

Utility and Comparative Efficacy of Recombinant Allergens Versus Allergen Extract

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Abstract Allergy immunotherapy (AIT) is the only disease-modifying therapy for the treatment of allergic diseases. Although its efficacy and utility are well-established, the potential for serious adverse events, cumbersome and lengthy treatment protocols, and variability of natural allergen preparations have limited its widespread application. Recent advances in recombinant technology have opened new avenues for the development of AIT vaccines. The purpose of this review is to highlight recent evidence on the use of novel recombinant vaccines and review the mechanisms, efficacy, safety, and limitations of AIT. Emerging evidence suggests that recombinant vaccines may provide a viable treatment alternative that improves on the limitations of natural extract therapy while maintaining efficacy.

Keywords Allergy immunotherapy · Recombinant allergy vaccines · T cell epitope therapy · Mechanisms of allergen immunotherapy · Mechanisms of recombinant allergy immunotherapy · Carrier-bound B cell epitopes · Hypoallergenic vaccines · Adverse effects of immunotherapy

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Introduction

Allergic diseases have increased in prevalence during the last century in both children and adults [1]. In particular, allergic rhinitis and asthma, which may be seasonally exacerbated or present throughout the year, are frequent causes of concern for patients. Although avoidance may be pursued for certain allergens, additional pharmacologic therapies are often required for symptom management. Allergen-specific immunotherapy (AIT) remains the only intervention with the potential to modify the disease process and offer a long-lasting therapeutic effect, even after cessation of treatment. First introduced over a century ago by Noon and Freeman [2, 3], AIT involves repeated administration of the sensitizing allergen resulting in a disease-modifying effect that exceeds the treatment period [4]. Although other routes of allergen delivery including bronchial, oral, and intranasal have been used, subcutaneous immunotherapy (SCIT) remains the predominant form of administration [5]. In recent years, sublingual immunotherapy (SLIT) protocols have also emerged, with both routes of administration proving to be effective [6].

AIT is indicated for patients who are unable to find symptomatic relief with avoidance or pharmacotherapy alone, or in those with side effects from or a desire to stop pharmacologic therapies, and guidelines supporting its use have been published by multiple organizations [7–9, 10•, 11•]. However, the use of natural allergen extracts as the active component of AIT limits its widespread use. Despite the efficacy of AIT for some allergen sources, results are less convincing for other allergens. Moreover, not only is treatment time-consuming and fraught with the potential for serious side effects, treatment outcomes can be unpredictable and often vary from patient to patient. Use of recombinant allergens is being examined as an alternative to natural extracts to improve treatment options for allergic patients.

In this review, we will highlight what is known about the mechanism of action of natural extract AIT along with its efficacy, limitations, and risk for adverse reactions. We will discuss more recent approaches to AIT using recombinant vaccines as a method to address these limitations. Specifically, we will review and evaluate the evidence using various types of recombinant vaccines to draw comparisons between the risks and benefits of natural extract AIT and recombinant AIT (Table 1). Although recombinant technology has been used to design AIT vaccines for aeroallergens, venoms, and foods, in this review, we will focus predominantly on studies using aeroallergen vaccines.

Mechanisms of AIT

CD4⁺ naïve T cells differentiate into distinct T cell subsets and effector cells depending on the cytokines, molecules, and cells present in the microenvironmental milieu [39]. During the initial sensitization phase, differentiation and clonal expansion of IL-4 and IL-13 producing allergen-specific Th2 cells essential for the induction of B cells producing allergen-specific IgE antibodies occurs [39]. Once a Th2 shift is established, sensitization and development of allergen-specific memory cells followed by inflammation and tissue injury caused by effector cell action ensues. In the context of allergy, these dysregulated inflammatory responses are due to a failure of immunoregulatory mechanisms that suppress reactions to harmless substances in the periphery. Indeed, non-allergic individuals maintain allergen-specific T regulatory cells (Treg) at a higher frequency than allergen-specific Th1 or Th2 cells; however, in allergy, a marked reduction in IL-10 in comparison with non-allergic subjects in both mouse models and humans is noted [40, 41•]. Therefore, the ultimate goal of allergy treatment is the induction of peripheral tolerance to the offending allergen [42].

Successful AIT is associated with a significant reduction in the immediate response to allergen provocation and late phase response in target tissues, as well as an increase in the allergen concentration required to elicit symptoms as compared to before AIT [41•]. While the mechanisms of AIT are incompletely defined, a significant amount is known about the immunologic modifications resulting in the development of peripheral tolerance to a particular allergen (Fig. 1). These include alterations in the responses and generation of T regulatory and B regulatory (Breg) cells, allergen-specific antibodies, and innate immune cells. In particular, the induction of peripheral T cell tolerance, characterized by the generation of allergen-specific Tregs able to produce anti-inflammatory cytokines such as IL-10 and TGF- β , plays a critical role in suppressing effector T cell responses by decreasing the Th2-driven state [43, 44]. The number of allergen-specific Bregs, which regulate the development, proliferation, and maintenance of CD4⁺

T cells as well as support Treg differentiation, also increases [41•, 45]. Breg-derived IL-10 also decreases IL-4-induced IgE-class switching and increases IL-4-induced IgG antibody production [39, 46]. Early in the course of treatment, AIT induces transient increases in IgE levels before eventually declining, whereas increases in specific IgG4 levels accompany clinical improvement [47]. Allergen-specific IgG4 serves as a tolerogenic antibody by competing with allergen-specific IgE binding, altering the heavy chain structure through Fab arm exchange to form monovalent IgG4 antibodies unable to cross-link allergens, and by its inability to activate complement [48]. AIT also affects mast cells and basophils by increasing their threshold for degranulation [41•].

