

Recombinant Allergens for Immunotherapy

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ABSTRACT

Many of the problems associated with using natural allergenic products for allergy diagnosis and treatment can be overcome using genetically engineered recombinant allergens. Over the past 10 years, the most important allergens from mites, pollens, animal dander, insects, and foods have been cloned, sequenced, and expressed. Allergens have diverse biological functions (they may be enzymes, enzyme inhibitors, lipocalins, or structural proteins). High-level expression systems have been developed to produce recombinant allergens in bacteria, yeast, or insect cells. Recombinant allergens show comparable immunoglobulin E (IgE) antibody binding to natural allergens and show excellent reactivity on skin testing and in in vitro diagnostic tests. Recombinant allergens will enable innovative new strategies for allergen immunotherapy to be developed. These include peptide-based vaccines, engineered hypoallergens with reduced reactivity for IgE antibodies, nucleotide-conjugated vaccines that promote Th1 responses, and the possibility of developing prophylactic allergen vaccines. (Allergy and Asthma Proc 23:5–8, 2002)

Allergen immunotherapy has been the mainstay for the treatment of allergic diseases for nearly 100 years, since Noon¹ first described the treatment in his article,

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“Prophylactic Immunization Against Hay Fever.” Immunotherapy involves the injection of progressively increasing doses of natural allergen extracts, usually out of season, until a maintenance dose is reached. Patients receive monthly maintenance doses and continuation of therapy is accompanied by symptomatic improvement and reduced medication use. Controlled trials have shown the efficacy of immunotherapy for seasonal pollen hay fever (reviewed in Bousquet *et al.*²) and immunotherapy is the treatment of choice for insect venom allergy. Immunotherapy also has been reported to reduce symptom scores, bronchial challenge, and medication use in asthma.² The efficacy of immunotherapy in asthma remains controversial; some studies have shown marginal improvements, compared with well-controlled pharmacotherapy.³ Durham and colleagues clearly established that immunotherapy for grass pollen hay fever has beneficial long-term effects and modulates immune responses for several years after treatment has been discontinued.⁴

The natural allergenic products used for immunotherapy are extremely heterogeneous. In addition to the protein allergens, they also contain many nonallergenic proteins and other macromolecules. Allergen manufacturers have significantly improved the quality and consistency of allergenic products and, in the United States, Food and Drug Administration (FDA) licensed standardized products are marketed for the major allergen groups: grass pollen, ragweed pollen, mites, and cat dander. However, it is inherently difficult to standardize mixtures of biological products and there is evidence that cross-contamination on natural allergenic products can occur. Recent studies suggest that the efficacy of immunotherapy is improved if patients tolerate maintenance doses of 5–20 μ g of major allergen.^{2,5} The problem with natural allergenic products is that the major allergen content, even among standardized products, can vary by severalfold. It is not possible to formulate precisely a natural extract with defined allergen concentrations. Many of these problems can be overcome by the use

of genetically engineered recombinant allergens. Moreover, the cloning of allergen sequences has allowed the structures of most important allergens to be determined and this has lead to innovative new approaches to immunotherapy.

RECOMBINANT ALLERGENS—STRUCTURE AND FUNCTION

Molecular cloning studies have determined the amino acid sequences of over 500 allergens from pollens, mites, animal danders, insects, molds, and foods. High-level expression systems vectors for producing recombinant allergens in bacteria or in eucaryotes have been developed. In most cases, the immunoreactivity of recombinant allergens is comparable with their natural counterparts, as assessed by immunoglobulin E (IgE) antibody binding *in vitro* and by skin testing (reviewed in Chapman *et al.*⁵ and Valenta *et al.*⁶). The tertiary structures of ~20 allergens have been determined by nuclear magnetic resonance spectroscopy, x-ray crystallography, or molecular modeling.⁵ The structural information has allowed amino acids involved in antibody binding and T-cell epitopes to be identified. Sequence homology searches have revealed the biological function of many important allergens and initiated studies on the role of biological activity in promoting IgE responses and contributing to inflammatory responses in the respiratory tract.⁷ Allergens belong to diverse protein families and may be enzymes, enzyme inhibitors, ligand binding proteins, or regulatory or structural proteins.⁵

