Novel Strategies for Allergy Immunotherapy

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Abstract This article reviews the literature on allergen modifications and novel routes of delivery for antigen-specific immunotherapy for allergic disease. Allergen modifications include the use of recombinant proteins, combining antigens with infectious carrier proteins, peptide immunotherapy, and genetic vaccines containing the code for allergenic proteins. Novel routes of delivery include oral immunotherapy, intra-lymphatic immunotherapy, epicutaneous immunotherapy, and oral mucosal immunotherapy. Allergen depot preparations, such as biodegradable, injectable microspheres and sublingual tablets, have also been developed. Current research in immunotherapy for allergic disease has focused on improving efficacy and patient adherence to therapy, while minimizing the risks of serious adverse events.

Keywords Immunotherapy · Peptide · Allergy · Subcutaneous · Sublingual · Delivery · Antigens · Recombinant · Epitopes · Oral mucosa · Asthma · Rhinoconjunctivitis · Antigen-presenting cells

Introduction

Allergy, when combined with its comorbidities, is the commonest chronic disease in the USA [1]. Unlike avoidance strategies and medications, allergen-specific immunotherapy is the only known disease-modifying treatment for allergic rhinitis and asthma [2]. Allergen-specific

immunotherapy involves the regular administration of allergenic extracts, to which the patient is sensitive, at a certain dose for approximately 3–5 years. When antigens interact with memory T cells, a downregulation of the allergic response occurs and the proallergic, T-helper cell type 2 (T_h2) phenotype shifts towards the T-helper cell type 1 (T_h1) phenotype [3]. Increases in the levels of IL-10, interferon- γ , and transforming growth factor β lead to decreased mast cell and eosinophil activation, and class switching results in downregulation of IgE production and increased IgG₄ production [4]. Patients treated with allergen-specific immunotherapy benefit from long-term symptom relief, decreased need for medications, and overall improvement in quality of life [5]. Allergen-specific immunotherapy also reduces the risk of new sensitizations and decreases the risk of asthma development [6, 7].

Since allergen-specific immunotherapy was described by Noon [8] in 1911, the primary delivery method has been through the subcutaneous route. Although it is generally considered safe, there is a risk of systemic reaction, which ranges from 0.05 to 3.2 % of injections [9]. Because of this, as well as the inconvenience of weekly physician visits, fear of needles, and discomfort from local reactions, alternative delivery routes have been sought. In 1998, the World Health Organization endorsed sublingual immunotherapy (SLIT) as an alternative to subcutaneous immunotherapy (SCIT) [10]. SLIT involves the placement of the same extract used in SCIT underneath the tongue once or twice daily. Because of its superior safety profile compared with SCIT, SLIT may be administered by the patient from home [11]. Over the past several years, additional modifications, both in the allergen delivered and in the route of delivery, have been investigated in order to improve the safety of, efficacy of, and adherence to immunotherapy.

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Allergen Modifications

Recombinant Allergens

Recombinant allergens are purified allergens made from allergen-encoding DNA. They were developed to improve standardization and quality control [12•, 13]. Recombinant wild-type allergens mimic the molecular and immunologic properties of natural allergens, and can produce large quantities of proteins, which are naturally in short supply, with consistent quality [12•, 14]. In addition, they can be produced as hybrid molecules that contain epitopes of several different allergens [15]. However, recombinant wild-type allergens share the same potential IgE-related side effects of natural allergens. Recombinant hypoallergens were developed with reduced allergic activity by methods such as introducing mutations into the allergen sequence, deleting sequences, oligomerization, and fragmentation, with an end result of affecting IgE binding, while leaving T-cell epitopes intact [12•, 16].

