

Genetically modified allergens

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Type I allergy, an immunoglobulin E (IgE)-mediated hypersensitivity disease, affects almost 25% of the population and shows a continuously increasing prevalence [1]. Allergic individuals can be discriminated from nonatopic persons by their increased tendency to produce IgE antibodies against harmless antigens (ie, allergens), which are presented to the immune system mainly through the respiratory or gastrointestinal tract. In sensitized individuals, the cross-linking of allergens with IgE antibodies that are bound by effectors (mast cells, basophils) elicits a plethora of allergic symptoms (eg, allergic rhinoconjunctivitis, asthma, dermatitis, food allergy, anaphylactic shock).

The only causative treatment of type I allergy, specific immunotherapy, is the administration of increasing doses of the disease-eliciting allergens to induce a state of allergen-specific nonresponsiveness [2]. Allergen immunotherapy was applied for the first time more than 90 years ago to treat patients with grass pollen allergy [3]. At that time, little was known about the immunologic mechanisms responsible for allergic reactions, and pollen-induced hay fever incorrectly was believed to be an intoxication with grass pollen toxins. Injections containing grass pollen extract were shown to improve allergic symptoms on pollen exposure, and immunotherapy also was used to treat allergy caused by other allergen sources.

This treatment has been used in patients with considerable success for a long time, although the molecular and cellular mechanisms involved were unknown

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Box 1. Disadvantages of the use of allergen extracts for immunotherapy*Composition*

- Variable and unpredictable amounts of different allergens
- Undefined nonallergenic materials
- Batch-to-batch variability

Possible contamination

- Endotoxins
- Allergens from other, unrelated sources

Impossible to produce patient-tailored immunotherapy

- Possible induction of new IgE sensitivities

Unpredictable therapeutic activity

- Variable allergen contents
- Variable immunogenicity and tolerogenicity of the components

Allergenic side effects

- Allergenic activity of natural allergen extracts

and are still not understood fully. One major change in the field of immunotherapy was the introduction of adjuvant-bound allergen extracts, which rendered allergen-specific immunotherapy safer by preventing the spread and systemic release of allergens [4]. Attempts were made to improve allergen extracts by reducing their anaphylactic side effects. Extracts were modified chemically by proteolytic digestion, coupling to polyethylene glycol or chemical denaturation [5–9]. Although some of these attempts to improve the safety of specific immunotherapy proved to be successful [10], it is technically difficult to produce chemically modified allergen extracts of consistent quality. Only allergoids produced by chemical denaturation, which had a reduced allergenicity, still are used for routine treatment. However, neither the use of adjuvants nor chemical modifications of allergen extracts can overcome the disadvantages of the allergen-specific immunotherapy related to the use of crude allergen extracts (Box 1).

Disadvantages of the use of allergen extracts for immunotherapy

Currently immunotherapy is performed using aqueous extracts of the allergen source, which are composed of a mixture of allergenic components and a large number of nonallergenic components [2]. The composition and allergen content of such crude extracts can vary widely, because they depend on several unpredictable factors (eg, heterogeneity of allergen sources, protein degradation). Studies have reported high levels of endotoxins in therapeutic allergen preparations [11] and contaminations with allergens from other sources (eg, presence

of mite allergens in animal hair dander extracts) [12]. Attempts to standardize allergen extracts regarding important allergens with the use of monoclonal antibody-based ELISA systems have been made, but the final composition of the extracts cannot be influenced [13]. Problems related to the composition of therapeutically used allergen extracts were demonstrated in a study investigating IgE and IgG antibody responses to different recombinant grass pollen allergens in patients undergoing grass pollen-specific immunotherapy. The investigators found large variations in IgG responses to individual grass pollen allergens [13].

Another concern regarding extract-based immunotherapy is the induction of IgE-sensitivities to previously unrecognized epitopes that may occur during treatment. Several studies have shown that extract-based immunotherapy induced new IgE specificities to allergens that were not recognized before therapy [14]. In 29% of patients who received birch pollen-specific immunotherapy [15] and in patients who received immunotherapy with grass pollen extract, de novo immune responses to new epitopes and allergens were found [16]. Although the clinical relevance of these findings is unknown, the data indicate that it would be preferable to treat patients according to their individual sensitization profile rather than with crude extracts. The only way to avoid the previously described problems of allergen extracts is through the use of recombinant allergen molecules for diagnosis and specific immunotherapy. Furthermore, the immunologic properties of recombinant allergen molecules can be modified by controlled methods [17]. [Box 2](#) summarizes the advantages of the use of modified recombinant allergens in immunotherapy.