Efficacy and Risk for Adverse Reactions with Natural Extract AIT

AIT is an effective treatment option in children and adults for multiple conditions including allergic rhinitis and conjunctivitis, allergic asthma, stinging insect hypersensitivity, and more recently atopic dermatitis [9, 49]. Meta-analyses support its use in allergic rhinitis leading to decreased need for pharmacotherapy and improved symptoms, as well as improved asthma symptoms with decreased bronchial hyperreactivity [14, 50–52]. AIT for pollen, specifically grass and birch, decreases the development of asthma in children with allergic rhinitis [53]. AIT may also prevent the onset of new sensitizations, especially in monosensitized individuals [54, 55]. The duration of treatment typically ranges from 3 to 5 years, although significant variability in dosing schedules exists. Even after discontinuation, AIT has a duration of efficacy of between 7 to 12 years [47]. The quality of the allergen extract impacts both diagnosis and treatment of allergic disease; but due to variations in the source material, allergen extracts prepared from natural allergen sources often vary greatly in their potency, stability, and level of contamination with allergens from other sources [56]. Methods for extract standardization to maintain batch-to-batch consistency have been proposed, and several allergens including house dust mite, cat, grass pollens, short ragweed, and hymenoptera venoms are standardized [57]. However, there are barriers to standardizing all products, including cost, regulatory factors, and differing in-house reference values among different companies [10•, 58]. Thus, considerable variation in non-standardized extracts remains a significant concern with regards to patient safety and efficacy [6, 58].

Adverse reactions to allergen immunotherapy are well-known and can be fatal. Several factors can influence the incidence of systemic reactions, including the rate of induction, use of a rush protocol, administration of premedication, degree of sensitization, and presence of asthma. The severity and type of reaction often vary depending on the route of administration, as well as the protocol used to advance

Table 1 Studies evaluating safety and efficacy of AIT with recombinant aeroallergens

Study	Allergen	Molecules studied	Control	Study type	Outcome with treatment
Recombinant wildtype					
Linhart et al. [12, 13]	Timothy grass	Hybrid with rPhl p 1, 2, 5, and 6	Individual Phl p peptides	In vitro assays and animal models	Stronger T cell responses, stronger and higher IgG1 responses, and IgG blocking immediate allergic reaction in mice and rabbits
Jutel et al. [14]	Timothy grass	Mixture of rPhl p 1, 2, 5a, 5b, 6	Placebo	RDBPC	↓ in symptom medication scores, ↑ RQLQ, ↑ IgG4
Pauli et al. [15•]	Birch	rBet v 1	Natural Bet v 1, birch pollen extract, placebo	Multicenter, RDBPC	↓ symptom and medication scores, ↓ skin sensitivity, ↑ IgG, no severe systemic adverse events with rBet v 1
Purohit et al. [16]	Birch	rBet v 1 trimer, mixture of two rBet v 1 fragments	Placebo	Three-arm, DBPC	Trend towards improvement in symptoms, strong IgG1 and IgG4, local reactions more common in trimer group, systemic reactions more common with fragments
Klimek [70]	Timothy grass	Mixture of rPhl p 1, 2, 5a, 5b, and 6	Placebo	RDBPC, dose-ranging safety study	No major side effects at doses up to 120 µg
Recombinant hypoallergenic					
Niederberger et al. [17]; Reisinger et al. [18]	Birch	rBet v 1 trimer, rBet v 1 fragments	Placebo	RDBPC, parallel group study	↓ symptoms and skin sensitivity, ↑ IgG, decreased rise in IgE on birch pollen exposure; ↑ IgG4 antibodies in serum and nasal secretions, ↓ nasal sensitivity to nBet v 1
Kahlert et al. [19]	Birch	Folding variant of rBet v 1	Natural Bet v 1, rBet v 1	In vitro	↓ IgE-binding inhibition assays, ↓ basophil activation, ↓ histamine release
Chen et al. [20]	Dust mite	Hybrid with Der p 1 and Der p 2 fragments	Natural Der p 1, rDer p 2	In vitro and animal models	Induced similar T cell proliferation vs. wild-type allergens, ↑ IgG antibodies in rabbits
Meyer et al. [21]	Birch	Folding variant of rBet v 1 at different doses	Placebo	DBPC	↓ symptoms, ↑ IgG at all doses, one EAACI grade II reaction in 160 µg, and one in 320 µg groups
Linhart et al. [22]	Timothy grass	Mosaic molecules derived from Phl p 1, 2, 5, and 6	Natural timothy grass pollen extract	In vitro and animal models	↑ IgE-binding inhibition by IgG induced by hypoallergenic molecules vs. wildtype allergen or extract-based vaccine
T cell epitope					
Norman et al. [23]	Cat	Short Fel d 1 peptides (Allervax® CAT)	Placebo	DBPC, parallel study at two centers	↓ nasal and lung symptoms with increasing dose, allergic side effects noted after first injection with high dose but required minimal or no treatment
Simons et al. [24]	Cat	Synthetic Fel d 1 peptides IPC-1 and IPC-2	Placebo	RDBPC, parallel study	Peptide immunotherapy did not reduce immediate- or late-phase reactivity or modify cat antigen-specific cytokine production
Maguire et al. [25]	Cat	Short Fel d 1 peptides (Allervax® CAT)	Placebo	Multicenter, RDBPC	Improvement in pulmonary function (FEV 1) and tolerance to cat, three patients required systemic epinephrine
Oldfield et al. [26]	Cat	Short, overlapping peptides derived from Fel d 1	Placebo	RDBPC	↓ late reaction, ↓ early reaction, ↓ INF-γ, ↓ IL4, ↓ IL13, ↑ IL-10
Worm et al. [27]	Cat	SPIRE Fel d 1 (Cat-PAD)	Placebo	RDBPC	No serious adverse events with intradermal or subcutaneous injection
Patel et al. [28], Couroux et al. [29]	Cat	SPIRE Fel d 1 (Cat-PAD)	Placebo	RDBPC, parallel group	↓ TRSS persisted for 2 years after initial therapy

