New millennium: The conquest of allergy

(Supported by a grant from Novartis Pharmaceutical Corp., East Hanover, NJ)

Series editors: Donald Y. M. Leung, MD, PhD, Stanley J. Szefler, MD, and Harold S. Nelson, MD

Recombinant allergens for diagnosis and therapy of allergic disease

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Many of the problems associated with using natural allergenic products for allergy diagnosis and treatment can be overcome with use of 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. In many cases the three-dimensional allergen structure has been determined and B-cell and T-cell epitopes have been mapped. These studies show that allergens have diverse biologic functions (they may be enzymes, enzyme inhibitors, lipocalins, or structural proteins) and that as a rule the allergen function is unrelated to its ability to cause IgE antibody responses. High-level expression systems have been developed to produce recombinant allergens in bacteria, yeast, or insect cells. Recombinant allergens show comparable IgE antibody binding to their natural counterparts (where available) and show excellent reactivity on skin testing and in in vitro diagnostic tests. Cocktails of recombinant allergens can be formulated with predetermined and uniform allergen levels, which could replace natural allergens and result in the development of innovative, patient-based tests for allergy diagnosis. Recombinant allergens also offer the exciting possibility of developing new forms of allergen immunotherapy, including the use of hypoallergens, allergens coupled to IgE suppressive adjuvants, and peptide-based therapies. The production of recombinant allergens as defined molecular entities makes it feasible to consider the possibility of developing prophylactic allergen vaccines. The introduction of recombinant allergens in research and in clinical trials should lead to significant improvements in allergy diagnosis and treatment. (J Allergy Clin Immunol 2000;106:409-18.)

Key words: *Recombinant allergens, mites, animal allergens, allergy diagnostics, allergy therapeutics, asthma, allergy vaccines, allergen immunotherapy*

Allergists have relied on natural allergenic products for the diagnosis and treatment of allergic diseases since the turn of the century. The quality of natural allergen extracts has improved during the past 20 years as knowledge of the protein chemistry of allergens has grown and has been applied by manufacturers to produce more consistent products. Allergen standardization is no longer based on protein nitrogen units or weight/volume estimates. Biologic and immunochemical procedures have been introduced by regulatory authorities in the United States, and in Europe, to improve standardization of natural allergenic products. There is also good evidence from clinical trials that measurements of major allergens can be used to provide optimal dosing for allergen immunotherapy.[1,2]

However, allergens prepared from natural source materials remain heterogeneous products containing many nonallergenic proteins and other macromolecules that are parenterally administered together with a few active components, the protein allergens. Allergens produced from natural source materials vary in allergen composition and content (compare cat dander and cat pelt extracts or mite body and whole mite culture extracts). Natural products are also at risk of being contaminated with allergens from other sources and can contain proteolytic enzymes.[3] The enzymes may be allergenic or nonallergenic, but in either case can cause degradation and loss of potency when administered together with other allergens during immunotherapy.[4,5] Most allergen sources contain multiple major and minor allergens and, even with use of mod-

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Supported by National Institutes of Health grants No. AI 32557 and AI 34607. L. K. A. was supported by Fundaçao de Amparo à Pesquisa de Estado de Sao Paulo (FAPESP), Sao Paulo, Brazil, and is a recipient of Young Investigator Award from FAPESP.

Presented as the Carl E. Arbesman Memorial Lectureship (M. D. C.) at the annual meeting of the American Academy of Allergy, Asthma, and Immunology, March 2000.

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Received for publication June 13, 2000; revised June 29, 2000; accepted for publication July 3, 2000.

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0091-6749/2000 $12.00 + 0 **1/1/109832**