Box 2. Advantages of immunotherapy with genetically modified recombinant allergens

Composition

- Highly purified proteins
- Exact amount of defined proteins
- No contaminants

Patient-tailored immunotherapy

- Administration of modified allergens according to the sensitization profile
- Avoidance of sensitization to new allergens

Predictable therapeutic efficacy

- Administration of the exact amount of modified allergens
- Predictable induction of immune responses

Side effects

- Controlled reduction of allergenic activity
- Reduced side effects

Production and modification of recombinant allergens

The principal steps in the production of recombinant allergens and modified allergen molecules are depicted in Fig. 1. During the past 15 years, the isolation and expression of cDNAs coding for allergens have led to the production of an increasing number of recombinant allergens [18–25]. Using this approach, the

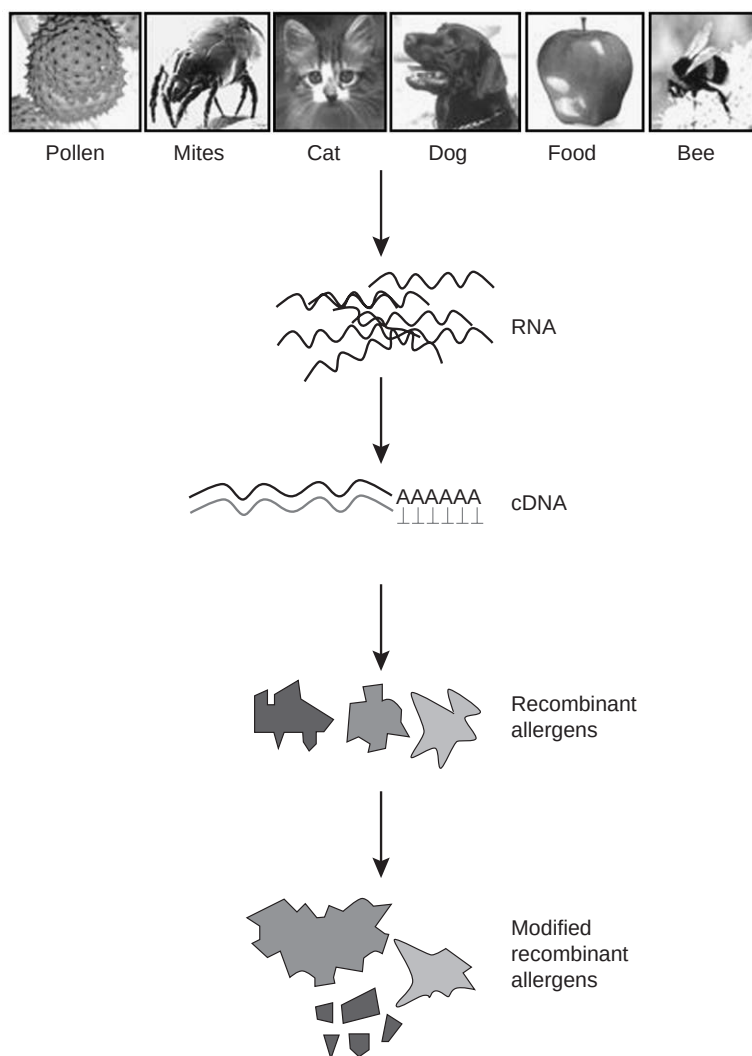


Fig. 1. Production of modified recombinant allergens. RNA is extracted from important allergen sources (eg, tree, grass, or weed pollen; mites; cat or dog tissues; food; bee venom) and reverse transcribed into cDNA. The cDNA is used for production of recombinant allergens. Genetically modified allergens then can be produced by various methods (eg, fragmentation, polymerization, point mutations).

allergen repertoires of the most common allergen sources have been reconstructed with recombinant allergens that closely mimic the properties of the corresponding natural allergens [26,27]. Immunologic studies (ie, IgE reactivity, T-cell proliferation) revealed that because of the large amount of cross-reactivity between allergens from different sources, a few recombinant allergens are sufficient to cover most of the disease-eliciting epitopes that are present in natural allergen extracts [27,28].