Table 1 (continued)

Study	Allergen	Molecules studied	Control	Study type	Outcome with treatment
Ellis et al. [30•]	Grass allergen	SPIRE Cyn d 1, Lol p 5, Dac g 5, Hol 1 5, and Phl p 5	Placebo	Multicenter, RDBPC	↓ TRSS 4 months after therapy
Contiguous overlapping peptides					
Pellaton et al. [31]	Birch	Bet v 1 (AllerT)	rBet v 1	Single center, open, and controlled	↓ IgE binding and absent basophil activation in mice; no reactivity to skin prick testing in humans
Spertini et al. [32•]	Birch	Bet v 1	Placebo	RDBPC, phase IIb	Improvement in RMSMS, quality of life, night time nasal symptom score; higher frequency of adverse events with 100 µg
Carrier-bound B cell epitope vaccines					
Focke [92]	Birch	B cell epitope-derived Bet v 1	rBet v 1	In vitro and animal models	Lack of allergenic activity, ↑ IgG, inhibition of IgE binding to allergen and blocking basophil degranulation
Campana et al. [33]	Birch	Three different rBet v 1 mosaic proteins	rBet v 1	In vitro and animal models	↓ IgE reactivity and allergenic activity, ↑ IgG comparable to rBet v 1 wildtype allergen
Niespodziana et al. [34]	Cat	Fel d 1-derived peptides fused to hepatitis B PreS	Fel d 1	In vitro and animal models	Lack of IgE reactivity and reduced allergenic activity on basophil activation tests
Marth et al. [35]	Birch	Bet v 1 peptides fused with hepatitis B PreS	Bet v 1	in vitro and animal models	lack of IgE reactivity and allergenic activity, ↑ IgG; ↓ T cell activation, ↓ IL-5, IL-10, ↑ IFN-γ
Banerjee et al. [36]	House dust mite	Der p 23 peptides fused with hepatitis B PreS	Der p 23	In vitro and animal models	Lack of relevant IgE reactivity, ↓ basophil activation tests; ↓ T cell proliferation, ↑ IL-10, ↑ IFN-γ
Focke-Tejkl et al. [37]	Timothy grass	Phl p 1, Phl p 2, Phl p 5, and Phl p 6 fused with PreS	Grass pollen allergen	In vitro and animal models	Lack of allergenic activity, induced T cell proliferation, ↓ release of proinflammatory cytokines, ↑ IgG
Ziegelmayer et al. [38•]	Timothy grass	Phl p 1, 2, 5, and 6 fused with hepatitis B PreS (BM32)	Placebo	RDBPC, phase II	↓ TNSS, ↓ TOSS, ↓ skin prick testing reactions; ↑ IgG, ↓ T cell activation, ↓ basophil activation, no immediate systemic side effects

DBPC double-blind, placebo controlled, *n* natural, *r* recombinant, *RCT* randomized controlled trial, *RDBPC* randomized, double-blind, placebo controlled, *ROLQ* rhinitis quality of life questionnaire, *RMSMS* rhinoconjunctivitis symptom and medication score, *SPIRE* synthetic peptide immunoregulatory epitopes, *TOSS* total ocular symptom score, *TRSS* total rhinoconjunctivitis symptom score