doi:10.1067/mai.2000.109832

TABLE I. Structure and biologic function of selected indoor allergens*

Source	Allergen	MW (kd)	Biologic function
House dust mite† *(Dermatophagoides pteronyssinus* and *Dermatophagoides farinae)*	Group 1‡§	24	Cysteine protease
	Group 2§	14	Epididymal or molting protein?
	Group 3	28-30	Serine protease
	Der p 4	56	Amylase
	Der p 5	14	Unknown
	Der p 6	25	Chymotrypsin
	Group 7	22-28	Unknown
	Der p 8	26	Glutathione-S-transferase
	Der p 9	24	Collagenolytic serine protease
	Der p 10	36	Tropomyosin
	Group 14	190	Apolipophorin-like protein
Euroglyphus maynei	Eur m 1	24	Cysteine protease
	Eur m 2‡	14	Epididymal or molting protein?
Blomia tropicalis	Blo t 5	14	Unknown
	Blo t 13	15-17	Fatty acid binding protein
Lepidoglyphus destructor	Lep d 2‡	14	Epididymal or molting protein?
Tyrophagus putresentiae	Tyr p 2‡	14	Epididymal or molting protein?
Mammalian allergens			
Cat, *Felis domesticus*	Fel d 1§	33-39	(Uteroglobin)?
	Albumin	66	Serum protein
Dog, *Canis familiaris*	Can f 1‡	16	Lipocalin (cysteine protease inhibitor?)
	Can f 2‡	18	Lipocalin
	Albumin	66	Serum protein
Rat, *Rattus norvegicus*	Rat n 1§	15-17	Lipocalin (pheromone binding protein)
Mouse, *Mus musculus*	Mus m 1	18	Lipocalin (odorant binding protein)
Cow, *Bos domesticus*	Bos d 2	19	Lipocalin
	Bos d 5	20	Lipocalin (β-lactoglobulin)
Horse, *Equus caballus*	Equ c 1	19	Lipocalin
	Equ c 2	18	Lipocalin
Cockroach allergens			
Blattella germanica	Group 1§	25-37	Unknown
	Bla g 2‡	36	Binding protein (inactive aspartic proteinase)
	Bla g 4‡	21	Lipocalin
	Bla g 5	23	Glutathione-S-transferase
Periplaneta americana	Group 1§	25-37	Unknown
	Per a 3§	72-78	Arylphorin-like protein
	Per a 7	33	Tropomyosin
Fungal allergens¶			
Aspergillus fumigatus	Asp f 1	18	Cytotoxin (mitogillin, a ribonuclease)
Alternaria tenuis	Alt a 1	30	Unknown
	Alt a 2	25	Enolase

MW, Molecular weight.

*Comply with criteria established by the WHO/IUIS Sub-committee on Allergen Nomenclature (see www.allergen.org).

†"Group" designation indicates that homologous allergens have been cloned from both *D pteronyssinus* and *D farinae*. Multiple isoform sequences of group 1 and group 2 allergens are deposited in GenBank. The group 14 cDNA includes sequences for the 177 kd, 40 kd, and 39 kd breakdown products (M-177, Mag3 and Mag1) that bind IgE and are found in natural extracts.

‡Three-dimensional structure defined by use of molecular modeling.

§Allergens for which one or more variants or isoforms have been reported.

[Three-dimensional structure determined by nuclear magnetic resonance (NMR) spectroscopy or by x-ray crystallography at high resolution. The Protein Data Bank files with the structural coordinates are as follows: Der p 2, 1A9V; Der f 2, 1AHK, 1AHM; Rat n 1, 2A2U, 2A2G; Mus m 1, 1MUP, 1DF3; Bos d 2, 1BJ7; Bos d 5, 1BEB, 1BSO, 1B0O; Equ c 1, 1EW3.

¶More than 20 allergen sequences have been cloned from *A fumigatus* and other *Aspergilli*. Multiple allergens have been cloned from other fungi, including *Alternaria, Cladosporium, Penicillium, Trichophyton, Candida, Malassezia,* and *Basidiomycetes* sp (see www.allergen.org for complete listing).

Abbreviations used
IUIS: International Union of Immunological Societies
NMR: Nuclear magnetic resonance spectroscopy
PAG: Pregnancy-associated glycoproteins
WHO: World Health Organization

ern techniques, it is difficult to standardize a mixture of different proteins. Recently, the number of allergenic products available in the United States has decreased as companies comply with Food and Drug Administration manufacturing practices.

We can improve on natural allergenic products by use of recombinant allergens. There has been tremendous