Recombinant wild-type birch pollen and grass pollens have been developed and tested in clinical studies. Pauli et al. [17] showed SCIT with recombinant wild-type birch pollen (rBet v 1) to be equally efficacious as that with commercial birch pollen extract and natural purified birch pollen allergen (nBet v 1). In this randomized, double-blind, placebo-controlled (DBPC) trial, 134 adults who had birch-pollen-induced rhinitis were randomized into four groups: commercial birch pollen extract, rBet v 1, nBet v 1, and placebo. Treatment consisted of 12 weekly injections followed by monthly maintenance injections for 2 years. There was a 59 % reduction in disease severity in patients treated with birch pollen extract and a 57 % reduction in disease severity in patients treated with rBet v 1 and nBet v 1 compared with placebo. Active treatment induced statistically significant increases in the levels of IgG₁, IgG₂ and IgG₄ Bet v 1 specific antibodies, which were higher in the rBet v 1 group than in the birch pollen and nBet v 1 groups. Wild-type rBet v 1 is also being studied in a phase III SLIT trial with a dissolving tablet (Oralair Birch) [18, 19].

A mixture of five recombinant grass pollen allergens (rPhl p 1, rPhl p 2, rPhl p 5a, rPhl p 5b, and rPhl p 6) has been studied in a randomized, DBPC study [20]. Sixty-two adults were randomized into two groups receiving either the recombinant grass pollen allergen mixture or placebo via SCIT for 20 months. Of those who completed treatment, there was a 39 % reduction in disease severity in actively treated patients compared with placebo. Actively treated patients had a statistically significant increase in the levels of allergen-specific IgG₁ and IgG₄ antibodies, and a significant decrease in allergen-specific IgE levels. However, seven of the 29 actively treated patients had systemic side effects, such as urticaria and asthma exacerbation.

Molecularly modified derivatives of recombinant, hypoallergenic birch pollen (Bet v 1) have been developed either by expressing three copies of the gene to produce a trimer or by cleaving the gene to create two recombinant peptides. Both the recombinant Bet v 1 trimer and fragments have been shown to be 100 times less allergenic by skin testing, while maintaining T-cell reactivity [21]. In a DBPC study, 124 subjects were randomized into three groups receiving placebo, recombinant Bet v 1 trimer, or recombinant Bet v 1 fragments. There were a high number of dropouts, and the results showed a trend towards improved symptoms, but did not show statistical significance in the combined symptom medication score. There were strong allergen-specific IgG₁ and IgG₄ responses in the treatment groups. A folding variant of hypoallergenic birch pollen, rBet v1-FV, has also been developed [22], with a recently completed multicenter, randomized DBPC study [23]. Clinical trials are ongoing using recombinant allergens for peach, crustaceans, dust mites, and nickel.

Peptide Therapy

Prior to class switching, the exogenous antigen is broken down into peptides within the antigen-presenting cell (APC). These peptides are presented on the APC surface by MHC class II molecules. Allergen-specific T cells recognize the T-cell epitope in the peptide, which initiates the secretion of T_H2 cytokines, leading to production of allergen-specific IgE by B cells. This allergen-specific IgE then binds to IgE receptors on the surfaces of mast cells and basophils. IgE antibodies on the mast cell cross-link with a B-cell epitope on the allergen, which causes degranulation and release of inflammatory mediators, resulting in the clinical symptoms of allergy [24].

In peptide immunotherapy, synthetic peptides, which are a fraction of the size of the allergen, contain T-cell epitopes to engage the T cells, leading to tolerance while eliminating the B-cell epitopes to avoid cross-linking of IgE. When T-cell epitopes are presented to T cells by nonprofessional APCs (such as epithelial or endothelial cells) in a noninflammatory condition, antigen-specific tolerance occurs [25]. In vitro studies have shown that regulatory antigen-specific T cells suppress antigen-specific T-cell proliferation, induced by IL-10, which downregulates the T_H2 response to allergens [24, 25]. Like recombinant allergens, synthetic peptides can be produced in a standardized fashion ensuring quality and consistency [26].

Clinical studies of peptide immunotherapy have focused on cat and bee venom allergies [27–29, 30•, 31, 32]. Early studies used T-cell lines from cat-allergic subjects to map T-cell epitopes for Fel d 1, the principal allergen of cat dander, to create two peptides of 27 amino acids each that contained multiple immunodominant T-cell epitopes

(Allervax[®] Cat). In a multicenter, DBPC study of cat-allergic patients who were chronically exposed to cats or in whom SCIT had failed, 133 subjects received placebo, low-dose therapy, or high-dose therapy [27]. All patients treated with Allervax[®] Cat reported an improvement in cat tolerance, whereas pulmonary function improved only in the high-dose group. Twelve patients reported early-onset allergic symptoms, likely secondary to IgE cross-linking, with three patients requiring epinephrine for treatment of systemic symptoms. Late-phase reactions, which are T-cell-mediated, occurred after 30 min but within 24 h more commonly, occurring twice as often in the patients treated with Allervax[®] Cat as in the patients treated with placebo.

