

MOLECULAR BIOLOGY OF ALLERGENS

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The identification and purification of allergens is important in the standardization of allergenic extracts used in the diagnosis of allergic disease as well as developing ways to improve treatment of these common diseases. The purification of allergens that consist largely of proteins or glycoproteins by physicochemical methods has proven difficult. Furthermore, amino acid sequence data are limited. The determination of both primary and tertiary structures of proteins are important not only for the understanding of their biologic activities but also for determining the immune response to these allergens. The advent of molecular biology and its application to the study of allergens during the past decade has lead to an ever expanding base of knowledge. Through the use of these techniques, the cDNA sequences and deduced amino acid sequences for a number of allergens have been obtained (Table 1). From these analyses, data regarding the functional activity of the proteins have been accumulated. Molecular methods allow for the expression of recombinant proteins in large quantities that can be purified easily. Other expression systems allow for the evaluation of the role of glycosylation of the proteins and its effect on their allergenic activity. Lastly, molecular biology techniques allow for studies of B-cell (IgE-binding) epitopes and T-cell epitopes, which, in turn, permit an evaluation of the

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Table 1. SOME MOLECULARLY CLONED ALLERGENS

Allergen	Homology/Biologic Activity	Reference
House dust mites		
House dust mite Group I <i>Der p 1, Der f 1, Eur m 1</i>	Cysteine protease	27,54,109
House dust mite Group II <i>Der p 2, Der f 2</i>	Lysozyme	18,111
House dust mite Group III <i>Der p 3, Der f 3</i>	Trypsin	105
House dust mite Group IV <i>Der p 4, Der f 4</i>	Amylase	104
House dust mite Group V <i>Blo t 5</i>	Unknown	6
House dust mite Group VI <i>Der p 6, Der p 6</i>	Chymotrypsin	104
House dust mite Group VII <i>Der p 7</i>	Unknown	103
	Heat shock 70 protein	2
	Tropomyosin	3
Other house dust mite allergens	Glutathione 5-transferase	103
Tree pollens		
Birch, alder, hazel, hornbeam e.g., <i>Bet v 1, Aln g 1, Cor a 1, Car b 1</i>	Pea disease-resistance protein	14,15,16,59
Birch <i>Bet v 2</i>	Profilin	114
Japanese Cedar <i>Cri j 1</i>	Pectate lysate	43
Grass pollens		
Grass pollen Group I e.g., <i>Lol p 1, Phl p 1</i>	Unknown	77,82
Grass pollen Group II e.g., <i>Dac g 2</i>	Unknown	87
Grass pollen Group V e.g., <i>Poa p (IX) V</i>	Unknown	68
Ragweed pollen <i>Amb a 1</i>	Pectate lysate	104
<i>Amb a 5, Amb t 5</i>	Unknown	38,84
<i>Amb a 6</i>	Plant lipid transferase	63
Animal allergens		
Cat allergen <i>Fel d 1</i>	Rabbit uteroglobin	42,69
Mouse allergen <i>Mus m 1</i>	Lipocalin	104
Rat allergens <i>Rat n 1</i>	Urinary prealbumin	104
<i>Rat n 2</i>	α 2u-globulin, lipocalin	104

Table continued on opposite page

Table 1. SOME MOLECULARLY CLONED ALLERGENS (*Continued*)

Allergen	Homology/Biologic Activity	Reference
Fungal allergens		
<i>Aspergillus fumigatus</i>		
<i>Asp f 1</i>	Mitogillin	7,70
<i>Alternaria alternata</i>		
<i>Alt a 6</i>	P2 ribosomal protein	1
<i>Alt a 7</i>	ycp 4 yeast protein	1
<i>Alt a 10</i>	Alcohol dehydrogenase	1
<i>Cladosporium herbarum</i>		
<i>Cla h 3</i>	P2 ribosomal protein	125
<i>Candida albicans</i>	Alcohol dehydrogenase	98
<i>Psilocybe cubensis</i>		
<i>Psi c 2</i>	Cyclophilin	51
Insect allergens		
Cockroach allergens		
<i>Bla g 2</i>	Aspartic proteases	8
<i>Bla g 4</i>	Calycine	8
<i>Bla g 5</i>	Glutathione transferase	8
<i>Bla g 6</i>	Troponin	8
Honey bee venom	Phospholipase A2	58
Wasp, hornet, yellow jacket venom	Pathogenesis-related	
e.g., <i>Dol m 5</i> , <i>Ves v 5</i> , <i>Pol e 5</i>	protein of tobacco leaf	36

T-lymphocyte response to allergens that may lead to improved therapeutic modalities.