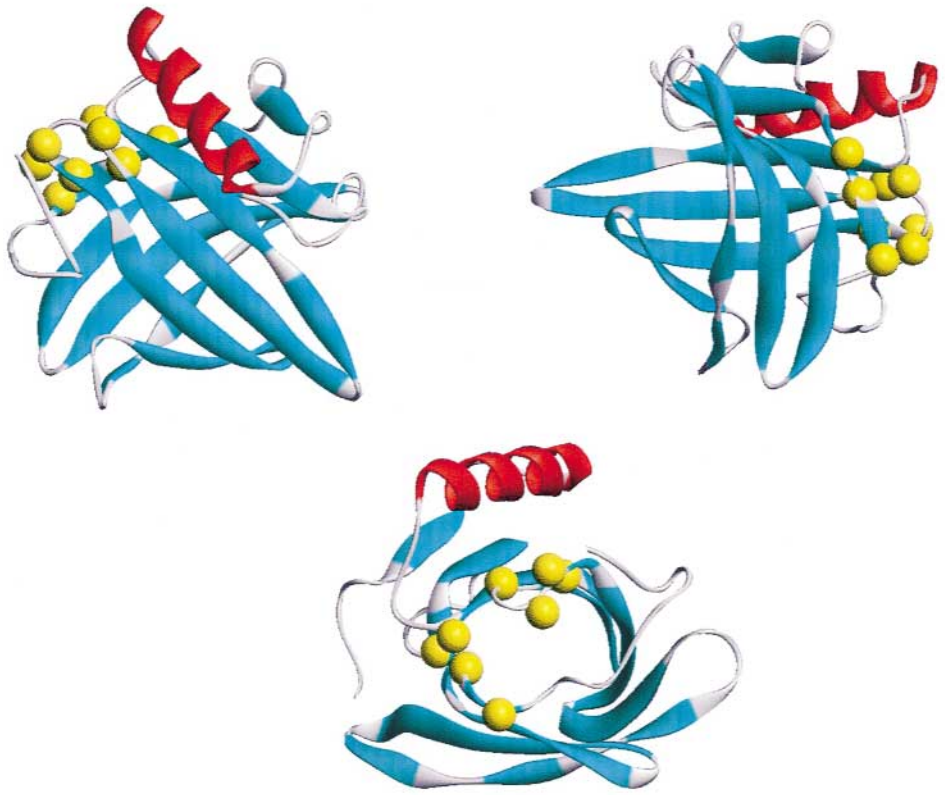

FIG 1. Tertiary structure of a lipocalin allergen: three spatial perspective views of rodent urinary allergen, Rat n 1 (chain A from 2A2U.pdb).[26] Spheres represent amino acids that form sequence motifs located in the three structurally conserved regions of lipocalins.[25] These residues are located at the base of the cup region of the molecule, which forms the ligand-binding pocket.

progress in the molecular biology of allergens over the past 10 years and all the important major (and minor) allergens have now been cloned[5-8] (Table I). Sequence homology searches have revealed the identity and putative biologic function of many allergens. Fully immunoreactive recombinant allergens are being expressed in bacteria, yeast, insect viruses, and plants. The three-dimensional structure of allergens, including Bet v 1, Der p 1, Der p 2, Bla g 2, Bos d 2, Equ c 1, and Ara h 1, has been determined by NMR techniques, x-ray crystallography, and computer-based molecular modeling.[9-16] For several allergens, the location of amino acid residues involved in antibody binding, as well as epitopes involved in T-cell activation, have been determined. More than 400 protein or nucleotide allergen sequences are now in the GenBank and other databases.[17] Allergens constitute one of the most widely studied families of proteins in biomedical research and it is anticipated that recombinant allergens will soon become incorporated into new products for allergy diagnosis and treatment.

This review draws mainly on studies of recombinant indoor allergens. Sensitization to these allergens is the strongest risk factor associated with asthma, and immunologic intervention to reduce the prevalence of asthma is a major challenge.[18] The premise here is that any new allergen-specific intervention will require the use of recombinant allergens with defined immunologic reactivity in terms of IgE antibody binding and T-cell reactivity.[18,19] Purified allergens (natural or recombinant) are also essential research tools to investigate the cellular mechanisms of immediate hypersensitivity and the molecular basis of inflammatory reactions. We conclude with discussion of potential strategies to use recombinant allergens in prophylactic vaccines to protect against allergic disease.

ALLERGENS BELONG TO SEVERAL PROTEIN FAMILIES AND HAVE DIVERSE BIOLOGIC FUNCTIONS

Sequence homology searches have revealed that allergens may fulfill a variety of biologic functions; they may be enzymes, enzyme inhibitors, ligand binding proteins (lipocalins), or structural proteins.[7,8,18,20,21] (Table I). Mites are a potent source of enzymatic allergens, most of which are involved in digestion and are excreted in the feces. These include Der p 1, Der p 3, and Der p 6, the cysteine, serine, and aspartic proteases, respectively. Mites also produce amylase (Der p 4) and glutathione transferase (Der p 8) allergens.[8]

Most animal allergens belong to the lipocalin (or calycin) family of proteins or are albumins. Lipocalins

