

Update review

Recombinant allergens for immunotherapy

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Recombinant allergens can be produced as defined molecules in consistent quality and unlimited amounts according to the corresponding DNA template. Furthermore, they can be modified to reduce their allergenic activity and to foster certain advantageous immunologic properties. Recombinant allergens equaling the natural allergens are available for diagnostic and therapeutic purposes, and modified versions have been developed with the aim to reduce IgE-mediated side effects during immunotherapy. First injection immunotherapy trials conducted with recombinant vaccines for birch pollen and grass pollen allergy show that recombinant allergen-based immunotherapy has vaccination characteristics and is clinically effective. The obtained results hold promise that recombinant allergen-based immunotherapy will improve current immunotherapy practice and may open possibilities for new treatment strategies and possibly even for prophylactic vaccination. (*J Allergy Clin Immunol* ■■■■;■■■;■■■-■■■.)

Key words: Recombinant allergens, immunotherapy

Traditional allergen extracts are prepared from natural allergen sources and accordingly have numerous disadvantages that cannot be overcome with existing technical means (Table I). Allergen extracts contain undefined amounts of nonallergenic materials, of which certain components have been shown to prime T_H2 responses.¹ In many cases, important allergens are present in small amounts or lacking, and their biological potency is subject to huge variability.² In contrast, recombinant allergens can be produced with high purity by using controlled production procedures that yield defined molecules with known molecular, immunologic, and biological characteristics.³ Recombinant allergens can be produced as molecules

Abbreviations used

rBet v 1: Recombinant Bet v 1

rBet v 1-FV: Folding variant of recombinant Bet v 1

rPhl p 1: Recombinant Phl p 1

that exactly mimic the properties of the natural allergens (ie, recombinant wild-type allergens); as modified variants with advantageous properties such as reduced allergenic activity or increased immunogenicity; or as hybrid molecules resembling the epitopes of several different allergens to include the relevant epitopes of complex allergen sources.^{4,5} Attempts are made to control the quality of traditional allergen extracts by using different standardization procedures (eg, testing of biological activity or IgE reactivity, measurement of certain allergens in the extract), but despite these efforts, extracts remain undefined mixtures of allergenic and nonallergenic materials. Their composition cannot be altered, and products from different companies and even batches from the same company are not comparable. There are reports that allergen extracts can be contaminated with allergens from other sources,⁶ and it has been found that patients developed new IgE reactivities under treatment with allergen extracts.⁷ Allergen extracts also cannot be manufactured to suit the individual patient's sensitization profile. Each of these problems related to allergen extracts can be easily overcome with recombinant allergens. Vaccines can be formulated containing defined molecules on the basis of mass units. Recombinant allergen-based vaccines do not contain irrelevant or unwanted components and in principle can be tailored to the patient's sensitization profile and sensitivity. Most recombinant allergens can be expressed in large amounts in *Escherichia coli* or insect cells at low cost without risk of containing infectious materials.

What has been said about the disadvantages of natural allergen extracts is even more true for allergoids, which are made from allergen extracts by difficult-to-control chemical modifications to reduce their allergenic activity. It is almost impossible to characterize allergoids properly because they are not only derived from poorly defined allergen extracts but also have lost IgE and antibody epitopes on which most existing standardization procedures rely. Especially for the preparation of defined hypoallergenic allergen derivatives, recombinant DNA technology offers a huge advantage over the traditional chemical modifications. Well characterized hypoallergenic

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TABLE I. Advantages of recombinant allergens over traditional allergen extracts

Disadvantages of natural allergen extracts
Contain numerous undefined components, some of which may promote allergic immune responses
Lack or contain low amounts of important allergens
Contain allergens with widely varying potencies and ratios
Can be contaminated with unwanted materials or allergens from other sources
Cannot be tailored to the patient's sensitization profile
May induce new sensitizations
Do not suit the international quality standards for vaccines
Cannot be compared between different products, batches
Do not allow the precise monitoring and investigation of mechanisms underlying treatment
Advantages of recombinant allergens
Represent molecules with defined physicochemical and immunologic properties that can be modified to foster advantageous characteristics
Amounts can be easily controlled on the basis of mass units
Potencies and ratios can be exactly adjusted for each molecule
Represent pure molecules
Vaccines can be exactly tailored according to the patient's sensitization profile
Fit the international quality standards for vaccines
Can be precisely compared to give consistent and reproducible products, batches
Allow the precise monitoring and investigation of mechanisms underlying treatment
Can be reproducibly modified to suit different treatment strategies

allergen derivatives have been developed by recombinant DNA technology for many of the most common allergens.^{4,8} Such molecules can be designed according to different vaccination or tolerance induction strategies to harbor all relevant T-cell epitopes and to preserve the capacity to induce beneficial IgG antibody responses.⁹ An overview of the many possibilities to produce new types of allergen modifications for the improvement of immunotherapy and eventually prophylactic treatment has been published.⁴