Use of recombinant allergens in diagnosis to improve specific immunotherapy

Studies have shown that a limited number of recombinant allergens is sufficient to diagnose grass or birch pollen allergy [29–31], and the replacement of conventional diagnostic tests based on crude allergen extracts was suggested. The fundamental advantage of using recombinant allergens for diagnostic purposes is that information about the individual sensitization profile of an allergic patient is provided [28], whereas conventional extract-based tests only determine if a patient is sensitized against a particular allergen source. Allergens that are exclusive to a certain allergen source can be used as marker allergens to indicate a genuine sensitization toward an allergen source. Such marker allergens include Bet v 1, which indicates a genuine sensitization to birch pollen [30], and Phl p 1 and Phl p 5, which are major timothy grass pollen allergens that can be used to confirm grass pollen sensitization [32]. When a diagnostic test is performed with allergen extracts, however, the presence of elevated levels of IgE to highly cross-reactive allergens that occur in many unrelated allergen sources may result in positive test results to all of these sources. Such patients could be treated inappropriately with specific immunotherapy that uses an extract from an allergen source that did not cause the sensitization. It has been suggested that cross-reactive allergens and species-specific marker allergens be used to identify the allergen source against which the patient genuinely was sensitized and to diagnose patients with polysensitization [32,33].

To facilitate allergen-specific diagnostic procedures, chip-based allergy tests using microarrayed recombinant allergens have been developed. These tests allow rapid determination of the sensitization profile against numerous allergens [34–36].

Development of modified allergens for immunotherapy by genetic engineering

The use of molecular cloning to decipher the DNA and deduced amino acid sequences for common allergens provides the basis for specialized and experimental forms of therapeutic strategies [17,37]. One example is the use of peptides containing T-cell epitopes for injection immunotherapy. These peptides lack B-cell epitopes and are believed to induce T-cell tolerance without causing IgE-mediated release of inflammatory mediators. Although mouse models showed that this form of therapy induced T-cell tolerance [38,39], therapy trials

with bee venom and cat allergen peptides yielded controversial results [40–42]. The systemic side effects observed during T-cell peptide immunotherapy possibly can be attributed to major histocompatibility complex-dependent activation of T cells [43]. Recent studies have yielded more promising results, especially concerning the suppression of T-cell-mediated late-phase responses [44,45].

Other concepts are based on the use of synthetic B-cell epitopes of allergens [46,47] or mimotopes [48] for the induction of blocking antibodies. B-cell epitopes, which are longer than T-cell epitopes (>20 amino acids) and are located on the surface of allergens, should induce blocking G antibodies. Based on structure analysis of allergens, it is possible to select peptides which lack IgE-binding capacity but upon immunization induce IgG antibodies that block IgE binding to allergens [46,47]. Mimotopes are peptides that are obtained from phage libraries expressing random peptides [48].

Another approach is the use of whole modified allergen molecules with reduced allergenic activity. Studies of the structure and IgE-binding sites of common respiratory allergens revealed that the presence of conformational epitopes on intact and folded allergens is of crucial importance for IgE recognition [27]. Therefore, allergenic activity and IgE binding can be abolished or greatly reduced by altering the structural fold of allergens. The fundamental advantage of such allergen derivatives, in comparison with T-cell peptides and B-cell epitopes, is that in most cases they preserve the repertoire of T-cell epitopes, which are necessary to induce T-cell tolerance, and the immunogenic structures that are essential for the antibody responses (Table 1) [49]. Appropriate modifications of allergenic molecules allow one to influence the magnitude and quality of the immune response.

Various strategies using genetic engineering have allowed the production of defined recombinant allergen derivatives with altered protein folds and IgE binding capacity. These strategies include the construction of allergen fragments, oligomers, mutants, deletion variants, hybrid molecules, and other variants (see Fig. 1).

Table 1

A comparison of candidate molecules for a new allergy vaccine

	Allergenic activity	Presence of T-cell epitopes	Induction of protective antibodies	Tolerance induction possible	Suitable for vaccination approaches	Induction of IgE against wild-type allergen
T-cell peptides	—	+	—	+	—	—
Unmodified (wild-type) recombinant allergens	+	+	++	+	+	++
B-cell peptides	—	—	+	—	+	—
Genetically modified recombinant allergens	—	+	++	+	+	+/-

—, none; +/-, weak; +, yes; ++, strong.