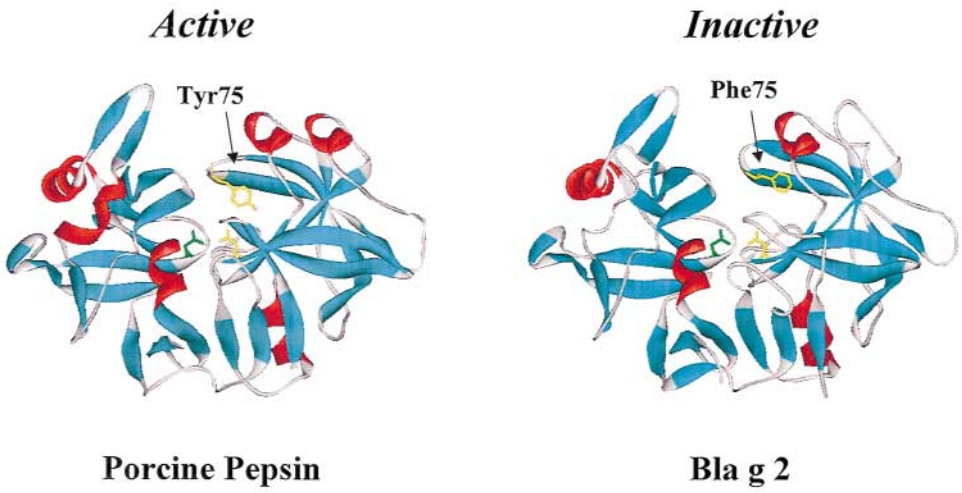

FIG 2. Comparison of the three-dimensional structure of an active aspartic protease (porcine pepsin) with the inactive cockroach aspartic protease allergen Bla g 2.[13,21,48,49] The active site of pepsin contains two identical catalytic triads (amino acid residues DTG) with the coplanar aspartate residues at positions 32 (in yellow) and 215 (green). In Bla g 2, the triad sequences are DST and DTS, which results in a loss of catalytic activity. The substitution of the tyrosine residue at position 75 for phenylalanine in Bla g 2 also contributes to the loss of aspartic protease activity in Bla g 2.[13,21]

are a family of ~20-kd proteins that bind and transport small hydrophobic chemicals. They include the rodent urinary allergens (Rat n 1 and Mus m 1) and allergens from dog (Can f 1 and Can f 2), cow (Bos d 2 and Bos d 5), and horse (Equ c 1 and Equ c 2)[22,23] (Table I). Cockroach allergen, Bla g 4, is also a lipocalin.[24] Although lipocalins show only ~20% amino acid sequence homology, they have a conserved tertiary structure comprising an N-terminal α-helix and an 8-stranded antiparallel β-barrel, with three structurally conserved regions (Fig 1).[25] The β-barrel encloses an internal cavity in which ligands are bound. The rodent urinary proteins are pheromone-binding proteins, whereas Bos d 5 (β-lactoglobulin) binds palmitate and retinol.[26,27] Dog allergen Can f 1 may act as a cysteine protease inhibitor and, recently, another cysteine protease inhibitor allergen, cystatin, has been cloned from cats.[28-30]

Among invertebrates the structural protein tropomyosin, is an important allergen in shrimp (Pen a 1), dust mite (Der p 10), and cockroach (Per a 7).[31-36] There is a striking lack of allergenic cross-reactivity between mammalian tropomyosins (found in dietary meats) and those of invertebrates. The invertebrate tropomyosins show ~80% homology, and cross-reactivity between these allergens may explain why patients on mite immunotherapy have food allergy symptoms on eating shellfish or snails.[37-39]

DOES BIOLOGIC FUNCTION PLAY A ROLE IN ALLERGENICITY?

The strongest evidence that biologic activity enhances the ability of a protein to induce IgE antibody responses has been obtained for Der p 1. Several in vitro studies have shown that Der p 1 can cleave the low-affinity IgE receptor CD23 from the surface of B cells and can cleave CD25, the α-subunit of the IL-2 receptor, from T cells.[40-44] Der p 1 is reported to cause detachment of bronchial epithelium and induce the release of proinflammatory cytokines (IL-6, IL-8, MCP1) from cultured bronchial epithelial cells.[45] Recent studies show that Der p 1 causes disruption of intercellular tight junctions and facilitates allergen traffic across the epithelium to dendritic antigen-presenting cells.[46] These studies suggest several mechanisms whereby Der p 1 could enhance allergenicity: by increasing the permeability of the respiratory mucosa, by enhancing antigen processing, by promoting IgE synthesis through the release of CD23, and by augmenting T_H2 responses through its effects on CD25. In addition to Der p 1, other mite protease allergens (Der p 3, Der p 6, Der p 9) are reported to cause detachment of pulmonary epithelial cells and release of inflammatory cytokines in vitro and to cause IgE-independent release of IL-4 and IL-13 from mast cells and basophils.[21,43,47]

Enzyme function per se is not necessary to induce IgE responses. The potent cockroach allergen Bla g 2 has sequence homology to aspartic proteases but has critical substitutions in the active site of the catalytic triad and in the "flap" region of the molecule, which suggests that the allergen is an inactive aspartic protease (Fig 2).[13,48,49] Bla g 2 was inactive in the milk clotting assay for aspartic proteases. Surprisingly, Bla g 2 is more closely related to a group of mammalian pregnancy-associated glycoproteins (PAG) that form an inactive subset of the aspartic protease family. The function of PAGs is unknown, but they are thought to be binding proteins and molecular modeling of Bla g 2 shows a well-defined ligand binding cleft.[21] The group 1 cockroach allergens (Bla g 1 and Per a 1) also have no apparent enzyme function and contain multiple 100 amino acid repeats whose

function is unknown.[50,51] The function of several other important allergens, including as Der p 2, the group 5 and 7 mite allergens, and Fel d 1, is entirely unknown.

