

Recombinant allergens: What does the future hold?

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This year we are celebrating not only the centenary of allergen-specific immunotherapy but also the 10-year anniversary of the first administration of recombinant allergen-based vaccines to allergic patients. By using recombinant DNA technology, defined and safe allergy vaccines can be produced that allow us to overcome many, if not all, of the problems associated with the use of natural allergen extracts, such as insufficient quality, allergenic activity, and poor immunogenicity. Here we provide an update of clinical studies with recombinant allergen-based vaccines, showing that some of these vaccines have undergone successful clinical evaluation up to phase III studies. Furthermore, we introduce a strategy for allergen-specific immunotherapy based on recombinant fusion proteins consisting of viral carrier proteins and allergen-derived peptides without allergenic activity, which holds the promise of being free of side effects and eventually being useful for prophylactic vaccination. (*J Allergy Clin Immunol* 2011;127:860-4.)

Key words: Allergy, allergen-specific immunotherapy, recombinant allergen

One hundred years ago, Leonard Noon¹ conducted and published the first clinical trial using grass pollen extract for desensitization of patients with hay fever. This study was conducted without knowledge regarding the pathomechanisms of the disease or the mechanisms underlying the treatment. However, the Noon study demonstrated clinical efficacy in the form of a reduction in symptoms and increased tolerance to allergen exposure determined by means of conjunctival provocation testing, and the duration of effects lasted for approximately 1 year.

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Abbreviation used

SIT: Allergen-specific immunotherapy

Shortly after the trial conducted by Noon, Cooke et al² conducted successful clinical trials in the United States and in 1935 published a classical work demonstrating that allergen-specific immunotherapy (SIT) induces a protective allergen-specific serum factor, today known as blocking IgG antibodies, which inhibits allergic patients' IgE antibody binding to the allergen.^{3,4}

Since then, numerous clinical trials have demonstrated the many advantages of SIT over conventional pharmacotherapy, which only ameliorates the symptoms of allergy. By contrast, SIT has been shown to be antigen specific, disease modifying, and long lasting.³ However, the insufficient quality of natural allergen extracts, such as the lack of important allergens, the instability and varying amounts of allergens, the presence of contamination and undefined nonallergenic materials, or the poor immunogenicity of certain allergens, represents a major hurdle for global allergy vaccination that cannot be overcome with vaccines based on natural allergen extracts.⁵ The second major problem is that the administration of allergens can induce severe and life-threatening side effects and hence requires cumbersome up dosing schemes based on either multiple injections or hospitalization for rush protocols. In particular, the latter disadvantages prevent the broad applicability of SIT.

Today, the allergen-encoding cDNAs coding for the clinically most relevant allergens have been isolated, and recombinant allergens equaling the natural allergens can be produced in large quantities and at consistent quality (Fig 1).⁴ As indicated in Fig 1, recombinant wild-type allergens, such as the corresponding natural allergen, contain mainly conformational IgE epitopes that are composed of different sequence elements (Fig 1, red and green). In the correctly folded allergen, these parts are close to the molecule's surface and thus can be recognized by IgE antibodies as part of conformational epitopes. Natural allergen extracts can thus be substituted by pure recombinant wild-type allergens or single recombinant fusion proteins consisting of several wild-type allergens.⁶ The use of recombinant wild-type allergens for the formulation of allergy vaccines basically allows elimination of all of the problems related to poor quality of natural allergen extracts but leave open the problem of potential side effects.

Seminal work carried out by Marsh et al⁷ has led to the conversion of natural allergens into "allergoids" with reduced IgE reactivity and allergenic activity, which still induced allergen-specific blocking IgG antibodies on immunization. With the aid of recombinant DNA technology, it is now possible to convert allergens into defined hypoallergens by means of manipulations that reduce or abolish the allergen's

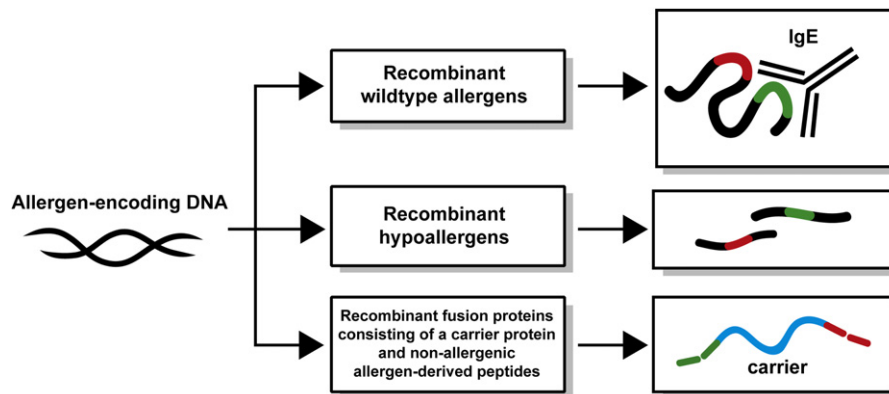


FIG 1. From allergen-encoding DNA to recombinant allergen-based vaccines. Allergen portions constituting conformational IgE epitopes on wild-type allergens are indicated in *red* and *green*, with the remaining allergen sequence in *black*. In fusion proteins large parts of the allergen are replaced by a foreign carrier protein (*blue*).