By splitting allergens into defined fragments or by deletion of IgE-reactive portions, reduced IgE reactivity and allergenic activity can be achieved. The reduced allergenicity of allergen fragments is based on the observation that IgE recognition is frequently dependent on the presence of conformational epitopes on intact and structurally folded allergens. One example is the production of two Bet v 1 fragments [50] that have a more than 100-fold reduced capacity to induce type I allergic reactions in skin prick tests and experiments involving basophil histamine release [51,52]. A similar reduction of IgE reactivity was noted for several other allergen fragments from pollen, mites, and molds [53–58].

The production of allergen oligomers shows that it is not necessary to completely destroy IgE reactivity to achieve a strong reduction of allergenic activity. Investigators produced a recombinant Bet v 1 trimer that exhibited a structural fold similar to the fold of allergenic Bet v 1 monomer and that maintained its IgE reactivity [59]. The trimer had greatly reduced allergenic activity in skin tests and experiments involving basophil histamine release [51,52]. It may be presumed that a reorientation of IgE epitopes on the trimeric molecule is responsible for the reduced allergenic activity. The Bet v 1 trimer shows that the immunologic properties of allergens can be altered by genetic engineering. It induced strong lymphoproliferative responses in T cells from patients allergic to Bet v 1 and diverted the patients' cytokine release profiles from T helper cell type 2 (Th2) toward Th1 [59]. The trimer may be a promising candidate for specific immunotherapy. A similar effect of genetic engineering on the immunologic properties of an allergen has been shown for Der f 1, the major house dust mite allergen [60].

The IgE reactivity of allergens may be altered by introduction of mutations by site-directed mutagenesis [61–65]. The disruption of calcium-binding domains on Bet v 4 by mutation of the acidic amino acids in the calcium-binding domains led to a reduced IgE-binding capacity [66].

Hybrid allergens may be constructed out of two or more immunologically unrelated allergens by fusion using the polymerase chain reaction. The fusion of weakly immunogenic allergens yielded hybrid molecules with strongly enhanced immunogenicity (stronger IgG antibody responses in animals and lymphoproliferative responses in peripheral blood mononuclear cell cultures), compared to equimolar mixtures of the individual allergens [67]. This observation suggests that the weak immunogenicity of certain allergens may be overcome by the hybrid-allergen approach [68].

Immunologic and clinical characterization of modified recombinant allergens

Genetically modified recombinant allergens can be evaluated by different *in vitro* and *in vivo* assays. The first step is the measurement of IgE and antibody reactivity. Allergenic activity may be evaluated by the induction of basophil degranulation and skin reactions.

Before modified allergens can be used for immunotherapy studies in humans, their *in vivo* allergenic activity needs to be evaluated by provocation testing. Different provocation tests (skin test, nasal provocation test, conjunctival provocation test) may be applied to determine the induction of immediate inflammatory reactions [51,52,69,70]. The results obtained by skin testing and those obtained by nasal and conjunctival provocation testing with genetically modified versions of Bet v 1 (the major birch pollen allergen) were comparable, suggesting that skin testing is appropriate for the evaluation of allergenic activity [69,70]. Such test systems involving a representative number of patients showed that modified birch, mite, weed, and cow dander allergens had strongly reduced allergenic activity [17]. Evaluation of allergen-mediated late effects on cell migration and activation and cytokine and mediator release may be performed in an *in vivo* skin chamber model [71].

Future perspectives

Genetically modified hypoallergenic allergen derivatives of the most common allergen sources likely can be developed. These molecules should offer considerable advantages over crude allergen extracts (see Box 2) and combine the advantages of peptide-based treatment and treatment with unmodified recombinant allergens (see Table 1). One double-blind, placebo-controlled, multicenter immunotherapy trial has been performed with recombinant Bet v 1 Trimer and Bet v 1 fragments [72]. The authors are convinced that this approach will provide better and safer tools for the treatment of allergic patients.

Summary

For more than 90 years, allergen-specific immunotherapy, the only causative allergy treatment, has remained basically unchanged. The development of molecular biology techniques has led to the preparation of individual recombinant allergens from the most important allergen sources. Recombinant allergens can be used to determine the individual sensitization profile of allergic patients and have allowed the development of novel therapeutic tools. This article summarizes data on genetically modified recombinant allergen derivatives with reduced allergenic activity, which may be used to improve the safety and efficacy of specific immunotherapy.

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