Overall, the evidence shows that allergens have diverse biologic functions that are not closely linked to their ability to induce IgE responses. In this regard, the potential effects of the enzyme function of Der p 1 on allergenicity may represent a special case. Der p 1 has properties that might be expected of any cysteine protease and the fact that mites produce several proteolytic enzymes may be coincidental. It has been argued that Der p 1 is an especially potent mite allergen because of its enzymic activity.[43] However, the group 2 allergens arguably elicit IgE responses in a higher proportion of mite-allergic patients (>90%) and environmental exposure to these allergens is 2- to 10-fold lower than to Der p 1. There is no direct evidence to support the idea that the effects of protease allergens facilitate or augment IgE responses to other allergens. Comparison of the tertiary structures of allergens shows that they have different structures and belong to different protein families, with little evidence to support the suggestion that allergens have common structural features or intrinsic properties linked to allergenicity.[47] The common features among allergens that appear to be responsible for IgE responses are the route of exposure, the aerodynamic properties of allergen-bearing particles, the level of environmental exposure, and the genetic predisposition of the host. This form of antigen presentation appears to preferentially stimulate T_H2 cells and lead to IgE production.

HIGH-LEVEL EXPRESSION OF IMMUNOREACTIVE RECOMBINANT ALLERGENS

A key advantage of recombinant allergens is that high-level expression of milligram or gram quantities of allergen can be obtained in bacterial, yeast, or insect virus systems. Recombinant allergens are effectively proteins that can be produced at will, under defined conditions, and purified with use of single-step procedures such as affinity chromatography. This has tremendous advantages in terms of quality control and standardization. Most recombinant allergens show comparable IgE reactivity to the natural allergen when compared by in vitro assays or by skin testing. The quality of these allergens has been confirmed in some cases by detailed structural analysis. For example, the IgE antibody reactivity of recombinant Der p 2 produced in *Escherichia coli* is indistinguishable from purified natural Der p 2, and the *E coli*–expressed allergen was used to determine the three-dimensional structure of Der p 2 by NMR and x-ray crystallography.[12,52] Similarly, the three-dimensional structure of birch allergen Bet v 1 was determined with use of recombinant allergen.[10]

Problems in recombinant allergen expression in a particular vector can be overcome by choosing a different expression system or by engineering the allergen sequences that enable the protein to assume the correct tertiary structure. Proteins typically may not fold properly when expressed in prokaryotic bacterial systems, but fold correctly when expressed in eukaryotic systems, such as yeast or baculovirus. Recombinant Fel d 1 chains expressed individually in *E coli* do not bind IgE as well as natural allergen but can be combined and refolded to produce immunoreactive recombinant Fel d 1.[53-55] However, refolding is not suitable for commercial production. Recently, recombinant Fel d 1 has been expressed from baculovirus as two chains with a peptide linker and shown to have comparable IgE and IgG antibody binding to natural Fel d 1 in ELISA and RIA.[56] Baculovirus-expressed Fel d 1 constructs have also been produced with an anti-CD64 antibody binding region to target the allergen to high-affinity Fcγ1 receptors on antigen-presenting cells and to facilitate more effective presentation to T cells.[56] This novel approach has for the first time produced fully immunoreactive recombinant Fel d 1, which can be scaled up with use of higher-level expression systems, such as the yeast *Pichia pastoris.* The first allergens to be expressed in *Pichia* were Cyn d 1, Alt a 1, Mus m 1, and Bla g 4.[57-60] The *Pichia* system is in widespread commercial use. *Pichia* gives high protein yields (50-250 mg/L), and the recent introduction of the pGAPZ vectors allows constitutive protein expression in fermentation systems.[61]

With allergens that are enzymes it can be important to express the proenzyme form of the sequence to obtain full IgE antibody binding. Mite group 1 allergens (Der f 1 and Der p 1) express poorly in bacteria. Fully immunoreactive group 1 allergens were first developed by expressing the proenzyme sequences of Der f 1 or Der p 1 in baculovirus or *Drosophila.*[62,63] These studies showed that it was critical to express the proenzyme form of the sequence to obtain correct folding for human IgE antibody binding. Recently, excellent production of recombinant Der f 1 and recombinant Der p 1 has been obtained with use of proenzyme forms expressed in *Pichia.*[64] Assessment of enzyme activity can be used as a further criterion to validate recombinant allergens that are enzymes.