The terminology for naming allergens is based on the recommendations of the International Union of Immunologic Societies Subcommittee for Allergen Nomenclature. This subcommittee proposes that purified allergens be identified by the source of the allergenic protein using the source's scientific name. The letters indicate the genus and the species and the number following is assigned in order of its acceptance by the Subcommittee. Nonetheless, confusion still occurs and some names as reported in the literature have not been recognized officially by the Subcommittee.

Figure 1 illustrates a typical approach to the cloning of allergens from a variety of sources. The reader is referred to the review articles by Stewart¹⁰⁴ and Donovan and Baldo.²⁹ In most instances, allergens are derived from cloning using cDNA-based libraries. Total RNA is extracted from the allergenic source, messenger RNA is purified, and double-stranded cDNA is prepared. The cDNA is then inserted into a suitable vector and expressed in a variety of systems. Clones expressing the allergenic protein of interest are identified by scrutinizing the cDNA library by monoclonal antibodies directed against particular allergens, sera from allergic patients, or oligonucleotide probes based on the known amino acid sequence of the native allergen. Once functional clones of interest have been obtained, the cDNA sequence is determined

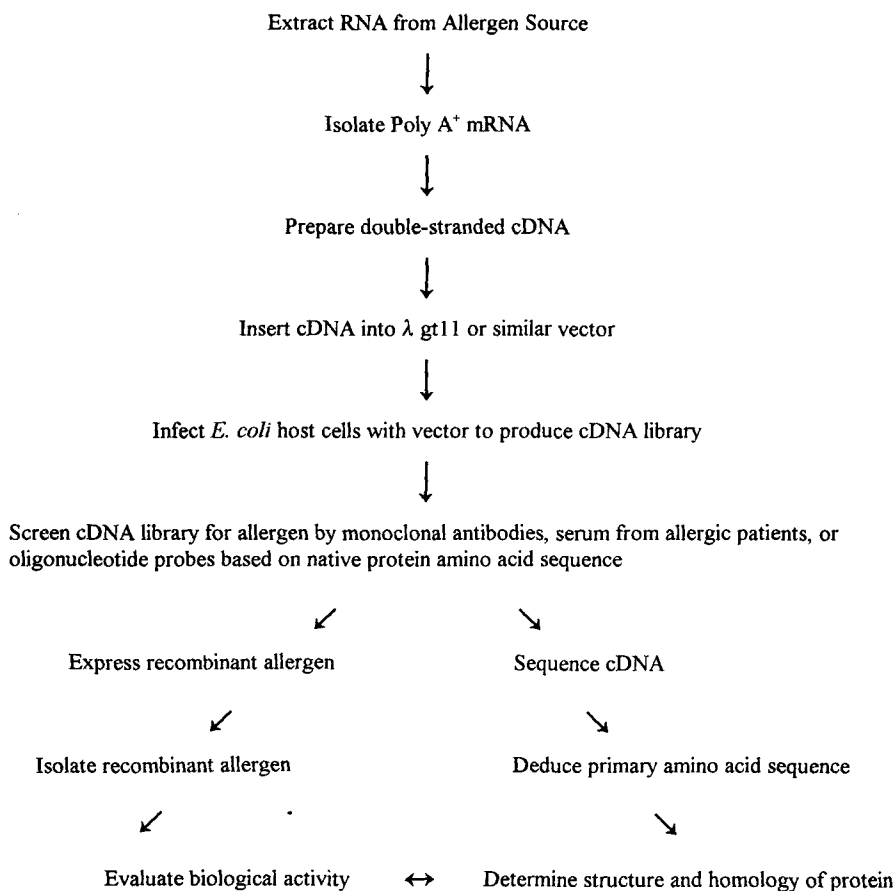


Figure 1. Scheme of the typical steps in cloning and characterizing allergenic proteins from a cDNA library.

and the sequence is analyzed by a variety of computer-based programs. These programs provide data regarding the possible functional activity of the protein as well as help determine its homology with other known proteins contained in the computer banks. Computer-generated information also can be used to deduce the amino acid sequence of proteins once the cDNA sequence has been generated.