CRITICAL STEPS IN THE DEVELOPMENT OF RECOMBINANT ALLERGEN-BASED VACCINES

Important questions that need to be addressed for the rational design of recombinant allergen-based vaccines are displayed in Fig 1. These issues are exemplified for 2 allergen sources, birch pollen and grass pollen, for which recombinant allergen-based vaccines have been developed and have entered clinical trials. The first important step is the selection of the predominant allergen sources. Selection criteria may include the frequency of sensitization, the clinical relevance, the magnitude of IgE responses, and the extent to which IgE epitopes are represented in a given allergen source. By using IgE

competition experiments in different populations, it has been demonstrated that the majority of IgE epitopes present in pollens of trees belonging to the order Fagales (eg, birch, alder, hazel, hornbeam, and so forth) and related plant food is represented by the major birch pollen allergen, Bet v 1.¹⁰ Similar experiments have identified a panel of recombinant timothy grass pollen allergens that contains the majority of IgE epitopes present in grass pollens.¹¹

For the identification of cDNAs coding for the biologically most active allergen isoforms, screening protocols relying on IgE antibodies from patients with allergy may be preferred over DNA-based strategies (eg, PCR-based cloning), which may also deliver cDNAs coding for allergen isoforms with low biological activity. The question of whether a panel of recombinant allergens resembles the IgE epitope complexity to which patients are exposed and sensitized can be addressed by IgE competition experiments if it is possible to obtain the natural allergens by extraction procedures. However, for many allergen sources (mites, molds, food), it is impossible to obtain suitable reference preparations from natural allergen sources to allow an accurate comparison of recombinant and natural allergens. Often important new allergens are discovered by molecular cloning techniques, not by protein chemical methods, because they are not present in natural allergen extracts. In these cases, it is important to obtain as complete a panel as possible of recombinant allergens via IgE-based screening approaches and to evaluate the relevance of the individual components. The evaluation of the individual molecules should be performed by IgE testing in large patient populations and most importantly by provocation testing because IgE serology is not always a good predictor for the allergenic activity and clinical relevance of a certain allergen molecule.

In our experience, skin testing is a useful method for the evaluation of the biological activity of recombinant allergens.^{12,13} Skin test studies performed in different populations may therefore be used for the identification of the clinically most relevant allergen molecules to be included in a therapeutic vaccine for a given allergen source. Lack of clinically relevant allergens will clearly reduce the efficacy of a vaccine, and inclusion of clinically not relevant molecules will make the production of the vaccine more complicated and increase costs. Information regarding the continuously growing number of recombinant allergen molecules can be extracted from databases.¹⁴ New diagnostic multiallergen tests consisting of microarrayed marker allergens have been developed to facilitate the diagnosis and accurate prescription of immunotherapy.¹⁵

CURRENT STATUS OF RECOMBINANT ALLERGEN-BASED IMMUNOTHERAPY

The selection of the relevant recombinant allergen molecules is the prerequisite for rationale vaccine development. Two types of recombinant allergen-based vaccines have been developed and tested in clinical trials. The