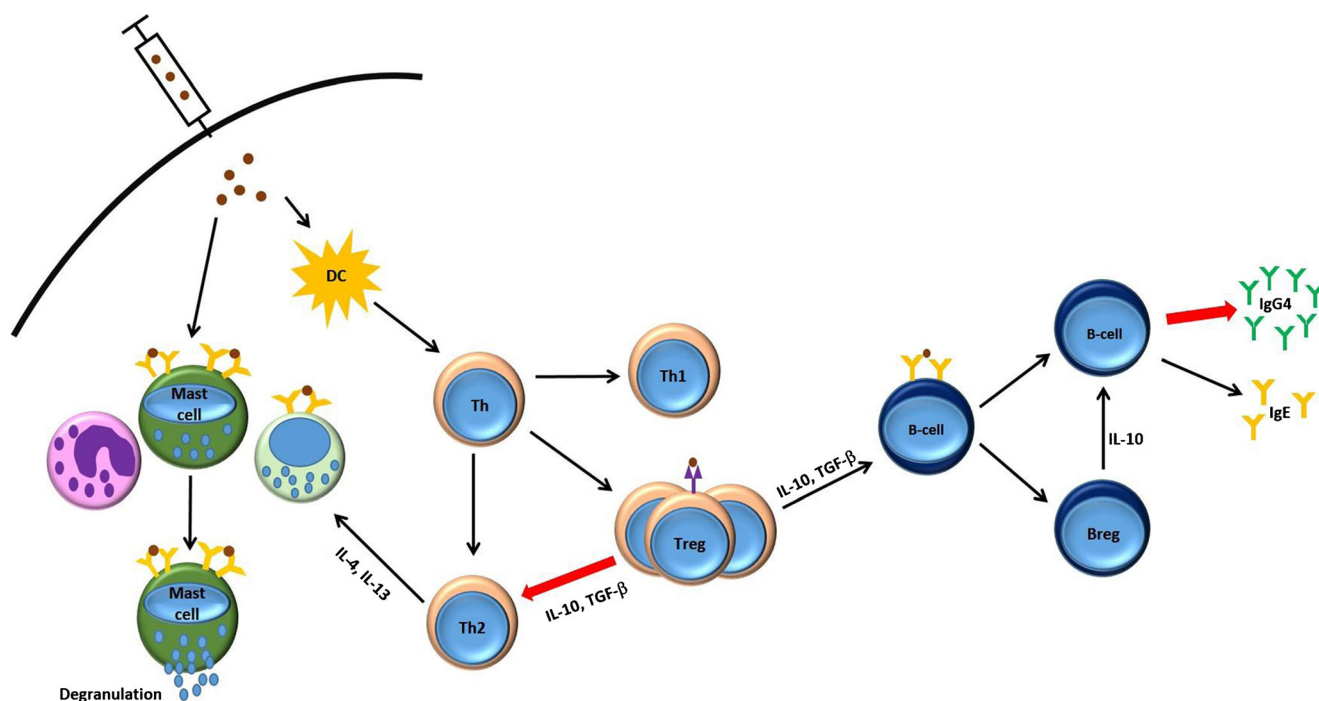


Fig. 1 Summary of sensitization and effects of AIT. Allergen is delivered subcutaneously and taken up by dendritic cells (DC) which present allergen to naïve T cells. In an allergic state, this leads to clonal expansion of allergen-specific Th2 cells which produce IL-4 and IL-13. These cytokines induce antibody class switching to IgE and expansion of allergen-specific B cell populations. IgE antibodies bind to receptors on mast cells, basophils, and Antigen Presenting Cells (APCs). Upon re-exposure, receptor-bound IgE molecules are cross-linked, resulting in

the activation and release of mediators that cause the development of type 1 hypersensitivity reactions. In AIT, continued administration of allergen leads to increased T regulatory (Treg) and Th1 cell populations. Tregs produce IL-10 and TGF- β which decrease effector activity of Th2 cells (red arrow) and also act on B cells. B regulatory cells (Breg) also secrete IL-10 which induces class switching to IgG4 (red arrow) instead of IgE. A shift also occurs in the Th2:Th1 ratio. Th1 cells produce IFN- γ which induces IgG class switching away from IgE

allergen exposure [59, 60]. Reactions are categorized as local or systemic. Local reactions to SCIT occur in up to 82% of patients and can include swelling, erythema, or pain at the injection site [9]. Local reactions to SLIT occur in up to 75% of patients and can include oropharyngeal pruritus or swelling most often occurring during the build-up phase or dose increases [8]. SLIT with aeroallergens has also been associated with development of eosinophilic esophagitis [61]. The risk of life-threatening systemic reactions such as anaphylaxis, particularly with SCIT, is especially concerning. Systemic reactions with SCIT occur at a rate of approximately 0.1% of injections with a near fatal reaction occurring in one per one million injections [62]. Alternatively, systemic reactions occur less frequently with SLIT at a rate near 0.056% per doses administered [63].