ALLERGEN COCKTAILS FOR DIAGNOSIS—IN VIVO AND IN VITRO

The advantages of recombinant allergens for diagnostic purposes are that they can consistently be produced at high purity. These reagents are specific and more amenable to standardization in mass units. Unlike natural allergenic products, which have variable content of specific allergens (even in "standardized" products), recombinant allergens can be produced at defined concentrations with verifiable protein content. Thus it is feasible to produce a vial of recombinant mite (or other allergen) with predetermined concentrations of allergen (Der p 1, Der p 2, Der p 5, Der p 7, etc). By careful allergen selection and careful formulation of the "cocktail," the allergenic activity of the natural product could be completely reproduced with recombinant allergens.[6-9]

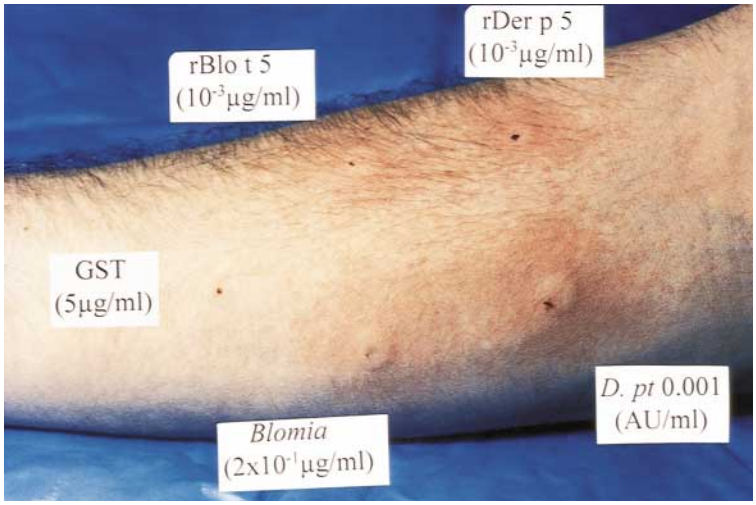

FIG 3. Immediate intradermal skin tests to recombinant *(r)* Blo t 5 and Der p 5. This patient had positive prick test result to 5 µg/mL recombinant Blo t 5 and to recombinant Der p 5 (not shown) and was given intradermal tests with serial 10-fold dilutions of the allergens to assess their potency. The patient had 8 × 8 mm wheals to 10^{-3} µg/mL each allergen, similar to *B tropicalis* or *D pteronyssinus* extract. Note that the potency of the recombinants can be directly compared in micrograms per milliliter, whereas the potency of the allergen extracts cannot (these are expressed in micrograms per milliliter of total protein or in arbitrary allergy units per milliliter). The patient was also prick tested with glutathione-S-transferase *(GST)* expression vector sequence as a negative control.

TABLE II. Recombinant allergens for diagnosis

Cat	Fel d 1, albumin
Mite	Groups 1, 2, 5, and 7
Cockroach	Bla g 1, 2, 4, and 5
Birch pollen	Bet v 1, Bet v 2
Grass pollen	Groups 1 and 5
Ragweed	Amb a 1, Amb a 2
Peanut	Ara h 1, 2, 3

The advantage of the recombinant product would be that each component would have a defined concentration and would be free of irrelevant proteins, macromolecules, or enzymes.[9] The recombinant product would also alleviate concerns about the possible presence of unidentified infectious agents in animal allergens.[55]

The purity of recombinant allergens can be established by SDS-PAGE, HPLC, and mass spectrometry. The allergens can be produced endotoxin free, which is especially important for use in cell cultures or for use in vivo. Antibody-binding activity of the recombinants can be evaluated with murine mAb in ELISA and with a large panel of human sera for IgE antibody measurements in vitro. For most major allergens the immunologic activity of recombinants has been directly compared with that of purified natural allergens. However, allergens are increasingly being identified and produced with use of cDNA cloning techniques alone. For many allergens the natural protein has not been isolated (examples include Bla g 4, Der p 5, and Der p 7). Our knowledge of *B tropicalis* allergens is based solely on recombinant allergens. *Blomia* is an interesting case in point because *B tropicalis* allergen is not available commercially and diagnosis of *Blomia* sensitivity could be made by use of the recombinant allergens that are currently available.

The biologic activity of recombinant Der p 2, Der p 5, Blo t 5, Bla g 2, Bla g 4, and Bla g 5 has been established by prick and intradermal skin testing of allergic patients and nonallergic control subjects.[24,48,65,66] Patients were prick tested with 5 µg/mL allergen and patients showing a >4 × 4 mm wheal were selected for quantitative intradermal testing, starting at 10^{-6} µg/mL and increasing in 10-fold dilutions (Fig 3). Excellent skin test reactivity has been observed with this protocol in a multicenter international study involving more than 120 patients, and patients responded to picogram quantities of recombinant Blo t 5 or recombinant Der p 5.[65,66] Further large clinical trials of recombinant allergens are required to establish optimal concentrations for skin testing, but these pilot studies suggest that good diagnostic efficacy could be obtained with concentrations of 5 to 20 µg/mL for prick testing.