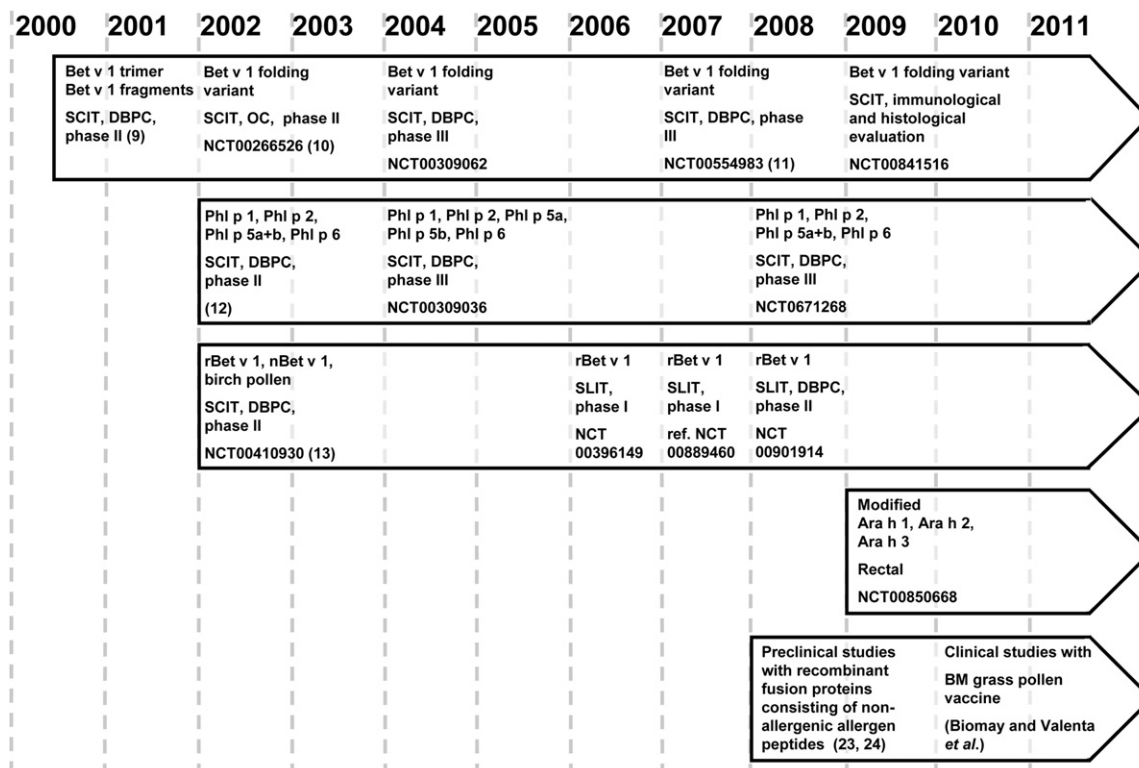


FIG 2. Currently ongoing development lines for recombinant allergen-based vaccines. NCT numbers identify the trials that are registered in the National Institutes of Health Clinical trial database (<http://clinicaltrials.gov>). DBPC, Double-blind, placebo-controlled; OC, open controlled; SCIT, subcutaneous immunotherapy; SLIT, sublingual immunotherapy.

IgE reactivity but leave the T cell-reactive sequences (Fig 1, black) almost unaltered. For example, fragmentation, reassembly in the form of mosaics and mutations, allows disruption of the conformational IgE epitopes or IgE-reactive portions.^{4,8}

Recombinant allergen-based vaccines were administered to allergic patients for the first time 10 years ago.⁹

STATE OF RECOMBINANT ALLERGEN-BASED CLINICAL IMMUNOTHERAPY STUDIES

Several clinical studies with recombinant allergens or allergen derivatives have been performed or are currently ongoing. These trials, which are graphically displayed in Fig 2, are mainly organized around a few strategic development lines that should lead to registered vaccines.