In turn, epitope mapping studies that look at IgE binding to peptide residues of the mature protein or the response of CD4⁺ T cells to the peptides can be determined. The IgE binding sequences may be conformational and, therefore, require intact proteins or large-chain polypeptides to be recognized. A number of studies, however, have indicated that IgE binding epitopes do exist on the primary structure of

the proteins. In contrast, the recognition of allergens by the T-cell receptor on CD4+ lymphocytes is governed by small peptide sequences. These approaches are especially useful for investigations into the treatment of allergic disease.

In this article, we discuss the characterization of a variety of allergens by molecular biology approaches as well as the IgE and T-cell responses to these allergens where they have been studied.

MOLECULAR BIOLOGY OF HOUSE DUST MITE ALLERGENS

Dust mites are important sources of allergens as they are ubiquitous in most environments. Further, sensitivity to the dust mites has been identified as an independent risk factor for the development of asthma. The most important family of dust mites are the Pyroglyphidae although other families such as Glycyphagidae, Acaridae, and Echymopodidae may be important in certain geographic areas. Among the Pyroglyphidae mites are *Dermatophagoides pteronyssinus*, *D. farinae*, *Euroglyphus maynei*, and *Blomia tropicalis*. Non-pyroglyphoid storage mites such as *Lepidoglyphus destructor* are important in certain regions as well.

At least seven groups of dust mite allergens have been identified. Within these groups, there is a high degree of amino acid and cDNA sequence homology and identity. Further, each group may have distinctive enzymatic activity. Shared sequence homology also may occur among groups of allergens.

Group I Mite Allergens

The group I allergens are among the most extensively studied allergens of any origin. These proteins have a molecular weight of approximately 25 kD.¹⁰³ Most dust mite-sensitive patients show IgE binding to these allergens. The group I dust mite allergens isolated from several species show similar amino acid sequences. Thomas et al¹⁰⁹ were among the first to report a molecularly cloned allergen. They used messenger RNA from *D. pteronyssinus* to produce a cDNA library using lambda gt 11 and expressed in *Escherichia coli*. The recombinant allergen was detected by a plaque immunoassay using rabbit anti-Der p 1 antisera. To verify that they had isolated the Der p 1 allergen, DNA from the clones was hybridized to a 17 mer oligonucleotide based on the known amino acid sequence of Der p 1. The expressed protein was a fusion protein with a 24-kD mature protein. This expressed protein, however, did not bind IgE.

Further work²² indicated that the amino acid sequence showed homology with cysteine protease. These are highly conserved proteins that have enzymatic activity. Similar proteins have been found in papain and actinidin of kiwi fruits. This protein is synthesized in the gut of the

dust mite and is found in extracts prepared from the body of the dust mite or from its feces.

Chua et al²¹ used a plasmid vector and expressed *Der p 1* in *Saccharomyces cerevisiae*. The recombinant *Der p 1* was purified by a monoclonal antibody armed affinity column. The *Der p 1* was synthesized from a pro-enzyme, which had a leader sequence of 18 amino acids, and a propeptide of approximately 80 amino acids as well as the mature protein. This recombinant allergen was capable of binding IgE antibodies.

Studies with *D. farinae*²⁷ resulted in the cloning of a similar protein. The recombinant allergen was screened by hybridization with two probes based on the cDNA sequence from *Der p 1*. This recombinant protein had 81% amino acid sequence homology with *Der p 1* and had a similar molecular weight. Kent et al⁵⁴ obtained a molecularly cloned group I allergen, *Eur m 1*, from *Euroglyphus maynei*. Two primers based on the *Der p 1* cDNA sequence were used to amplify *E. maynei* genomic DNA by the polymerase chain reaction (PCR). The deduced amino acid sequence showed 78% homology with *Der p 1* and *Der f 1*.