For all the main sources of allergens (mites, pollens, animal dander, cockroach, and foods), recombinant allergens can be identified that could be used in cocktails for diagnostic purposes (Table II).[7,9,16,22,23,55,67] It is envisaged that recombinants would initially be used in in vitro tests, where interference or nonspecific binding by nonallergen proteins in natural products is a particular problem. Here, recombinants have advantages because they can be loaded with greater efficiency onto capture supports because they are pure proteins. Recombinants would also provide greater specificity and fewer problems with spurious cross-reactivities than would natural allergen extracts. Exciting new developments are possible with recombinants in microchip technologies or rapid screen-

ing tests for allergy diagnosis. With use of these technologies, home-based tests for allergy diagnosis could be developed, along the lines of glucose, cholesterol, or HIV tests, to allow patients to assess their allergy status before visiting the allergist. These tests typically take 5 to 10 minutes to perform and single tests could be envisaged to cover principal allergen groups: indoor, outdoor, foods, venoms, etc. Recombinant allergens are essential for the development of these innovative technologies.

WORLD HEALTH ORGANIZATION/ INTERNATIONAL UNION OF IMMUNOLOGICAL SOCIETIES INITIATIVES TO ESTABLISH RECOMBINANT ALLERGEN STANDARDS

The World Health Organization (WHO)/International Union of Immunological Societies (IUIS) Allergen Standardization Committee is embarking on a new program to produce purified natural and recombinant allergen standards. The aim of this program is to produce reference standards that can be used by industry, academia, and regulatory agencies. The references will serve as standards for in vitro assays, such as ELISA, for immunoblotting, for comparison of allergenic products, and for research. For indoor allergens, the standards will be used to calibrate environmental assays and allergen control devices. In principle, such assays could also be applied to pollen and fungal allergens. In conjunction with the WHO/IUIS initiative, monoclonal and polyclonal antibodies will be identified that can be used together with purified allergens to provide reference assays.[68] The development of WHO/IUIS standards and antibodies will enable allergen levels in different allergenic products to be directly compared and environmental allergen measurements to be standardized. The strategy of the committee is to adopt validation procedures for the standards that are internationally acceptable, so that the standards can be used worldwide.

THERAPEUTIC APPLICATIONS OF RECOMBINANT ALLERGENS: PROSPECTS FOR SAFE, EFFECTIVE ALLERGY VACCINES

Allergen immunotherapy relies on the use of high-quality natural allergenic products. However, apart from improved standardization and quality control, there have been few significant innovations in allergen immunotherapy in recent years. A number of studies have defined optimal dosing requirements for maintenance therapy with natural extracts (approximately 5-20 μg major allergen), but the treatment remains essentially unchanged since the time of Noon. It is possible that natural allergen products are advantageous for immunotherapy because they may contain substances that confer an adjuvant effect for down-regulating IgE responses or because they contain peptides derived from naturally digested allergens that have effects on T cells.[69] However, there is little evidence to support these possibilities. Attempts to introduce modified allergenic extracts in the United States (polymerized allergens or formaldehyde treated "allergoids") failed because the effects of the modifications could not be defined to the satisfaction of the Food and Drug Administration.

The advent of recombinant allergens offers exciting new prospects for developing innovative allergen-specific treatments in which the allergens are molecular entities and any substitutions, deletions, or modifications can be precisely defined at the level of specific amino acids. There are several approaches to using recombinant allergens in immunotherapy. Cocktails of recombinant allergens could be used either together with natural allergenic products, or preferably to replace those products, in conventional immunotherapy protocols. The quality of the treatment would be improved with use of a recombinant cocktail containing uniform allergen levels.

However, the real gains to be made with recombinants come from the use of these molecules in new forms of treatment. Recombinant allergens can be engineered to produce "hypoallergens" that show reduced binding to IgE antibodies but retain T-cell epitopes. Hypoallergens have been developed for group 2 mite allergens, grass allergen Phl p 5, and peanut allergens Ara h 2 and Ara h 3.[67,70-74] Reduced immediate skin test reactivity to dimers and trimers of recombinant Bet v 1 has been reported and natural isoforms of Bet v 1 with reduced IgE binding have been identified.[75,76] The rationale for using hypoallergens is that higher doses of allergen could be used for treatment with a reduced risk of adverse reactions. Another approach is to use recombinant allergens and adjuvant(s) for immunotherapy, with the aim of down-regulating T_H2 responses. The adjuvants that are currently receiving most attention are the immunostimulatory sequences of CpG nucleotides, which act as T_H1 adjuvants and are being tested in animal models and in clinical trials.[77] Other potential adjuvants include allergens coupled to IL-12 or IL-18.[78-80] Immunotherapy trials with Amb a 1 coupled to CpG sequences for treating ragweed-allergic patients are under way. Successful immunotherapy is associated with a boost in IgG4 responses. Thus another approach would be to couple recombinant allergens to lectins that preferentially stimulate the production of allergen-specific IgG4.[81] The use of allergen peptides to elicit T-cell anergy or hyporesponsiveness was pursued in the early 1990s with Fel d 1 and Amb a 1 peptides. Although Fel d 1 peptides showed symptomatic improvements in clinical trials, high doses of peptide were required, some adverse reactions were reported, and the peptides failed to make phase 3 clinical trials.[82,83] Interest in peptide-based therapy has been rekindled by the recent observations that intradermal injection of short (14 residue) Fel d 1 peptides elicits late-phase reactions in the lungs of cat-allergic patients with asthma.[84] Administration of multiple overlapping peptides spanning the Fel d 1 sequence–induced hyporesponsiveness of several months' duration in the lung and may provide a viable approach to peptide-based immunotherapy.[85] Peptide-based therapy is also being