With safety and efficacy concerns for current modalities of AIT, attempts have been made to reduce the allergenicity of immunotherapy preparations while maintaining their immunogenicity. Reactions occur due to IgE cross-linking on the surface of mast cells and basophils, followed by degranulation. By using highly reproducible, known allergen epitopes or sequences with decreased cross-reactivity, the desired immunogenic effects can be maintained while simultaneously decreasing adverse reactions [64]. This was first attempted

by chemically modifying allergens to create allergen polymers, called allergoids, which reduce conformational IgE epitopes, while preserving T cell epitopes [65]. Although studies on the use of allergoid SCIT demonstrate its effectiveness [66], concerns that the low allergenicity of allergoids may be associated with reduced immunogenicity remain [65]. Recent advances in recombinant technology allow new avenues to increase efficacy and reduce side effects from allergen immunotherapy.

AIT with Recombinant Allergens

Recombinant allergen-based vaccination strategies arose from a strong need to improve safety and enhance efficacy of AIT. By using the allergen-encoding cDNA, recombinant allergens that exactly mimic the properties of natural allergens can be produced with high purity to yield defined molecules with known molecular, immunologic, and biologic characteristics [67]. Thus, compared to natural allergen extracts, recombinant allergen extracts can be formulated with high quality and precise quantity of relevant allergen, as well as high technical reproducibility of the mixture. [48]. Moreover, the pivotal role of the T cell in driving immune responsiveness to allergen,

together with the distinct differences between T and B cell epitopes, can further facilitate the development of refined therapeutics for redirecting the cellular immune response towards peripheral tolerance [68•]. Tolerance induction is likely dependent on both the immunogenicity and allergenicity of the vaccine used for immunotherapy, which is defined by the specific epitopes present on the macromolecule. B cell epitopes, which are specifically recognized by antibodies, are located on the three-dimensional structure of the antigen molecule, whereas T cell epitopes must first be phagocytosed by antigen-presenting cells (APCs) and presented in the context of MHC II on the cell surface [42]. Activation through B cell epitopes typically results in antibody class switching, whereas activation of T cell epitopes stimulates the production of T cell subsets that regulate immune responses [42]. Thus, using recombinant technology, it is possible to generate peptides containing short linear allergen-derived B and T cell epitopes for use in immunotherapy to specifically target these cells, but due to their small size and lack of secondary and tertiary structures, have a reduced ability to bind IgE [42]. Recombinant allergens are divided into two broad types: those that equal natural allergens or those that are genetically engineered or modified which include hypoallergenic vaccines and peptide vaccines [67].

Recombinant Wildtype Allergens

Recombinant wildtype allergens contain mainly conformational IgE epitopes composed of different sequence elements that when folded correctly, are recognized by IgE antibodies in a specific three-dimensional array of peptide residues [69]. Natural allergen extracts can be substituted by pure recombinant wildtype allergens or by single recombinant fusion proteins consisting of several wildtype allergens [12]. The advantages of recombinant wildtype allergens are that they can be produced with defined quality and quantity in a reproducible production process that allows the formation of vaccine batches with consistent properties and potencies [69]. Moreover, these allergens can be produced as hybrid molecules incorporating the epitopes of several allergen molecules, thereby facilitating vaccine production and increasing immunogenicity [69]. Although the use of recombinant wildtype allergens allows elimination of the problems related to poor quality and contamination of natural extracts, it does leave open the problem of potential side effects similar to those of natural allergens.

Initial trials involving immunization of mice with recombinant timothy grass pollen containing four of the most recognized allergens (Phl p 1, 2, 5, and 6) induced higher IgG1 titers than treatment with equimolar mixtures of individual allergen or timothy grass pollen extract [12, 13]. This was followed by a randomized, double-blind, placebo-controlled trial in 62

patients with allergic rhinitis treated with recombinant timothy grass with five allergens (Phl p 1, 2, 5a, 5b, 6) [14]. Treated patients demonstrated a 36% reduction in symptoms and medication usage and an increase in allergen-specific IgG1 and IgG4 [14]. Another multicenter, randomized, double-blind placebo-controlled trial comparing recombinant birch pollen allergen vaccine, standard birch pollen extract, natural purified birch pollen allergen, and placebo in 134 patients with birch allergy demonstrated significant reductions in rhinoconjunctivitis symptoms, rescue medication use, and skin sensitivity in the three treatment groups [15•]. Although there was no significant difference in clinical markers between the three treatment groups, there was a greater increase in birch-specific IgG levels and a greater decrease in skin test reactivity in the recombinant birch allergen group than either the standard or purified extract-treated groups [15•]. In both studies, the rate of systemic reactions was low, though a greater number of local reactions with recombinant birch pollen immunotherapy versus either birch extract or purified extract were noted [14, 15•]. The safety of recombinant grass therapy was further established in a randomized, double-blind, placebo controlled study which demonstrated grade I and II systemic reactions in 16% of the patients during the build-up phase of treatment at doses up to 120 µg; similar to rates in other clinical trials with commercially available SCIT preparations that often contain much lower allergen contents [70]. Collectively, these studies suggest that although immunotherapy with recombinant wildtype vaccines results in increased immunogenicity as compared to natural extracts, there may not be significant gains in terms of clinical efficacy or safety.