In addition to the known biologic activity of the group I allergen cysteine proteases, further investigations on the biologic activity of *Der p 1* have been conducted. Hewitt et al⁴⁸ found that *Der p 1* cleaves the low affinity IgE receptor (CD23) from the surface of human B lymphocytes. They hypothesized that this may promote and enhance IgE antibody responses by ablating an important feedback inhibitory mechanism that normally limits IgE synthesis. Because soluble CD23 promotes IgE production, fragments of CD23 released by the *Der p 1* allergen may enhance IgE synthesis directly.

Investigations of the IgE binding epitopes of *Der p 1* have been conducted.^{40, 41} Peptide fragments were generated using fragments of cDNA encoding four portions of *Der p 1*. An amino acid sequence of 30 residues was necessary for consistent IgE binding.⁴¹ IgG bound to some epitopes but these had little or no IgE binding.⁴¹ Further investigations⁴⁰ found that most IgE binding was located on a flexible loop connecting two domains of the molecule, which appeared on amino acid residues 81–94 and 101–111. Synthetic peptides ranging from 19 amino acids (residues 52–71) and 16 amino acids (residues 111–133) of the *Der p 1* molecule have been found to trigger histamine release from basophils of patients with dust mite allergy.⁵³

In studies of the T-cell response to group I allergens, peripheral blood mononuclear cells from house dust mite-sensitive patients were found to have stimulation indices of 10 vs. 2 for non-house dust mite allergic patients. Patients with asthma had higher stimulation indices than did patients with rhinitis alone.⁷⁴ The T-cell recognition of epitopes depends on the context of the MHC class 2 molecules. Yssel et al¹²³ found that for HLA-DR7 restricted cells, amino acid sequences of the *Der p 1* molecule 45–67 and 117–143, were capable of triggering a T-cell proliferative response whereas in HLA-DR2-DRW11 or DR8 molecules recognized amino acid sequences 94–104. Others have found⁴⁶ that there is restricted use of T-cell receptor (TcR) beta chain variable region 3

(V β 3) gene elements in the human polyclonal T-cell response to house dust mite allergens. Further, a peptide analog of TCR-V β 3 CDR2 specifically inhibits the polyclonal proliferative response of human V β 3 positive T cells to crude dust mite extracts.

Using *Der f* 1, Bonno et al¹³ found that peripheral blood mononuclear cells from untreated house dust mite allergic patients when stimulated by the allergen induced significant CD25 on CD4+ T lymphocytes but there was little induction on CD8+ T cells. In patients who were treated with immunotherapy to house dust mite there was a change to CD8+ CD25+ cells. The number of CD4+ CD25+ cells was correlated with the severity of the patient's underlying illness. These data suggested that immunotherapy may induce specific CD8+ T cells in patients treated with *D. farinae* extracts.

Group II Mite Allergens

The group II allergens are considered major allergens in most individuals sensitive to house dust mites. These are 14-kD proteins that possibly have lysozyme activity. The complete amino acid sequences are known for *Der p* 2, *Der f* 2, and *Lep d* 2. There is a leader protein with 1-17 amino acids and a mature protein with 129 residues for *Der p* 2. Chua et al¹⁸ cloned *Der p* 2 in *E. coli* using lambda gt11 vector and screened with human serum for IgE binding. They found the cDNA-deduced amino acid had sequence homology with biochemically purified *Der f* 2. Because *Der p* 2 expressed *E. coli* was unstable and was difficult to purify, the same investigators¹⁹ purified the recombinant allergen by using the pGEX-1 vector and glutathione agarose affinity chromatography. Using cDNA constructs to express large overlapping peptides with amino acids of 69 residues, it was found that the IgE binding activity to *Der p* 2 is highly conformational and restricted.²⁰

Molecular cloning of *D. farinae* group II allergens also has been performed. Trudinger et al¹¹¹ used PCR technology based on primers from *Der p* 2 sequences to obtain the DNA encoding for *Der f* 2. The deduced amino acids showed 88% homology between *Der f* 2 and *Der p* 2. Other investigators¹²⁴ used the vector pUEX-1 and expressed the recombinant *Der f* 2 allergen in *E. coli*. The library was screened with rabbit antisera to *Der f* 2. The recombinant *Der f* 2 allergen showed positive skin test reactivity in one patient and released histamine from the basophils of six mite allergic individuals.