The advantages of recombinant allergens are that they have defined purity, biological activity, and composition; they can be formulated precisely; engineered and targeted; and they can be produced under GMP conditions as biopharmaceuticals. Cocktails of two to four recombinant allergens can be formulated at defined concentrations and used for allergy diagnosis. The use of selected, pure recombinant allergens also will allow rapid *in vitro* tests to be developed for diagnostic purposes. In addition, there are exciting prospects for using recombinant allergens in immunotherapy, but before considering these possibilities, it is worth briefly considering the immunologic mechanisms to be targeted if immunotherapy is to be successful. Generally, it is accepted that immunotherapy modulates allergen-specific T-cell responses, resulting in altered patterns of cytokine production. T-cell strategies for immunotherapy include clonal deletion by apoptosis (in early life), induction of anergy and linked suppression by T regulatory cells, and immune deviation from Th2 to Th1 phenotypes.^{8–10} Recent evidence suggests that successful immunotherapy is associated with increased interleukin 10 (IL-10) production, with an associated decrease in IL-4 and IL-5.^{4,11,12} The production of IL-10 enhances the production of IgG4 antibodies, which increase during pollen immunotherapy and are associated with clinical improvement. Reductions in the prevalence of IgE responses after high-dose natural allergen exposure also are associated with increased IgG4 produc-

tion.¹³ Several modifications of recombinant allergens are being investigated as vehicles to alter T cell and antibody responses: peptide based vaccines, hypoallergens, plasmid DNA vaccines, and high-dose immunization regimens.

PEPTIDE-BASED VACCINES

The rationale for T-cell peptide-based vaccines is that administration of high-dose peptide will induce anergy or unresponsiveness in allergen-specific T cells in the absence of co-stimulatory signals that provide T-cell help for antibody production. In animal studies, mice immunized with a major T-cell epitope of Der p 1 were refractory to subsequent challenge with Der p 1 that was administered in Freund's adjuvant. Moreover, T-cell responses to an unrelated antigen (ovalbumin) were suppressed also, providing evidence for "linked suppression" of T-cell responses.^{9,14} In the 1990s, extensive clinical trials of two immunodominant T-cell epitopes of cat allergen Fel d 1 met with mixed success. High doses of peptide (75–750 μ g) administered over a 4-week period reduced nasal and lung symptom scores and increased tolerance to cat allergen exposure. However, ~20% of patients developed late reactions to the peptides and a few patients had adverse reactions that required systemic epinephrine.^{15–17} The 27 amino acid peptides used in these studies were large enough to elicit IgE responses in some patients and, ultimately, the trials were discontinued. Interest in T-cell-based peptides recently has been rekindled by the observations that intradermal injection of multiple, small (12–14 amino acid) Fel d 1 peptides into the skin induced hyporesponsiveness to bronchial challenge with whole allergen.¹⁸ Cat allergen is an attractive target for developing peptide-based therapies because only one major allergen Fel d 1 has been identified. The approach is less feasible for multiallergen systems such as dust mites and pollens, which would involve the synthesis of large numbers of peptides.

ENGINEERED HYPOALLERGENS

The aim of this study was to generate allergen variants that show reduced binding to IgE, yet retain the majority of T-cell epitopes, so-called "hypoallergens." The approach was first developed for dust mite allergen Der p 2, in which it was found that genetically engineered mutants without disulfide bonds showed 10–100 reduced IgE antibody binding *in vitro* and on skin testing.¹⁹ Subsequent studies on other group 2 allergens confirmed these observations and the approach has since been extended to include important grass pollen, tree pollen, and peanut allergens.^{20–28} Point and deletion mutants of grass pollen allergen Phl p 5 showed 10- to 100-fold reductions in IgE reactivity but had conserved T-cell epitopes. Similarly, point mutants, fragments, or dimers and trimers of Bet v 1 showed markedly reduced skin test reactivity without affecting *in vitro* T-cell proliferation.^{23–27} Promising results have been obtained using hypoallergens in preclinical stud-

ies and it will be interesting to see the results of ongoing clinical trials of these modified allergens. The advantages of these products, as compared with chemically modified allergoids, are that the precise amino acid changes in the allergen molecules can be defined and monitored.

