of products that can be added to a therapeutic mixture may be limited, depending on the potency of stock allergen products used and the volume administered to the patient [36].

Standardized allergen products allow for a more accurate target for dosing, while nonstandardized products usually require dosing based on the highest tolerated dose. For many patients, because of occurrence of adverse reactions, dosage adjustments are required. Dosage adjustments of individual allergenic components are not feasible when large numbers of nonstandardized allergen products are included in the treatment course. In these cases, the maximal dose of all allergens in a mixture is defined by the ones to which the patient is most sensitive. Treatment mixtures can be divided into two or more vials to allow for treatment with multiple allergen vaccines and for more control in dose adjustments, especially during the build-up phase of immunotherapy.

Modified Allergens

The use of modified allergens to increase the safety of immunotherapy is a well-established practice. Alum-precipitated allergen vaccines have been commercially available for many years and produce less systemic reactions than do unmodified allergen vaccines [37]. However, long-term use of alum-precipitated allergen vaccines may cause subcutaneous lesions presumably due to a type IV hypersensitivity response to the aluminum component [38]. Allergoids are allergens polymerized by formalin or glutaraldehyde and have reduced allergenicity but maintain their immunogenicity (*ie*, T-cell reactivity). Allergoids can be administered using accelerated dosage regimens, permitting patients to reach maintenance doses in significantly less time with less than 1% occurrence of systemic reactions [39,40]. Another method of reducing allergenicity and maintaining the immunogenicity of allergens is to prepare peptide epitopes. Enzyme-digested allergens or recombinant allergen fragments that have decreased IgE binding but intact T-cell epitopes were shown to suppress allergic symptoms upon allergen exposure [41,42]. The wide use of such products has been limited because of the limited number of products available, the lack of significant increases in efficacy over conventional allergen vaccines, or difficulties in product standardization. Many other methods of allergen modification were shown to be effective in animal models, but none was ever studied extensively in humans. These include urea-denaturation, polyethylene glycol conjugation, and D-glutamic acid:D-lysine polymerization [43].

New Approaches to Immunotherapy

Recent studies on cytokines and DNA vaccines suggested novel approaches to increasing the effectiveness and safety of conventional immunotherapy. The adjuvant activities of these molecules may offer potentially useful strategies. Peptides representing T-cell epitopes of allergens are being used in attempts to dampen T_H2 responses by inducing T_H2/T_H0 cell anergy. Anti-IgE therapy targets 1) the high-affinity IgE receptor on effector cells, such as basophils and mast cells, in an allergen nonspecific manner to prevent its interaction with IgE and 2) the low-affinity IgE receptor on B cells to downregulate the ability of these cells to produce IgE.

IL-12

IL-12, a cytokine that reduces IL-4 production and enhances interferon- γ production, administered simultaneously with allergen or as an allergen IL-12 fusion protein, induces a shift from a T_H2 -type to a T_H1 -type immune response in sensitized mice [44,45]. Significant reduction in IL-4, IL-5, and IgE production was observed, and the administration of IL-12 also prevented allergen-induced eosinophil infiltration into the bronchoalveolar area and reduced inflammatory mediator levels in bronchoalveolar lavage fluids.

Immunostimulatory sequences

Immunostimulatory sequences (ISS) or CpG motifs have been studied as a potential adjuvant for allergen immunotherapy in animal models [46,47]. Allergen- or covalently conjugated ISS-DNA was effective in inducing a T_H1 response and suppressed IgE antibody formation after challenge with allergen. The conjugate also induced high-titer allergen-specific IgG responses. Allergen-ISS conjugates are less allergenic as determined by histamine release assays using human basophils from patients with ragweed allergy, suggesting that this approach to immunotherapy may offer a safe alternative to currently practiced methods. The immune response modifiers resiquimod (R-848) and imiquimod are potent inducers of interferon- γ , IL-12, and tumor necrosis factor- α . R-848 administered orally or subcutaneously showed many of the activities possessed by ISS and also may prove to be useful as vaccine adjuvants [48].

Peptide immunotherapy

The development of allergic disease most likely is initiated and regulated by select T-cell recognition of major histocompatibility complex class II molecules that contain specific 13- to 25-amino acid peptides derived from the larger allergen molecules. The T-cell-recognized determinants, or epitopes, are distinct from those recognized by B cells and antibody including IgE. Specific T-cell anergy, or unresponsiveness, occurs if antigen or allergen is administered under conditions without a second T-cell activation signal. Immunization with T-cell-specific monomeric peptides does not elicit IgE-mediated side effects because these peptides do not bind to IgE; and even if they did, they are too small to cross-link surface IgE molecules. Peptide immunotherapy, therefore, may permit administration of higher, more effective doses of allergen with a lower risk of a systemic reaction.

Successful conventional immunotherapy was correlated with a decrease in allergen-specific T-cell responses to allergens [9]. Peptides containing T-cell-reactive determinants were identified for grass pollen, dust mite, and cat [49]. The efficacy and safety of peptide-fragment immunotherapy were demonstrated with bee venom phospholipase A2 and cat allergen peptides in humans. While human clinical trials with cat allergen peptides did demonstrate reduced sensitivity on exposure to cats in an actively treated group versus placebo, the occurrence of unusual side effects likely will limit the usefulness of the procedure.

Anti-IgE

Monoclonal antibodies directed to human IgE have been produced and humanized by Genentech (South San Francisco, CA). These antibodies (rhuMAb-E25) bind to the Fc portion of human IgE molecules and appear to prevent binding to the high-affinity IgE receptor on mast cells and basophils. Clinical trials with anti-IgE have shown a reduction in allergic sensitivity in patients both with asthma and

allergic rhinitis [50]. This reduction does not appear to be antigen specific. This treatment results in passive immunity since there is no evidence that a sustained immune response occurs after prolonged administration. Anti-IgE therefore needs to be injected at 2 to 4 week intervals for its efficacy to be maintained. The role of anti-IgE therapy for treatment of allergic disease remains to be defined. For example, the possible use of anti-IgE as a pretreatment to conventional immunotherapy to reduce adverse IgE-mediated reactions should be investigated.

Conclusions

The basic approach to allergen immunotherapy has not changed in more than 90 years. Its use, however, has evolved over time to incorporate knowledge gained about the immunochemistry of allergens, the mechanisms of immunotherapy, and the pathophysiology of allergic diseases. The standardization of allergen vaccines and the determination of optimal effective doses have led to more consistent studies demonstrating the efficacy and safety and to more general acceptance of allergen immunotherapy. Allergen immunotherapy will continue to play an important role in the treatment of allergic disease, and recent studies suggest that immunotherapy should be used earlier in allergic patients.

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