RECOMBINANT HYPOALLERGENIC BET V 1 FOR SUBCUTANEOUS IMMUNOTHERAPY

In the first clinical immunotherapy study with recombinant allergen preparations, 2 different hypoallergenic derivatives of the major birch pollen allergen Bet v 1 and placebo were compared.⁹ In a series of skin test studies, both preparations had shown a more than 100-fold reduced allergenic activity.⁴ In a double-blind, placebo-controlled study performed in 124 patients, the 2 preparations were adsorbed to aluminum hydroxide and administered as a single preseasonal treatment course for birch pollen allergy by using 8 doses increasing weekly up to a maximum maintenance dose of 80 µg of protein. Active treatment induced robust induction of IgG1, IgG2, and IgG4 antibodies directed against the Bet v 1 molecule in the peripheral blood and in nasal secretions. These IgG antibodies blocked allergen-induced basophil degranulation and were associated with the ability of patients to tolerate higher allergen concentrations in nasal provocation tests. Because of the encouraging results, the development of a hypoallergenic vaccine for birch pollen allergy based on a folding variant obtained by means of denaturation of rBet v 1 was started. This folding variant basically resembled the features of the originally tested Bet v 1 hypoallergens and has now been moved successfully up to a double-blind, placebo-controlled phase III trial in 226 patients, which was performed over a treatment course of 18 months.^{10,11} As reported by Rak¹¹ at the 27th Congress of the European Academy for Allergology and Clinical Immunology in Warsaw in 2009, a statistically significant and clinically relevant reduction of symptom medication scores was observed in the actively treated group compared with scores seen in the placebo-treated group. Again, the vaccine was reported to have a good safety profile and induced strong increases in Bet v 1-specific IgG1 and IgG4 antibody levels. According to the entries in the www.clinicaltrials.gov database (Fig 2), another phase III study enrolling 800 patients was initiated in 2007, and therefore a registered vaccine can be expected on the European market.

RECOMBINANT WILD-TYPE TIMOTHY GRASS POLLEN ALLERGENS FOR SUBCUTANEOUS IMMUNOTHERAPY

In a first study initiated in 2002, a group of 62 patients with grass pollen allergy was treated either with a combination of the major recombinant grass pollen allergens Phl p 1, Phl p 2, Phl p 5a, Phl p 5b, and Phl p 6 or with placebo.¹² After 18 months of treatment, actively treated patients fared significantly better regarding the primary end point of the study, the symptom medication score, and had high IgG antibody levels against natural grass pollen allergens.¹² A phase III study using the mixture of 5 recombinant grass pollen allergens was started in 2004, and another phase III study was scheduled for 2008 (Fig 2).

RBET V 1 FOR SUBLINGUAL IMMUNOTHERAPY

Immunotherapy with wild-type rBet v 1 has also been examined, with one of the aims being to establish that it can be effective and further developed for tablet-based sublingual immunotherapy. This proof-of-principle phase II, multicenter, double-blind, placebo-controlled injection immunotherapy trial comparing rBet v 1, natural Bet v 1, birch pollen extract, and

aluminum hydroxide was performed over a period of 2 years in 134 patients.¹³ One of the goals of this trial was to investigate whether rBet v 1 can substitute a natural birch pollen extract and hence could be used for sublingual immunotherapy. This study found in both study years a significant reduction in symptoms, medication consumption, and skin sensitivity in the rBet v 1-treated group, which was associated with increases in Bet v 1-specific IgG antibodies. In the rBet v 1-treated group no IgE specificities against new birch pollen allergens were induced, whereas 3 of 29 birch pollen extract-treated patients had IgE antibodies against minor birch pollen allergens.¹³ On the basis of the encouraging results obtained with rBet v 1, a series of clinical trials with rBet v 1 tablets for SLIT has been initiated (Fig 2).¹⁴

RECOMBINANT MODIFIED PEANUT ALLERGENS FOR RECTAL IMMUNOTHERAPY

The National Institutes of Health's clinical trial database also contains information regarding a study that intends to use the recombinant modified peanut allergens Ara h 1, Ara h 2, and Ara h 3 encapsulated in heat/phenol-killed *Escherichia coli*. This phase I study should recruit healthy volunteers to receive 4 escalating doses of the modified peanut preparation rectally at weekly intervals. Thereafter study participants with peanut allergy should be treated.

STATE AND POSSIBLE IMPROVEMENT OF RECOMBINANT ALLERGEN-BASED APPROACHES

On the basis of the clinical trial results obtained with recombinant hypoallergens and wild-type allergens, the induction of IgG antibodies that block allergic patients' IgE binding to the natural allergens and allergen-induced allergic inflammation has been identified as one key mechanism common to all forms of injection immunotherapy. Furthermore, these IgG antibodies inhibited IgE-facilitated allergen presentation and boosting of IgE responses induced by natural allergen exposure.^{9,15} Because similar results were obtained for birch and grass pollen allergens, it is possible that recombinant allergen-based vaccines can be developed for the most common allergies, including respiratory, food, and venom allergies. It will only be necessary to identify the clinically most relevant allergens of each of the allergen sources and convert them into hypoallergens by using established methodologies to achieve this goal.