Using fusion proteins from subcloned cDNA, Kobayashi et al⁵⁷ performed IgE epitope mapping studies with *Der p* 2. Eleven of twelve sera had IgE antibodies that reacted with peptide fragments 41-80, 64-105, 81-129 by Western blotting as well as to the full-length 129 amino acid residue.

T-cell epitopes of *Der p* 1 have shown that approximately 18% of patients have stronger proliferative response to *Der p* 1 than to *Der p* 2.⁷⁴ T-cell epitope mapping studies that used 15-19 mer peptides spanning

the entire *Der p 2* protein to stimulate peripheral mononuclear cells of house dust mite individuals showed that all the peptides were antigenic for at least one of the individuals. There was substantial variability in the number and location of the epitopes (approximately 2.3/patient). The most frequently detected residues were 111–127, which were recognized by 67% of the patients. There was no correlation between the epitope recognized and the presence of any particular HLA-DDQ antigens.⁷⁵

Group III Mite Allergens

The group III dust mite allergens also have been purified, and their sequence has been identified. These are fecal enzymes that have trypsin-like activity. Their molecular weight is between 30 and 31 kD. There is variability, however, in the frequency of sensitivity to these proteins ranging from approximately 100% of patients being sensitive to *Der p 3* to only 10% being sensitive to *Der f 3*. The sequence homologies of *Der p 3* and *Der f 3* are similar to that of crayfish trypsin. At least nine isoforms of *Der p 3* exist, with isoelectric points ranging from 4 to greater than 8. Two isoforms of *Der f 3*, with isoelectric points from 4 to 5, have been identified.¹⁰⁵

Group IV Mite Allergens

Group IV allergens have been studied less extensively. These appear to be intermediate to minor allergens. These have molecular weights around 60 kD, and have amylase activity.¹⁰⁴

Group V Mite Allergens

Group V allergens show IgE binding in approximately 50% to 70% of sera from house dust mite allergic patients. *Der p 5* originally was cloned by Tovey et al.¹¹⁰ The cloned cDNA sequence encoded for a polypeptide 148 amino acids. The molecular weight ranges between 14 to 17 kD. More recently, Arruda et al.⁶ cloned a group V allergen, *Blo t 5* from *Blomia tropicalis*. This recombinant protein had 40% sequence homology with the allergen described by Tovey et al. Approximately 70% of the *Blo t 5* positive sera bound to the cloned allergen.

Group VI Mite Allergens

Group VI allergens appeared to be minor allergens showing IgE binding in about 40% of house dust mite allergic individuals.¹⁰⁴ These

are 25-kD proteins that have serine protease activity, similar to chymotrypsin.

Group VII Mite Allergens

Group VII allergens are 22-kD proteins that are recognized by about 50% of dust mite allergic patients by skin testing.¹⁰³ A cDNA encoding for one of these proteins has been obtained. The molecular mass of that protein was approximately 22 kD. There is at least a potential glycosylation site within the molecule; however, its functional activity is not known.

Other Mite Allergens

A number of other mite allergens also have been identified but their significance is not entirely understood. One of these, a *D. farinae* allergen, however, has 65% homology with the human heat shock proteins 70 family. This allergen has been obtained in recombinant form.² It showed IgE binding in approximately 10% of sera from dust mite sensitive patients.

Aki et al³ also have cloned a 32.9-kD protein that shows sequence homology to tropomyosin. The recombinant molecule showed weaker IgE binding than did the native protein, although 80% of allergic patients had IgE antibodies to the tropomyosin in their serum. Five of thirteen patients showed positive interdermal skin test to the recombinant tropomyosin. The significance of this protein is that it may cross-react with tropomyosin, which recently has been identified as a major allergen in shrimp.

A glutathione-5 transferase has been cloned that has a molecular weight of 25.6 kD.¹⁰³ The biologic significance of this allergen, however, remains unknown.