Hypoallergenic Vaccines

An alternative approach to avoid IgE-mediated adverse effects is to modify the structure of IgE epitopes to bypass IgE binding without compromising desired T cell reactivity by creating hypoallergenic recombinant allergens using techniques such as fragmentation, oligomerization, and mutation. The rationale for such preparations being that a higher dose of allergen could be used for treatment with a reduced risk for adverse reactions [71]. However, although hypoallergenic vaccines nearly abolish IgE-mediated side effects, the presence of T cell epitopes have the potential to induce late phase reactions [72].

In a randomized, double-blind, placebo-controlled multicenter study, 124 patients were treated with placebo or hypoallergenic derivatives consisting of three copies of the gene in sequence to form a trimer of Bet v 1 [17]. Vaccination with recombinant birch-pollen derivatives induced a strong and significant rise in IgG antibodies to both Bet v 1 and Bet v 1-related food allergens, along with a decrease in the seasonal

rise of IgE response after birch pollen season [17, 73]. Additional studies with Bet v 1 derivatives also demonstrated a trend towards improvement in the subjects' well-being and clinical scores and a decrease in skin and nasal sensitivity, though there was no significant difference in the combined symptom medication score [16, 18]. However, there was a significant increase in reactions in the treatment groups as compared to the placebo group, with systemic reactions occurring more frequently in the Bet v 1 derivative-treated group [16]. Another approach using a stable recombinant Bet v 1 folding variant demonstrated decreased total symptom score and increases in IgG1 levels in all treatment groups at doses of 20, 80, and 320 µg as compared to placebo, with grade II reactions occurring at the highest dose of allergen [19, 21]. SLIT with recombinant Bet v 1 therapy has also been shown to decrease symptom scores as compared to placebo, with predominantly local reactions commonly associated SLIT [74].

A hypoallergenic grass pollen vaccine consisting of a mosaic molecule with two non-IgE reactive Phl p 2-derived peptides was also created [75]. This mosaic molecule lacked IgE reactivity and had strongly reduced allergenic activity as determined by basophil activation assays and skin testing in grass-allergic patients [75]. More recently, treatment of mice with hypoallergenic hybrid proteins containing the primary sequence of four major timothy grass pollen allergens (Phl p 1, 2, 5 and 6) induced higher levels of allergen-specific IgG4 with decreased allergenic activity compared to wild type allergens [22]. Similarly, a hypoallergenic mosaic protein for dust mite allergens, rDer p 2 consisting of reassembled elements of Der p 1 and Der p 2 designed to have alternate folding was created [20]. In vitro assays using serum from dust mite-allergic patients demonstrated decreased IgE binding and decreased basophil activation, as well as reduced in vivo allergenicity in immunized mice with rDer p 2 as compared to natural Der p 2 [20]. Moreover, immunization of rabbits with rDer p 2 induced allergen-specific IgG antibodies that were able to inhibit allergic patients' IgE binding [20]. Clinical trials for grass and dust mite recombinant hypoallergenic vaccines have not been done. Together, these studies suggest that the spatial arrangement of IgE epitopes on proteins may have an important influence on its allergenic activity while still maintaining good immunogenicity [76]. Though in the absence of additional clinical studies, whether or not recombinant hypoallergenic vaccines will offer therapeutic or safety benefit over natural extract vaccines is unclear.

T Cell Epitope Vaccines

T cell epitope peptide therapy harnesses the established immunologic dogma that dominant T cell epitope peptides can induce anergy of allergen-specific T cells if delivered in a way that fails to activate the T cells [68, 77]. As previously discussed, T cells play a role in allergic response mostly by

responding to short peptides bound to MHC molecules rather than the tertiary structure of an allergen, allowing for delivery of T cell epitopes in a vaccine that induces subsequent T cell tolerance without unwanted IgE reactions [78]. The administration of short soluble peptides that are unable to cross-link allergen-specific antibodies may exploit a tolerance pathway since T cell recognition occurs in the absence of inflammatory signals [79]. However, the absence of conformational epitopes on short peptides may explain the relatively weak humoral mechanistic signals seen in peptide immunotherapy as compared to AIT with whole allergen [77].

Early studies used two, small, 27 amino-acid polypeptides from the major cat allergen, Fel d 1 (Allervax® Cat) to show modest improvement in nasal and respiratory symptoms after four, weekly subcutaneous injections [23, 25]. In a subsequent multicenter, randomized, double-blind, placebo-controlled study of 133 cat allergic patients who previously failed conventional cat immunotherapy, those receiving a total of eight subcutaneous injections (twice weekly for 2 weeks followed by twice weekly for 2 weeks 4 months later) of Allervax® CAT demonstrated improved pulmonary function and increased tolerance to cat exposure [25]. Of note, skin reactivity to whole cat extract did not change before and after treatment, adverse reactions requiring systemic epinephrine occurred in three patients, and late onset reactions consisting of predominantly respiratory symptoms occurred twice as frequently in the active groups [25]. These reactions declined with subsequent injections, suggesting the development of tolerance [25]. However, further investigations with Allervax® CAT failed to show clear clinical or safety benefits [24, 26, 80].