Clinical studies with the recombinant hypoallergens showed that these molecules had reduced IgE-mediated side effects or even lacked these effects altogether. It thus seems that anaphylactic side effects can be avoided with hypoallergen-based vaccines. However, late-phase side effects, most likely caused by the activation of allergen-specific T cells, were observed.¹⁶ In fact, an atopy patch test study performed with non-IgE-reactive rBet v 1 fragments revealed that these derivatives can induce T cell-dependent delayed-type hypersensitivity reactions in the skin.¹⁷ Therefore there seems to be room still for improvement regarding the elimination of side effects, but this might require attempts to eliminate allergen-specific T-cell epitopes from the vaccine. However, mechanistic clinical trials will be necessary to study the importance of antibody-based and T cell-targeting SIT approaches for different forms of SIT (eg, food allergy, chronic allergic inflammation, and prophylactic

vaccination). One possible approach to maintain the beneficial effect of inducing allergen-specific blocking IgG and to avoid the problem of T cell-mediated side effects is introduced in the next section.

VACCINES CONSISTING OF NONALLERGENIC PEPTIDES FUSED TO CARRIERS: TOWARD SIDE EFFECT-FREE THERAPEUTIC AND PREVENTIVE ALLERGY VACCINES

With the aim of focusing blocking IgG antibodies toward sequences within conformational IgE epitopes, we have developed carrier-based allergy vaccines.^{18,19} For this purpose, non-allergenic peptides with a length of approximately 20 to 35 amino acids derived from conformational IgE epitopes (Fig 1, *red and green*) were chemically coupled to a carrier protein (ie, keyhole limpet hemocyanin). According to the hapten-carrier principle used by the Nobel laureate Benacerraf, which led to the discovery of T-cell help,²⁰ robust IgG antibody responses can be induced against small molecules, such as peptides, with T-cell help derived from the carrier molecule. Using keyhole limpet hemocyanin-coupled nonallergenic peptides from the major timothy grass pollen allergen Phl p 1, the major birch pollen allergen Bet v 1, and, recently, the major olive pollen allergen Ole e 1, we could induce allergen-specific blocking IgG responses on immunization of animals, suggesting that it might be possible to use this principle for allergy vaccination.^{18,19,21} In fact, it was also demonstrated that a nonallergenic peptide from the major house dust mite allergen Der p 1 induced allergen-specific IgG antibodies on vaccination of nonallergic subjects after chemical coupling to the bacteriophage protein Q β .²²

However, chemical coupling of allergen-derived peptides is a cumbersome process that can lead to a variety of reaction products that cannot be well defined. A possible solution to this problem is the expression of recombinant fusion proteins consisting of a nonallergenic carrier protein and allergen-derived peptides without allergenic activity using *E coli* as the expression system. By using the rhinovirus-derived coat protein VP1 and the PreS domain of hepatitis B as carrier proteins, non-allergenic fusion proteins for vaccination of grass pollen and cat allergy have been recently developed^{23,24} and produced (unpublished data; Biomay AG, Vienna, Austria). It could be shown that by applying a high-cell-density cultivation process (fed-batch fermentation), these molecules can be manufactured at high quantities under the conditions of good manufacturing practice. These vaccines are nonallergenic and induce allergen-specific IgG-blocking antibodies similarly to recombinant hypoallergen-based vaccines. However, because these vaccines contain only minor portions of the allergen sequences, the presence of allergen-specific T-cell epitopes and thus the potential to induce T cell-mediated side effects should be strongly reduced. The pre-clinical evaluation of a vaccine consisting of PreS of hepatitis B²⁴ fused to nonallergenic peptides from the 4 major timothy grass pollen allergens Phl p 1, 2, 5, and 6, designated BM32, has been finished. The lack of induction of IgE- and T cell-mediated inflammation of the candidate vaccine will be studied by means of skin prick and atopy patch testing in mid-2011 and, provided that these data confirm *in vitro* and animal data, be followed by a safety and dose-finding immunotherapy trial in patients with grass pollen allergy at the end of 2011.

If one looks into the crystal ball and tries to predict what recombinant allergens hold for the future of SIT, one can safely say that the technology for producing high-quality vaccines devoid of anaphylactic activity is now well established. Therefore vaccines for the most common allergen sources based on hypoallergenic molecules can be developed and made available to the patients with sufficient efforts by the pharmaceutical industry. Moreover, there is an advanced technology on the horizon (ie, fusion proteins consisting of carrier-bound allergen-derived peptides) that promises to avoid both IgE- and T cell-mediated side effects⁵ and therefore might be used for broad therapeutic allergy vaccination and eventually be useful for prophylactic vaccination.

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