TREE POLLENS

Birch Pollen Allergens

Birch (*Betula verrucosa*) pollen allergen, *Bet v* 1, was one of the first tree pollen allergens to be cloned molecularly.¹⁶ A cDNA library was constructed in *E. coli* using lambda gt 11 as the vector and messenger RNA from birch pollen. The library was screened with serum from birch pollen allergic individuals. A 17.4-kD protein was isolated. The deduced amino acid sequence showed homology with similar proteins from alder, hazel, and hornbeam. The amino acids also showed 55% sequence identity and 70% sequence similarity with pea disease resistant gene. Elsayed and Vik³⁵ purified two birch pollen isoallergens of *Bet v* 1 that are homologous to the cloned allergen by physicochemical methods.

Using hybridization techniques, Valenta et al¹¹³ found that a cDNA probe from *Bet v 1* bound to mRNA from alder, hazel, and hornbeam. In Southern blots, highly homologous genomic DNA digest hybridized with *Bet v 1* cDNA. Analysis of mRNA in various tissues of birch trees showed that *Bet v 1* is synthesized in tissues other than pollen such as leaves and callus.⁸³ In situ hybridization studies showed that *Bet v 1* genes were expressed in late bi-cellular and mature pollen but not in sporophytic anther tissues at any developmental stage.¹⁰⁷

The IgE binding epitopes of *Bet v 1* have been localized to the N-terminal portion of the protein.¹⁰⁴ Seven distinct epitopes have been found on *Bet v 1*, which are scattered over the entire molecule.³⁴ Synthetic peptides based on these epitopes did not inhibit IgE binding, which suggests that T-cell and IgE antibodies recognized different structures on the *Bet v 1* molecule. T-cell lines and clones derived from nonallergic individuals also recognized similar epitopes of *Bet v 1*, but the cytokine patterns produced by cell lines from allergic individuals differed in that they generated Th2 profiles (IL-4, IL-5) versus Th1 (IL-2, interferon- γ) from nonallergic individuals.³⁵

Breiteneder et al¹⁵ cloned the major allergen *Aln g 1* from alder (*Alnus glutinosa*). The deduced amino acid sequence of this allergen shared 87% homology on *Bet v 1*. In addition, IgE binding epitopes were similar to that found with *Bet v 1*.

Four recombinant isotypes of *Cor a 1*, the major allergen of hazel pollen (*Corylus avellana*), have been identified.¹⁴ Using a primer based on the internal amino acid sequence, the investigators were able to amplify *Cor a 1* cDNA by the PCR technique. The deduced amino acid sequence showed similarity with *Bet v 1*, *Aln g 1*, and *Car b 1* (from hornbeam). The isoforms, however, had differing IgE binding activity. It is interesting to note that in tree pollen allergic patients with hazel nut intolerance, IgE to *Cor a 1* was found in 100% of patients.⁴⁹

The major allergen of hornbeam, *Car b 1* (*Carbinus betulus*) has been obtained.⁵⁹ Using PCR-based cloning and sequence analysis, three isoallergens were isolated. The molecular weight ranged from 17.27 to 17.217 kD. The deduced amino acid sequence showed approximately 75% homology to *Bet v 1*.

Thus, a number of allergens derived from the tree pollens of birch, hazel, alder, and hornbeam show similar amino acid homology. This may explain why patients who have tree pollen sensitivity have difficulty with the ingestion of hazel nuts, which contain proteins similar to this group of allergens.

A second group of allergens, *Bet v 2*, have been generated in recombinant form from cDNA libraries. Valenta et al¹¹⁴ identified a 14-kD birch pollen protein that had 30% identity and 40% homology with human profilin. Profilins regulate actin polymerization and participate in signal transduction. Profilins can be found in pollens of trees, grasses, and weeds. Further investigation of the cDNA sequence showed 80% identity between *Bet v 2* and *Phleum pratense* profilins.¹¹² Valenta et al¹¹² showed that 20% of all pollen allergic individuals have IgE to profilin.

The same group of investigators has shown that the recombinant *Bet v 2* is capable of inducing positive skin tests and basophil histamine release.^{106, 112, 115, 118}

The *Bet v 2* allergen has been found only in pollen and is not distributed in tissues in contrast with the *Bet v 1* allergen. Using the combination of recombinant *Bet v 1* and *Bet v 2* in immunoassays, virtually 100% of birch pollen allergic patients can be identified.¹¹⁷

Seiberler et al⁹⁷ have cloned two additional birch pollen allergens that have a molecular weight of approximately 20 kD. They showed sequence homology with calmodulin. These allergens, however, appear to be fairly minor in that only 5% to 10% of birch pollen allergic sera will bind IgE to these allergens.