Subsequently, smaller peptides, including a mixture of 12 peptides, each 16–17 amino acids in length, have been investigated to avoid the possibility of early-phase reactions [28]. Most recently, a seven-peptide vaccine (ToleroMune Cat or Cat-PAD) from Fel d 1 has been created by analyzing MHC binding in the previously mentioned 12-peptide mixture, and eliminating those peptides that did not bind to a panel of common HLA-DR molecules [29, 30]. This new vaccine also incorporated thioglycerol, which prevents dimerization of peptides containing cysteine residues, thereby decreasing the chance of IgE cross-linking. In a randomized DBPC parallel-group clinical trial, 202 subjects were exposed to cat allergen before and after treatment with either placebo, 6 nmol Cat-PAD every 4 weeks for 12 weeks (four injections), or 3 nmol Cat-PAD every 2 weeks for 12 weeks (eight injections) [30]. There was a significant reduction in the total rhinoconjunctivitis symptom scores after allergen exposure with the 6-nmol dose compared with the 3-nmol dose and placebo. The treatment effect persisted for 1 year after the start of treatment. One limitation of peptide therapy is the degree of polymorphism in a population, producing a large diversity in T-cell peptide epitopes and making it challenging to develop small, specific synthetic peptides.

Adjuvants and Coupled Allergens

Allergen-specific immunotherapy has traditionally used aluminum hydroxide as an adjuvant. Developments have been made to couple allergens with immunostimulatory molecules, often derived from viruses and bacteria, to further activate and modulate the innate immune system [33–37].

The innate immune system provides a nonspecific first line of defense, by recognizing pathogen-associated molecular patterns that are not present on host cells. One example is unmethylated cytosine–guanine dinucleotides (CpG), which are found in bacterial DNA. CpG or CpG-ODN

(synthetic oligodeoxynucleotides that contain CpG motifs) is recognized by Toll-like receptor (TLR) 9, an innate pattern recognition receptor, on B cells, dendritic cells, and other cells, resulting in the induction of T_H1 and regulatory T cells with suppression of the T_H2 response [34]. A randomized, DBPC phase II trial in which 25 adults with ragweed allergy received either a vaccine consisting of Amb a 1, a ragweed-pollen antigen, conjugated to a CpG-ODN allergen immunostimulatory conjugate or placebo before the ragweed season, showed improved nasal symptom scores and overall quality of life scores in the actively treated group compared with the placebo-treated group during two ragweed seasons [35]. Although the phase III trial was discontinued when these results were not confirmed, CpG immunostimulatory sequences are still being included in the development of other vaccines, including DNA vaccines.

Other TLR ligands that have been shown to induce the T_H1 pathway and inhibit the T_H2 pathway include lipoproteins, polyinosinic–polycytidylic acid, and lipopolysaccharides [38]. Monophosphoryl lipid A, a derivative of lipid A from *Salmonella* Minnesota R595 lipopolysaccharide, is a TLR-4 agonist. Pollinex Quattro vaccines combine chemically modified grass, ragweed, or tree allergens with monophosphoryl lipid A [39]. Clinical trials in adults and children have shown symptom improvement [40–42]. Pollinex Quattro is available in Europe, and the FDA recently lifted a clinical hold, allowing a phase III study to proceed in the USA [43].

Other viral and bacterial proteins or by-products also have the ability to skew the immune system towards a T_H1-dominated pathway. Both virus-like particles, which resemble viruses but do not contain viral genetic protein, and viral capsids have been studied. CYT003-QbG10 is an A-type CpG-ODN packaged into a virus-like particle without an allergen [44]. In a randomized, double-blind phase IIb study, 299 patients with perennial allergic rhinoconjunctivitis caused by house dust mite (HDM) allergy received six weekly injections of low-dose CYT003-QbG10, high-dose CYT003-QbG10, or placebo. The treatment was well tolerated, and symptoms and overall quality of life improved in patients treated with high-dose CYT003-QbG10 compared with placebo. Also, the high-dose group tolerated a tenfold improvement on the conjunctival provocation test, whereas the placebo group experienced no change [44].