Thus, a new generation of vaccines consisting of short synthetic peptide immunoregulatory epitopes (SPIRE) selected for their ability to bind commonly expressed HLA-DR molecules was designed to induce tolerance via binding to MHC II on APCs with subsequent upregulation of Tregs [79]. The first of these was a Fel d 1 peptide vaccine consisting of seven peptides with overlapping T cell epitopes known as Cat-PAD and was shown to be safe and well-tolerated when administered either intradermally or subcutaneously [27]. In a follow-up randomized, double-blinded, placebo-controlled study, four injections of Cat-PAD given 4 weeks apart resulted in a significant change in total rhinoconjunctivitis symptom scores, both at 18–22 weeks and 1 year after treatment [28]. Moreover, there were no significant differences in the number of adverse reactions between the treatment and placebo groups [28]. These patients continued to show clinical benefit when exposed to Fel d 1 2 years after the start of treatment [29]. Similarly, subjects receiving ragweed vaccine containing T cell epitopes of Amb a 1 (ToleroMune Ragweed) also had reduced total rhinoconjunctivitis symptom scores when exposed to controlled amounts of ragweed pollen as compared to placebo controls [81].

Additional evidence for the safety and efficacy of SPIRE vaccines was more recently demonstrated in a multicenter,

randomized, double-blind, placebo-controlled study that evaluated three regimens of recombinant grass-pollen AIT versus placebo in 282 patients with grass-pollen-induced allergy [30•]. Patients receiving eight intradermal doses 2 weeks apart of the lowest dose (6 nmol) of grass-SPIRE showed a significantly greater reduction in mean total rhinoconjunctivitis symptom scores as well as total non-nasal symptom scores during rye grass challenge as compared to the placebo group; however, there was no difference in rye grass-specific skin prick test reactivity or IgG4 levels between the groups [30•]. Moreover, although the number of local injection-site reactions was more common in the patients receiving grass-SPIRE, the proportion of patients reporting severe adverse reactions were similar between the treatment and placebo groups [30•]. Interestingly, this study recapitulated findings by others that increasing the cumulative dose of allergen did not lead to a greater treatment effect [30•, 43, 82].

Despite their clinical efficacy, changes in IgE and IgG levels are less apparent with short peptide AIT, likely because SPIRE peptides are too short to bind to immunoglobulin on the surfaces of B cells [30•, 83]. However, peptide mixtures have been shown to decrease inflammatory cytokine levels and increase IL-10 levels in allergen stimulated blood lymphocytes [26, 84, 85]. Though these studies demonstrate both the short- and long-term safety and efficacy of SPIRE therapy, there are possible drawbacks. Approaches based on a restricted set of peptides requires predetermination of the patient's T cell epitope response, which can vary greatly as a result of HLA-genotype variability and render treatment with just a few peptides difficult [68•, 72, 86]. Moreover, short T cell epitope-based therapies fail to induce allergen-specific blocking IgG, and it is not yet clear whether the production of allergen-specific IgG4 is necessary for clinical efficacy or an epiphenomenon occurring as a result of AIT [68•, 72].

Contiguous Overlapping Peptides

As an alternative approach, vaccines utilizing long contiguous overlapping peptides (COPs) are under investigation. COPs encompass the complete allergen sequence with overlaps ensuring the presence of all possible T cell epitopes, thus avoiding the selection bias inherent in allergen fragments and selected dominant epitopes [72]. While their short nature and lack of original three-dimensional structures allows for undetectable IgE binding and a low side effect profile, their longer length allows for the induction of IgG4 antibodies [68•]. Additionally, in contrast to short synthetic peptides, COPs more efficiently recruit allergen-specific T cells [87].

Using three long synthetic peptides mapping the entire sequence of PLA2 from bee venom, 16 patients with bee venom allergy underwent immunotherapy in a 1-day dose escalating protocol followed by five subsequent maintenance doses [86]. COP-AIT induced T cell anergy, increased total IL-10 and

IFN- γ secretion, and increased allergen-specific IgG4 levels [86]. Treatment was well-tolerated except for the development of erythema in two patients more than 2 h after administration [86]. A combination of three COPs derived from major birch allergen Bet v 1 (AllerT) demonstrated more than a tenfold lower ability to bind IgE versus recombinant Bet v 1 and did not induce the activation of basophils from sensitized mice or birch allergic patients [31]. Moreover, AllerT did not elicit positive skin prick reactions in birch allergic patients, even at fivefold higher concentrations than recombinant Bet v 1 and did not induce anaphylaxis in sensitized mice [31]. In a multicenter, randomized, double-blinded, placebo-controlled, phase IIb trial, 240 patients with moderate to severe allergic rhinoconjunctivitis to birch pollen were randomized to receive five subcutaneous injections of either placebo, 50-, or 100- μ g of AllerT prior to the onset of birch pollen season [32•]. Rhinoconjunctivitis symptom and medication scores, as well as quality of life, and nighttime nasal symptom scores showed statistical improvement in both treatment groups, with sustained efficacy during a second pollen season [32•, 88]. Repeated administration of Bet v 1 COPs lead to a significant increase in allergen-specific IgG4 levels in all but six patients in both treatment groups [32•, 89]. There was no difference between the three groups in the number of local reactions, though in the Bet v 1 COP treatment groups, two patients reported serious adverse reactions, one requiring intramuscular epinephrine, and two others reported delayed reactions consisting predominantly of respiratory tract-related symptoms [32•].