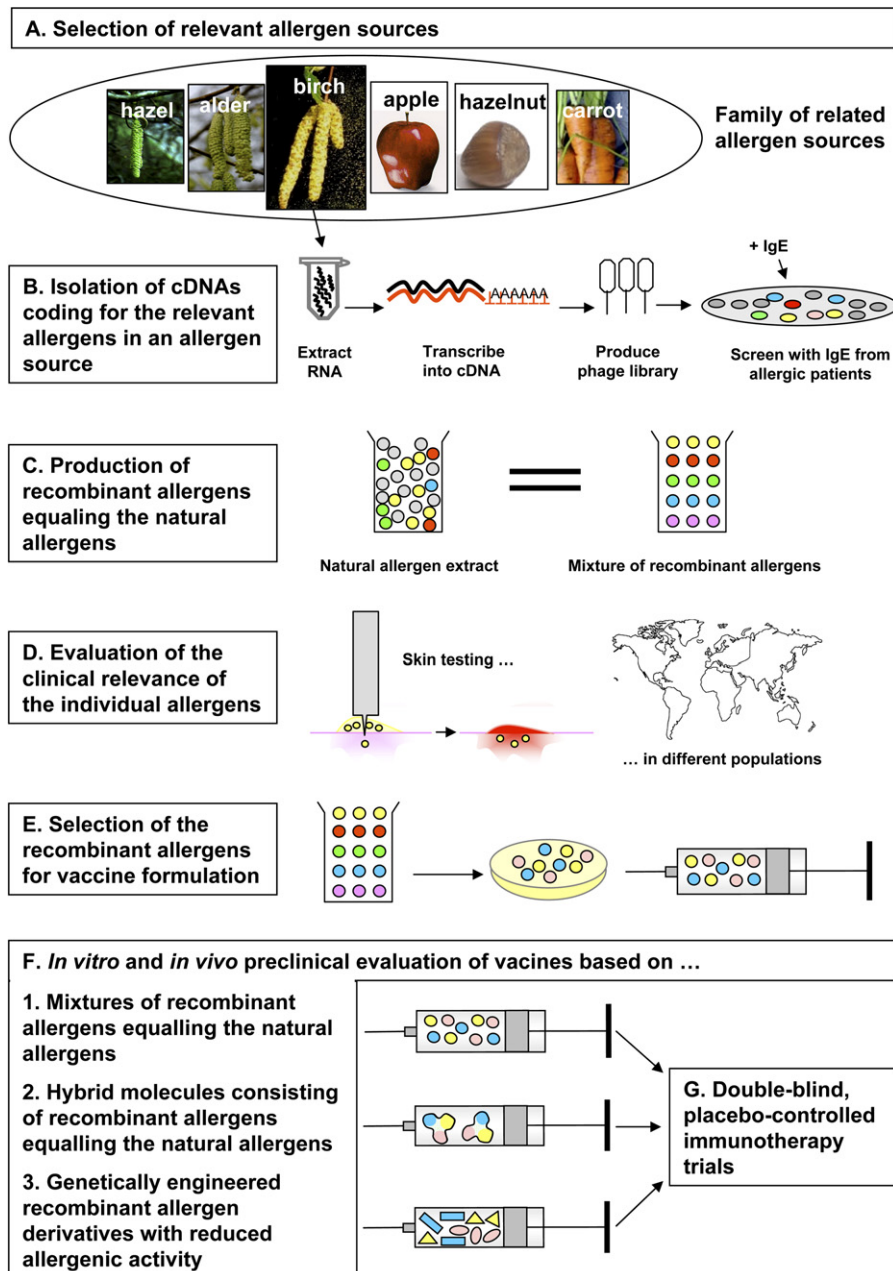


FIG 1. Steps toward recombinant allergen-based vaccines.

first type is based on the use of recombinant allergens that equal the natural allergens (ie, recombinant wild-type-based vaccines), and the second type is based on genetically engineered/modified recombinant allergens that exhibit reduced allergenic activity (Table II).

In fact, the first immunotherapy trial with recombinant material has been performed with genetically modified hypoallergenic derivatives of the major birch pollen allergen, Bet v 1, and several analytic studies have been performed.¹⁶ In this multicenter, randomized, double-blind, placebo-controlled study, 124 patients with birch pollen allergy received a single preseasonal course of

injection immunotherapy with a mixture of 2 recombinant Bet v 1 fragments or a recombinant trimer of Bet v 1 adsorbed to aluminium hydroxide. Preclinical testing has shown that recombinant Bet v 1 (rBet v 1) fragments and rBet v 1 trimer had a more than 100-fold reduced allergenic activity compared with Bet v 1.¹⁷⁻²⁰ Because of the hypoallergenic nature of the derivatives, patients were able to tolerate maintenance doses of 80 µg active ingredient per injection, and most of the patients reached cumulative doses of more than 150 µg active preparation in the short treatment period. Despite the fact that only 1 pre-seasonal treatment was given, patients developed robust

TABLE II. Immunotherapy trials with recombinant allergen-based vaccines

Molecules	Allergen source	Treatment groups	No. of patients	Application	Adjuvant	Study design	References
Type I recombinant wild-type allergen							
rPhl p 1+rPhl p 2+ rPhl p 5a+rPhl p 5b+ rPhl p 6	Grass pollen	Allergen mix, placebo	n = 62	Subcutaneous	Alum	DBPC, randomized, single-center	25
rBet v 1	Birch pollen	rBet v 1, nBet v 1, birch pollen extract, placebo	n = 147	Subcutaneous	Alum	DBPC, randomized, multicenter	27
Type II recombinant hypoallergenic allergen derivatives							
rBet v 1 fragments rBet v 1 trimer	Birch pollen	rBet v 1 fragments, rBet v 1 trimer, placebo	n = 124	Subcutaneous	Alum	DBPC, randomized, multicenter	16, 21, 22, 23
rBet v 1-FV	Birch pollen	rBet v 1-FV reference group birch pollen extract	n = 51	Subcutaneous	Alum	Open, single-center	24

Alum, Aluminium hydroxide; DBPC, double-blind, placebo-controlled.