PLASMID DNA VACCINES

Injection of bacterial DNA into animals results in immune responses characteristic of Th1 lymphocytes. Moreover, injection of a bacterial plasmid containing the coding sequence for another protein will preferentially skew the antiprotein response to a Th1 phenotype. These observations were exploited by Raz *et al.*, who showed that murine IgE responses to β -galactosidase could be abrogated by injection of plasmids containing the antigen.²⁹ The active “immunostimulatory” component of the plasmid DNA is a particular cytosine-guanine (CpG) nucleotide motif and subsequent studies have shown that conjugation of CpG oligonucleotides to allergen can down-regulate IgE responses. As indicated previously, the mechanism of action is thought to be through immune deviation from Th2 to Th1. Animal experiments have shown that plasmid DNA vaccines can suppress ongoing IgE responses, reduce allergen-induced histamine release, and bronchial hyperreactivity.^{29,30} There is also evidence that conjugation of multiple CpG motifs reduced the allergenicity of Amb a 1 in animal models, while enhancing Th1 responses to the allergen.³¹ Phase 1 trials of CpG conjugates of Amb a 1 and Cry j 1 are being carried out in several U.S. centers. There also is interest in extending the approach to other allergens, including foods.²⁸

HIGH-DOSE “PROPHYLACTIC” VACCINES

Recent evidence suggests that natural exposure to high levels ($>20 \mu\text{g/g}$) of cat allergen Fel d 1 leads to a reduction in the prevalence of IgE responses and is associated with boosting of allergen-specific IgG4.¹³ This “modified Th2” response may explain why the overall prevalence of IgE responses to cat is low (*e.g.*, compared with mite or pollen allergens) despite the fact that cat allergen is ubiquitous in the environment. An obvious question is whether this kind of natural (“protective”) immunization can form the basis of a new therapeutic strategy. Would high-dose (mg) immunization in infancy (aged <12 months) induce the production of IgG1 and IgG4 antibodies while protecting against the production of IgE antibody? This approach to prophylactic treatment could not have been considered previously because the levels of Fel d 1 in natural allergen products are too low. Cats are an attractive target to test this approach for “proof of principle” because the allergen is widely distributed in the environment and difficult to avoid (many patients who develop a cat allergy have never owned a cat). Although there are obvious ethical considerations to be considered in this approach, the concept is important because if IgE responses in humans can be suppressed by

high-dose immunization (either using allergen alone or using allergen + CpG nucleotides or other adjuvants), allergy treatment would be changed radically to a true preventative therapy.

CONCLUSIONS

The use of recombinant allergens will facilitate the development of innovative approaches to allergen immunotherapy. The introduction of recombinant allergens into clinical practice will require scientific studies, clinical trials, and regulatory approvals, as well as substantial commercial investment. At present, most recombinant allergens are being produced for research purposes only. An important element will be to manufacture these proteins under good manufacturing practice or good clinical practice conditions so that they can be incorporated into clinical trials. Most biotechnology companies develop a single protein product for treatment, *e.g.*, recombinant insulin or growth hormone. One of the challenges for allergen biotechnology will be to bring together the production of several recombinant proteins into a single product, *e.g.*, for dust mite or pollen allergens. Only a small number of allergens are of sufficient importance to make such an investment worthwhile and one can envisage the gradual introduction of selected recombinant proteins over the next 5–10 years. This scenario is well timed to allow the first recombinant allergen vaccines to be marketed in 2011 on the 100th anniversary of Noon’s¹ classic article on immunotherapy!

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