Recombinant allergens for specific immunotherapy

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Recombinant DNA technology provides the means for producing allergens that are equivalent to their natural counterparts and also genetically engineered variants with reduced IgE-binding activity. The proteins are produced as chemically defined molecules with consistent structural and immunologic properties. Several hundred allergens have been cloned and expressed as recombinant proteins, and these provide the means for making a very detailed diagnosis of a patient's sensitization profile. Clinical development programs are now in progress to assess the suitability of recombinant allergens for both subcutaneous and sublingual immunotherapy. Recombinant hypoallergenic variants, which are developed with the aim of increasing the doses that can be administered while at the same time reducing the risks for therapy-associated side effects, are also in clinical trials for subcutaneous immunotherapy. Grass and birch pollen preparations have been shown to be clinically effective, and studies with various other allergens are in progress. Personalized or patient-tailored immunotherapy is still a very distant prospect, but the first recombinant products based on single allergens or defined mixtures could reach the market within the next 5 years.
(J Allergy Clin Immunol 2011;127:865-72.)

Key words: *Recombinant allergen, allergen immunotherapy, allergen vaccines, personalized immunotherapy, subcutaneous immunotherapy, sublingual immunotherapy, hypoallergenic variants*

Abbreviations used
DBPC: Double-blind, placebo-controlled
SMS: Symptom-medication score

The first allergens to be cloned with recombinant DNA technology were Dol m 5 from the white-faced hornet *(Dolichovespula maculata)*,[1] Bet v 1 from birch pollen *(Betula verrucosa)*,[2,3] and Der p 1 from the house dust mite *Dermatophagoides pteronyssinus*.[4] Now some 20 years later, several hundred have been cloned and expressed in various systems, including bacteria, yeasts, insect viruses, and plants.

One of the main advantages of recombinant proteins is that they can be fully characterized in terms of their physical, chemical, and immunologic properties and presented as chemically defined entities (Table I), with all batches stemming from 1 master cell bank (Fig 1). Preparations for specific immunotherapy and diagnosis can then be formulated with consistent high pharmaceutical quality to meet more stringent specifications than can normally be achieved with products based on extracts of natural source materials. In the latter case the relative concentrations of various allergens are dictated by the source material, except in those instances in which some postextraction purification is undertaken. In practice, it is usually only realistic to define the activity of an allergen extract in terms of its total IgE-binding activity and the concentration of 1 major allergen. Recombinant products, on the other hand, can be defined with respect to the concentration and activity of each component and the optimal dose for the required application. In addition, recombinant preparations contain only allergens and none of the nonallergenic proteins and polysaccharides present in extracts of natural source materials. Some of the difficulties posed by working with natural source materials, such as the need to demonstrate the lack of contamination of pollen preparations with foreign pollens or pesticides, can be avoided.[5] Recombinant forms of animal allergens might very well find greater acceptance than extracts of natural tissue, thus increasing the practice of immunotherapy for cat allergy, for example. Preparations derived from raw materials that are difficult to collect (eg, yellow jacket and hornet venoms) could be replaced by recombinant products.

The availability of high-quality recombinant allergens is providing new opportunities to obtain a detailed understanding of the nature of sensitization and the cross-reactivity between different allergens, thereby allowing more informed choices to be made regarding strategies for allergen-specific immunotherapy. The use of recombinant DNA technology does not stop with allergens *per se*; it also provides the means to genetically engineer allergen variants embodying features such as reduced IgE reactivity or enhanced immunogenicity. This is attractive from the point of view of enhancing the safety of immunotherapy and facilitating administration of higher doses.

In some instances allergenic source materials contain just 1 major or dominant allergen, such as Fel d 1 from cat *(Felis domesticus)* and Bet v 1 from birch pollen. However, in most cases several allergens are involved. For example, 11 different allergens have been characterized and cloned from sweet grasses, including timothy grass *(Phleum pratense)* and rye grass *(Lolium perenne)*,[6] and for the house dust mites *D pteronyssinus* and *Dermatophagoides farinae*, the number is in excess of 20.[7] Efforts to develop therapeutic preparations are being focused on those major allergens that account for the larger part of the IgE reactivity to the particular source material. If this approach does not achieve consistent clinical benefit, then recombinant products would be placed at a disadvantage, unless of course customized or personalized products can be developed. The first products to reach the market will be for allergies to grass pollen, tree pollen (birch), and house dust mite, with short ragweed *(Ambrosia artemisiifolia)*, wall pellitory (*Parietaria judaica* or *Parietaria officinalis*), Japanese cedar *(Cryptomeria japonica)*, and cat *(F domesticus)* likely