Late phase respiratory events have been reported with both short T cell epitope peptides as well as with longer allergen peptides [25, 26, 32•]. Although the mechanism of these late phase allergic reactions is unclear, most are likely dependent on T cell activation rather than IgE binding or mast cell degranulation [80]. Of note, reactions occur mainly after the first injection, followed by progressive T cell tolerance on subsequent injections, thus arguing for early T cell activation [26, 89]. However, the ability of long-peptide therapy to induce allergen-specific IgG may provide a clinical benefit to short T cell epitope peptide vaccines.

Carrier-bound B Cell Epitope Vaccines

B cell epitope-based allergy vaccines contain intrinsically non-IgE reactive peptides of 20–40 amino acids in length which are derived from the IgE-binding sites of major allergens [90–93]. These peptides are covalently linked to a protein carrier unrelated to the allergens and provides carrier-specific T cell help to render them immunogenic enough to induce a robust IgG antibody response while still blocking IgE binding [90, 91]. Because carrier molecules without allergen-specific T cell epitopes can be chosen, the presence of allergen-specific T cell epitopes in the vaccine is reduced, thus

avoiding T cell-mediated side effects and offering a major advantage over T cell epitope containing peptides or hypoallergens. Furthermore, as IgE antibodies preferentially recognize conformational IgE epitopes, it is possible to identify peptides that are part of the IgE-binding site but do not react with IgE antibodies and therefore do not induce IgE-mediated reactions. Vaccines based on carrier-bound allergen peptides should allow the elimination of both IgE and T cell-mediated side effects, while still inducing robust allergen-specific IgG responses. Because of their hypoallergenic nature, it is possible to inject high doses into patients and combined with its good immunogenicity; SCIT may be performed with only a few injections per year [72]. Moreover, these carrier protein-bound peptides can be expressed in *Escherichia coli* in large amounts and purified to homogeneity, resulting in an easy to produce and standardize product.

A hypoallergenic grass pollen vaccine, designated BM32, was derived from non-allergenic peptides from major IgE-binding sites of four timothy grass pollens (Phl p 1, 2, 5, and 6) fused to the hepatitis B-derived PreS domain [37]. BM32 did not show detectable IgE reactivity with sera or induce activation of basophils from grass-allergic patients collected during the peak of pollen season [37]. Moreover, BM32 induced significantly lower T cell proliferation and proinflammatory cytokine release as compared to natural grass pollen-allergens and was also able to induce high levels of allergen-specific IgG antibodies upon immunization of rabbits [37]. In a randomized, double-blind, placebo-controlled phase II study investigating safety and dose-dependent effects of three different doses of BM32 versus placebo, 71 grass-pollen allergic patients were randomized to receive three injections at 1-month intervals of either placebo or 10, 20, or 40 µg of BM32 [38•]. There was a significant decrease in the total nasal symptom scores after treatment in the 20- and 40-µg treatment groups and a dose-dependent reduction in total ocular scores and skin prick sensitivity in all treatment groups [38•]. Additionally, AIT with each of the doses of BM32 induced significant increases in allergen- and carrier-specific IgG antibodies, which inhibited both basophil degranulation and T cell proliferation in the sera of allergic patients [38•]. The majority of treatment-related adverse reactions were local injection site late phase reactions, with no significant difference between the occurrence of reactions in the placebo and treatment groups, though there was a dose-dependent trend to higher intensity of reaction in the treatment groups [38•]. Of note, no systemic reactions were observed [38•].

The concept of using PreS as a carrier for B cell epitope vaccines has been applicable to different allergens as well. Animal and in vitro studies using fusion proteins containing Der p 23, Fel d 1, and Bet v 1 demonstrated reduced allergenic activity, strong induction of allergen-specific IgG, and reduced T cell activation along with skewed responses towards a Th1 and Treg phenotype [33–36]. Though these studies

provide evidence for clinical efficacy after a short course of treatment, long-term clinical efficacy studies have yet to be completed.

Conclusion

Although AIT with natural extracts has a long history of efficacy, the potential for life-threatening side effects, concerns for the variations in amounts, potencies, and immunogenicity of individual allergen molecules, and a cumbersome and lengthy treatment protocol have limited its wide spread use. Recombinant vaccines offer new alternatives for AIT eliminating the problems of variability, as well as reducing allergenicity for an improved safety profile. The increased immunogenicity of recombinant vaccines may also reduce the treatment duration without compromising long-term clinical efficacy; however, the occurrence of late phase reactions and poor IgG responses may limit the use of some therapies. Although in vitro and animal studies comparing natural extracts and recombinant vaccines suggest that recombinant vaccines may serve as viable replacements for natural extracts, head-to-head comparison clinical studies have not been done and may be needed to demonstrate a clear advantage for recombinant therapies.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflicts of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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