Combination allergen–infectious disease vaccines, coupling an allergen with a viral capsid, represent an innovative direction in immunizing children against both common viral infections and allergens. A vaccine composed of viral capsid protein (VP1) of human rhinovirus and a peptide derived from major timothy grass pollen allergen (Phl p 1) has been studied in a mouse model, showing a VP1-specific

T_H1 response, inhibited basophil degranulation, and immunity to rhinovirus exposure [45].

Biodegradable microspheres, such as poly(D,L-lactico-glycolic acid), can be used to encapsulate an allergen for delivery via injection or ingestion. The benefit of microsphere delivery is a controlled release of the allergen over a period of time, which may result in less frequent treatments, as well as avoiding the safety risks associated with subcutaneous bolus injections of an allergen [46].

Genetic Vaccines

DNA vaccines have been shown to produce an antigen-specific T-cell immune response, and have been extensively studied in the realm of antiviral and cancer therapy. Within the field of allergy, DNA vaccination has been shown in a mouse model to modulate IgE response and shift the immune system towards a T_H1 -dominated pattern, thereby limiting the clinical presentation of allergy [47, 48, 49, 50].

The mechanism of DNA vaccination is in vivo transfection, whereby plasmid DNA encoding an allergen is injected into the skin or muscle. The DNA is then taken up by APCs, as well as non-APCs (epithelial cells, muscle cells), where it is transcribed and translated into the allergen. The endogenously produced allergen is presented on the APC surface by MHC class I and class II molecules, leading to antigen-specific $CD4^+$ and $CD8^+$ T-cell responses, in a T_H1 -biased manner. This results in secretion of interferon- γ , induction of cytotoxic T lymphocytes, B-cell proliferation, and class switching to IgG, which competes with IgE. Immune complexes of IgG and allergen also serve to activate APCs and amplify the immune response. In addition, the uptake of plasmid DNA and expression of foreign antigen may cause apoptosis in the transfected cell, which again contributes to T_H1 -biased immunity [47].

Genetic vaccination experiments have also used recombinant technology and peptide therapy research to produce hypoallergenic DNA vaccines. Theoretical risks associated with DNA vaccines include side effects from long-term, uncontrolled expression of the encoded antigen, possible generation of anti-DNA antibodies, autoimmunity, and inclusion of the DNA into the genome, causing potential oncogenic consequences [49]. RNA vaccines, which degrade more quickly within the cell and cannot be incorporated within a genome, were developed to address these concerns. In a mouse model, RNA vaccines have been shown to induce T_H1 -weighted immune responses similar to those induced by DNA vaccines [51, 52].

Novel Routes of Delivery

Oral Immunotherapy

SCIT is not recommended for the treatment of food allergy because of a high rate of systemic reactions with peanut allergen injection [53]. Oral immunotherapy (OIT) has been shown to be a safer route for desensitization, with an increasing body of literature over the last 5 years. Peanut [54, 55, 56], egg [57–59], and milk [60, 61, 62] are the antigens which have been most studied.

One recent multicenter, DBPC trial examined 28 children with peanut allergy who received OIT with peanut flour or placebo over 1 year in three phases (initial escalation, buildup, and maintenance) [54]. After 1 year of therapy, an oral food challenge was performed. Although three of 19 subjects receiving peanut OIT withdrew early because of adverse effects, the remaining 16 subjects were able to ingest a maximum cumulative dose of 5,000 mg (approximately 20 peanuts), compared with the nine patients receiving placebo, who ingested a median cumulative dose of 280 mg, or one peanut. The OIT patients also exhibited a decrease in skin reactivity, decreased T_H2 cytokine production, and increases in the levels of peanut-specific IgG and IgG₄.