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Disclosure of potential conflict of interest: The authors have declared that they have no conflict of interest.
Received for publication October 18, 2010; revised December 16, 2010; accepted for publication January 21, 2011.
Available online March 9, 2011.
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0091-6749/$36.00

doi:10.1016/j.jaci.2011.01.047

TABLE I. Advantages and disadvantages of proteins produced with recombinant DNA technology

Advantages
Molecules with defined amino acid sequence
Preparations of consistent pharmaceutical quality
All batches of one allergen derive from the same master cell bank
Avoidance of possible contamination and the risk of infectious agents
Dosage in mass units in respect of all components: absolute standardization
Inclusion of only the relevant proteins
Optimization of the dosage of all components of a preparation
Possibility to tailor preparations to a patient's sensitization profile
Precise monitoring and investigation of mechanisms underlying treatment
Option to create genetically engineered variants (eg, with reduced IgE reactivity)
Disadvantages
Each allergen has to be developed by using a specific approach.
For those allergens occurring in many isoforms, there is a need to choose the most relevant.
It might be necessary to include >1 isoallergen in cases of limited identity.
There are high development costs in relation to limited market potential.

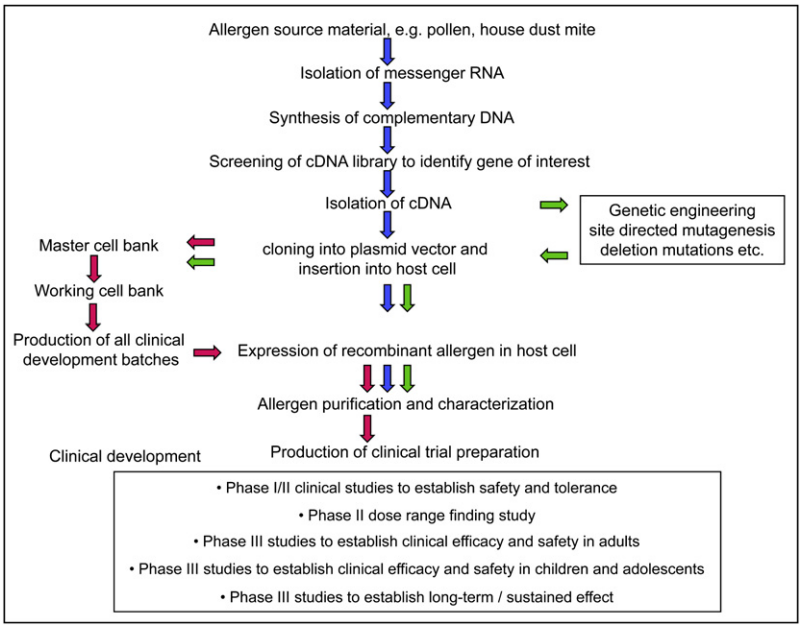

FIG 1. Development of recombinant allergen products for specific immunotherapy. *Blue arrows*, Cloning, expression, and characterization of recombinant allergen. *Green arrows*, Development and characterization of hypoallergenic variants. *Red arrows*, Production of clinical trial preparations and future products all stemming from a single master cell bank.

to follow. The complete development program for such preparations is depicted in Fig 1.

WILD-TYPE RECOMBINANT ALLERGENS

The term wild-type recombinant allergen is often used to describe the equivalent of the natural unmodified allergen in respect to both structural and immunologic characteristics. In practice, it might not always be possible to achieve the desired result with a prokaryotic bacterial expression system, such as *Escherichia coli*, and indeed, different production conditions can have a strong influence on the characteristics of the recombinant allergen, as shown recently for an isoform of the birch pollen Bet v 1.[8] It might be necessary to accept some structural differences or alternatively to undertake modifications to achieve satisfactory expression of the recombinant protein or to switch to eukaryotic systems, such as the yeasts *Saccharomyces cerevisiae* and *Pichia pastoris*, or baculovirus. Furthermore, bacterial expression systems lack the ability to perform protein glycosylation, and therefore allergens that normally occur as glycoproteins are expressed devoid of carbohydrate moieties. In the case of the glycoprotein Phl p 1, the group 1 allergen of the grass *P pratense*, expression without the carbohydrate component appears not to have any appreciable effect on IgE antibody binding or T-cell reactivity[9,10] or on the ability to induce allergen-specific IgG1 and IgG4 responses.[11] On the other hand, whereas approximately 50% of sera from subjects with grass pollen allergy showed IgE reactivity toward natural glycosylated Phl p 13, only 21% reacted with the