TABLE III. Cat as a potential target for development of a prophylactic allergy vaccine

	Natural exposure	Cat vaccine*
Dose	μg/y†	mg ± adjuvant
Route	Inhalation	Inhalation (or parenteral)
Immune response		
Antibody	IgG1, IgG4, IgE	IgG1 (IgG4)
T-cell phenotype	T_H2	T_H1? modified T_H2?

*Recombinant allergens included in this vaccine would be Fel d 1 and cat albumin.[55] The route of administration could be by nasal drops or by parenteral administration, beginning at age 3-6 months.
†At high exposure (median 270 μg/g Fel d 1) IgE sensitization to cat is reduced.[90,91]

pursued as an approach to treatment of peanut allergy. Here conventional immunotherapy is not recommended because the risk of adverse reactions is too high. Short peptides derived from peanut allergen sequences may provide a safe alternative approach.

The ability to produce essentially unlimited amounts of recombinant allergens makes it possible to consider whether it would be possible to use prophylactic vaccination in allergic disease. Although this may be considered a radical and contentious approach, the idea of postnatal immunization to prevent the development of IgE responses is now within the realm of possibility and amenable to investigation.[86-88] The rationale here is that immunization of at-risk individuals with high-dose allergen (or allergen in T_H1 adjuvant) would generate T_H1-type responses (ie, IgG1 antibody without IgE antibody) to the allergen. If allergen was administered along with other vaccines within 1 to 2 years of birth, the development of IgE responses might subsequently be partially or completely suppressed. With use of natural allergens, it is not possible to produce extracts that contain the milligram amounts of allergen that would be necessary for this kind of trial. This approach assumes that neonatal infants are "immunologically naive," that is, they do not make IgE responses or significant T-cell responses to allergen.[89] The approach also assumes that the development of early high-affinity IgG1 (and possibly IgG4) antibody responses to allergen would abrogate subsequent development of IgE responses after natural allergen exposure. The latter aspect is the most difficult because it not clear whether mounting an IgG response to allergen in the serum (ie, a systemic antibody response) would have local effects that would prevent IgE responses to allergen from developing in the respiratory tract.

Let's take cat allergen, for example, in considering these theoretic prospects for prophylaxis. Natural exposure to cat involves inhalation of nanogram or microgram amounts of Fel d 1 and elicits a T_H2 response (IgG1, IgG4, and IgE antibody) in allergic individuals (Table III). At very high levels of exposure (median 270 μg/g Fel d 1), there is evidence that sensitization for IgE is reduced and that nonallergic individuals have IgG1 and IgG4 responses to Fel d 1 but not IgE responses.[90,91] Cat is a ripe target for a potential vaccination strategy because only one major allergen has been identified (Fel d 1) and it is very difficult to avoid environmental exposure to cat allergen. A potential "cat vaccine" would contain milligram quantities of recombinant Fel d 1 (with or without adjuvant) administered intranasally (or possibly parenterally), with the aim of producing a T_H1-type (IgG1) response. The rationale here is not necessarily that IgG1 would exert a "blocking" effect but that IgG1 would be a readout of a T-cell response that does not provide help for IgE production and would not switch to IgE, either systemically or locally, after natural exposure to cat.

There are obvious ethical issues and potential pitfalls to consider before trials of prophylactic vaccines could be initiated, as well as practical issues relating to production of recombinant allergen under good manufacturing practice conditions. However, the development of recombinant allergens has generated new immunologic approaches to allergy treatment that could not previously have been considered with use of natural allergens.

CONCLUSIONS

Over the next 5 years it can be anticipated that recombinant allergen production will be optimized and that there will be widespread use of recombinant allergens for research purposes and clinical trials. Ultimately, this will require production of allergens under good manufacturing practice conditions to allow the use of these reagents for in vitro and in vivo diagnostic tests. Manufacturers will then be able to apply for regulatory approval to use recombinant allergens for both diagnostic and therapeutic applications. There have already been trials of allergen peptide vaccines, and trials with allergen-oligonucleotide conjugates for ragweed hay fever (with use of natural Amb a 1) are in progress. Thus it can be predicted that there will be a steady incorporation of recombinant allergens into immunotherapy trials, which should result in more effective vaccines for established allergic disease and the longer-term prospect of prophylactic immunization.

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