Safety and permanent tolerance are the main limitations of OIT. Many of the clinical trials performed exclude subjects who have had anaphylactic reactions to the food [54, 55–59, 60, 61, 62]. Side effects during immunotherapy, although usually mild, are largely unavoidable. Yu et al. [56] reported on the safety of peanut OIT, with 84 % of all symptoms reported as mild, requiring no treatment or treatment with antihistamines. However, severe reactions do occur, often after exercising, bathing, OIT on an empty stomach, concurrent febrile illness, or poorly controlled asthma [54, 55]. As such, OIT still requires periods of close observation or inpatient stay during periods of escalation.

Developments are currently being made to improve safety. OIT using extensively heated milk or egg in children with milk or egg allergies has been shown in clinical trials to be effective in increasing tolerance [63, 64]. Tolerance is defined as a permanent unresponsiveness to an allergen after therapy has been completed, whereas desensitization requires continued therapy to maintain unresponsiveness. Few studies have been able to demonstrate tolerance, especially for long periods of time. Keet et al. [60] reported an open-label trial whereby 30 children with cow's milk allergy were randomized into three groups: SLIT alone, SLIT followed by OIT to a target of 2 g milk protein (OITA), or SLIT followed by OIT to a target of 1 g milk protein (OITB). After 60 weeks of maintenance therapy, one of ten patients in the

SLIT-alone group, six of ten patients in the SLIT/OITB group, and eight of ten patients in the SLIT/OITA group were not reactive to challenge with 8 g of cow's milk protein. These 15 patients were taken off therapy and after 1 week were given another food challenge. Two patients (from the SLIT/OITB group) failed the food challenge. The 13 patients who passed underwent another food challenge 5 weeks later, where four patients failed (one from the SLIT/OITB group and three from the SLIT/OITA group). This demonstrates that although SLIT and OIT showed efficacy with desensitization to cow's milk allergy, tolerance after therapy was not easily achieved, and further studies with longer periods of follow-up are necessary.

Intralymphatic Immunotherapy

Intralymphatic injections of anticancer therapy and antiviral vaccines have demonstrated strong immune responses to small amounts of antigen in animal models and humans [65–67]. The adoption of intralymphatic injection in allergen-specific immunotherapy has many potential benefits, including enhanced immunogenicity by direct injection, decreased number of systemic side effects, reduction in the treatment dose, and a decrease in the overall length of therapy [65].

In an open-label study, 165 patients with grass-pollen-induced rhinoconjunctivitis were randomized to receive either SCIT with pollen extract over 3 years or three intralymphatic injections over 2 months. Intralymphatic immunotherapy (ILIT) was found to induce allergen tolerance faster than SCIT (4 months vs 1 year) and was found to cause fewer allergic side effects [68]. ILIT-induced tolerance was also found to be as long-lasting as SCIT.

More recently, a randomized, DBPC trial compared ILIT with a modified recombinant major cat dander allergen (MAT-Fel d 1) with ILIT with placebo in 20 cat-allergic patients [69•]. MAT-Fel d 1 was modified to enhance presentation through the MHC class II pathway. Subjects received three injections of MAT-Fel d 1 or placebo over 3 months. ILIT with MAT-Fel d 1 increased nasal tolerance by 74-fold, compared with placebo, which increased nasal tolerance by threefold. ILIT with MAT-Fel d 1 also stimulated regulatory T-cell responses and increased the levels of cat-dander-specific IgG₄ [69•].

Epicutaneous Immunotherapy

Epicutaneous immunotherapy (EPIT), or transcutaneous immunotherapy, offers a promising noninvasive route of allergen delivery [65]. The repeated application of adhesive skin patches effectively strips the skin of the stratum corneum, allowing penetration of the allergen into the

epidermis [65, 70]. This process also stimulates the release of proinflammatory cytokines by keratinocytes [71].

Senti et al. [72] reported a DBPC trial of 132 patients with grass pollen rhinoconjunctivitis, randomized to receive six weekly patches of placebo or low, intermediate, or high doses of allergen. Only the high-dose group showed a statistically significant reduction in hay fever symptoms of 30 % in the first year and of 24 % in the second year of follow-up. The higher dose was also associated with more adverse events, most commonly pruritus, erythema, and eczema at the site of the patch. There was also an 8.3 % dropout rate because of systemic side effects.