IgG (IgG₁, IgG₄, IgG₂) antibody responses against natural Bet v 1, Bet v 1-related pollen allergens from alder (Aln g 1) and hazel (Cor a 1), and food allergens (ie, apple, Mal d 1; carrot, Dau c 1; and celery, Api g 1).^{16,21} These antibodies but no relevant rises of allergen-specific IgA were detectable in serum and nasal secretions and were associated with a reduction of immediate-type cutaneous and nasal sensitivity to Bet v 1 and inhibited Bet v 1-induced basophil degranulation *in vitro*.^{16,22} Interestingly, 1 treatment course was sufficient to induce a blunting of seasonal pollen exposure-induced rises of allergen-specific IgE in both actively treated groups but not in the placebo group.¹⁶ Trimer-treated patients exhibited a reduction of Bet v 1-specific T_H2 responses which may be due to the high treatment dose or the intrinsic ability of the Bet v 1 trimer to induce higher T_H1 responses than the recombinant wild-type allergen.²³ Although patients had received only 1 treatment course, there was a trend toward clinical improvement regarding respiratory birch pollen allergy and birch pollen-associated oral allergy syndrome. Similar results were obtained with a folding variant of rBet v 1 (rBet v 1-FV) adsorbed to aluminium hydroxide that had been prepared to reduce the allergenic activity of the allergen.²⁴ A study was conducted in a group of 24 patients with birch pollen allergy receiving rBet v 1-FV versus a reference group composed of 27 individuals receiving a birch pollen extract-based vaccine as a pre-seasonal injection course. Patients received maintenance doses of as much as 80 µg rBet v 1-FV, reaching a cumulative dose of 157.7 µg, and developed Bet v 1-specific IgG₁ and IgG₄ responses. Notably, they showed a 53% lower symptom medication score compared with a parallel group treated with birch pollen extract.²⁴

As far as recombinant allergens equalling the natural allergens are concerned, there are currently data from 2

immunotherapy trials available. The first study performed with recombinant wild-type allergens was performed as randomized, double-blind, placebo-controlled study with an equimolar mixture of 5 aluminium hydroxide-adsorbed recombinant timothy grass pollen allergens—Phl p 1, Phl p 2, Phl p 5a, Phl p 5b, and Phl p 6—in patients with grass pollen allergy (maintenance dose, 40 µg total protein consisting of 10 µg rPhl p 1, 5 µg rPhl p 2, 10 µg rPhl p 5a, 10 µg rPhl p 5b, and 5 µg rPhl p 6).²⁵ Sixty-two subjects were randomized, of whom 29 actively treated and 28 placebo patients could be fully analyzed. Patients received a pre-seasonal up-dosing treatment, and treatment was continued with approximately monthly injections for a period of 2 years. Similar to the results of trials with genetically modified allergens, actively treated patients developed robust IgG₁ and IgG₄ antibody production against natural grass pollen allergens, and clinical improvement was observed in these patients.²⁵

The first results from the largest trial performed with a recombinant wild-type allergen²⁶ were presented in June 2006 at the 25th European Academy of Allergy and Clinical Immunology congress in Vienna. In a multicenter, double-blind, placebo-controlled trial, 147 patients with birch pollen allergy were randomized in 4 groups: 1 group receiving recombinant major birch pollen allergen, Bet v 1a; 1 purified natural Bet v 1; 1 birch pollen extract; and 1 placebo.²⁷ Patients received aluminium hydroxide-adsorbed preparations in a pre-seasonal build-up phase and monthly injections with a maximal maintenance dose of 15 µg Bet v 1 for a period of 2 years. In both years, actively treated patients showed a significant clinical improvement in both symptom and medication scores over placebo. In addition, a strong induction of Bet v 1-specific IgG₁, IgG₄, and IgG₂ antibody responses and a reduction of cutaneous sensitivity to birch pollen—

derived Bet v 1 and rBet v 1 were observed. This study was the first to demonstrate in a direct comparison that a single recombinant allergen is as effective as treatment with a natural extract for the treatment of respiratory allergy.²⁷

In conclusion, the clinical studies performed with recombinant wild-type allergens and genetically modified hypoallergenic allergen derivatives indicate that these molecules can be used for immunotherapy for allergen sources containing 1 predominant allergen as well as for complex allergen sources, provided that the relevant allergens have been identified and included in the vaccine.

FUTURE PERSPECTIVES

Encouraging results have been obtained with recombinant allergen-based vaccines in the first proof of principle studies conducted for birch and grass pollen allergy. It is therefore likely that recombinant allergen-based therapeutic vaccines can be developed according to the same principles—that is, use of recombinant wild-type allergens and hypoallergenic derivatives for the most common allergen sources. Future work will have to focus on the identification of the relevant allergens in these allergen sources, the thorough preclinical characterization and selection of the candidate vaccines, and the rational design of immunotherapy studies involving objective as well as subjective parameters for measuring clinical efficacy. With the use of defined molecules instead of crude allergen extract-based mixtures, it will be possible to decipher more precisely the mechanisms underlying immunotherapy and to develop new forms of immunotherapy and perhaps prophylactic immunotherapy strategies.

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