Viaskin[®] is an epicutaneous delivery system that allows delivery of allergen into the epidermis without disrupting the stratum corneum [73]. The allergen is placed on a polyethylene membrane within an occlusive chamber that adheres to the skin. Perspiration releases the allergens, which diffuse through the epidermis to the APCs [73]. EPIT testing with pollen, HDM, ovalbumin, and peanut in a mouse model has demonstrated efficacy similar to that of SCIT [73]. In a study of 18 children with cow's milk allergy randomized to receive either active epicutaneous delivery with cow's milk powder or placebo, safety was demonstrated, with a trend towards clinical efficacy [74]. A multicenter, DBPC phase II study (NCT01197053) of EPIT in children with peanut allergy is currently under way [75].

Allergy Immunotherapy Tablets

Over the past decade, tablets containing freeze-dried allergenic proteins have appeared on the market. They are designed to disintegrate immediately when interacting with saliva in the sublingual space, thus exposing amino acids and polypeptides to Langerhans cells in the oral mucosa. The potential advantages of allergy immunotherapy tablets (AIT) over liquid SLIT preparations are the standardization of dosing and easier application of extract proteins to the mucosa. The first AIT was Grazax[®], developed in Europe by ALK-Abello (Horsholm, Denmark). In Europe, grass pollen is associated with over 50 % of allergic rhinitis cases, being the commonest cause of respiratory allergy [76]. Grazax[®] contains 75,000 standardized quality tablet (SQ-T) units, which corresponds to approximately 15 µg of the major antigen, *Phleum pratense*, and has demonstrated the best efficacy-to-tolerability ratio [77, 78].

Several multicenter, randomized, DBPC phase II and phase III trials have been completed demonstrating the safety and efficacy of AIT with Grazax[®] for seasonal allergic rhinoconjunctivitis in both preseasonal and co-seasonal therapy, as well as with 3 years of continuous use [79, 80•]. Grazax[®] and a five-grass-pollen tablet (Oralair, Stallergenes, Antony, France) have demonstrated reductions in symptom scores of 37 % and reductions in

medication scores of 53 % [81]. Significant symptom reduction scores have been reported in pediatric patients with grass-pollen-induced rhinoconjunctivitis [82•]. With use of a timothy grass tablet containing 75,000 standardized quality tablet units developed by Schering-Plough/Merck, similar safety and efficacy for grass AIT was determined for North American children, adolescents, and adults who began treatment 16 weeks before the season started and continued treatment during the season [83, 84]. Local side effects, such as oral itching, mild swelling, and throat irritation, were common, particularly at the beginning of therapy. However, postmarketing surveillance data for Grazax[®] demonstrated a rate of systemic reactions of 0.7 per 1,000 treatment years [85]. Asthma prevention trials are also currently under way for grass AIT.

Other AIT products currently being developed include HDM, ragweed, birch pollen, Japanese cedar, and cat dander. An HDM tablet is currently in phase III trials. Two studies performed on children, adolescents, and adults with allergic rhinoconjunctivitis and asthma demonstrated that the HDM tablet was well tolerated [86, 87]. The side effects were similar to those resulting from grass AIT, but severer adverse reactions, such as vomiting, mouth edema, and throat tightness, were also noted. In addition, treatment with dust AIT at the highest dose for 40 weeks led to a significant reduction in inhaled corticosteroid use after 1 year. A similar tolerability profile was noted for a ragweed tablet, which is currently in phase III trials [88•].

Oral mucosal immunotherapy is an attractive alternative because of the higher density of Langerhans cells in the vestibule and gingiva compared with the sublingual mucosa [89]. Current testing is under way on toothpaste preparations containing oak, birch, and cat allergens (AllerDent, Allovate, New York, NY, USA).

Conclusions

Therapeutic approaches to immunotherapy are primarily aimed at reducing the risk of adverse events, while maintaining or improving immunogenicity and ultimately achieving tolerance. Several different antigen modifications are being developed along with innovative routes of delivery. Although this review has highlighted separate approaches, the combination of these approaches is at the forefront of allergen-specific immunotherapy research today.

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- Of major importance

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