Japanese Cedar Pollen Allergens

Cri j 1, the major allergen of Japanese cedar (*Cryptomeria japonica*), has been cloned molecularly.⁴³ This allergen showed sequence homology with mountain cedar pollen. There was limited homology with ragweed allergens, *Amb a 1* and *Amb a 2*. The amino acid sequence also demonstrated similarity to bacterial pectate lysates. A T-cell epitope has been identified residing between peptides 335–346.⁵² A single amino acid substitution at residue 339 from threonine to valine altered the T-cell response with increased interferon- γ production with no significant change in the proliferative response or IL-4 production by T-cell clones.

Olive Pollen Allergens

The allergen, *Ole e 1*, has been purified immunochemically from olive (*Olea europaea*) pollen. This is a 20-kD protein whose amino N-terminal amino acid sequence is known.¹⁰⁴ Using monoclonal antibodies and immunocytochemical techniques, Rodriguez-Garcia et al^{88, 89} localized the *Ole e 1* protein to the rough endoplasmic reticulum of the mature pollen grain. This acts as either a storage or synthesis site for the protein. *Ole e 1* is distributed in the pollen of several Oleacea species.⁸⁹ The functional activity of this protein is not known.

GRASS POLLEN ALLERGENS

Grass pollens are a significant source of allergen exposure. They have a world-wide distribution, and sensitivity to their allergens is a major cause of allergic disease. The grasses are occasionally broken into two groups, those found primarily in temperate areas and those with a more tropical and subtropical distribution. The temperate grasses include rye grass (*Lolium perenne*), timothy grass (*Phleum pratense*), Kentucky blue grass or June grass (*Poa pratensis*), and orchard or cocksfoot

(*Dactylis glomerata*). The more subtropical and tropical grasses include Bermuda grass (*Cynodon dactylon*) and Johnson grass (*Sorghum halepense*). Allergens from grass pollens have been divided into seven groups based on their physiochemical and immunochemical similarities. These are groups I, II, III, IV, V (IX), X and the proflins. Major grass allergens are found in group I and group V. There is marked heterogeneity within groups in terms of amino acid sequences of the proteins. The amino acid sequences of the allergens have been derived by immunochemical as well as molecular biology techniques. Because up to 75% of patients in cool climates have grass pollen allergy, the study of these allergens is important.

Group I Grass Pollen Allergens

The group I grass pollen allergens are approximately 27-kD acidic glycoproteins.^{104, 108} Based on the deduced C-terminal amino acids of the *Lol p 1* from rye grass, there is marked sequence homology with group II and group III. Rye grass allergens as well as cross-reactivity based on the N-terminal amino acid sequences with other group I allergens from Kentucky blue grass (*Poa p 1*) and Bermuda grass (*Cyn d 1*). The *Lol p 1* allergen has been localized to the cytosol of the pollen.^{100, 108}

Perez et al⁷⁷ extracted mRNA from rye grass pollen and used the cloning vector PTZ18r-BSTX1 to produce a cDNA library. This was screened by a 20-mer oligonucleotide probe corresponding to an amino acid sequence of the mature *Lol p 1* allergen. Two isoforms that had numerous nucleotide differences were isolated. Both had asparagine-linked glycosylation sites. Griffith et al⁴⁵ used different cDNA technology to obtain a full-length cDNA by anchored PCR. A lambda gt11 vector was used to express the recombinant protein in *E. coli*. The deduced amino sequence resulted in a calculated molecular weight of 26.6 kD. The recombinant protein contained a 28 amino acid peptide fragment that was homologous with peptides from *Lol p 2*. Further, the C-terminal had sequence homology with *Lol p 2* and *Lol p 3*. Using human antibodies to perform epitope mapping on *Poa p 1* from Kentucky blue grass and comparing this with *Lol p 1* from rye grass, Lin et al⁶⁰ found extensive allergenic cross-reactivity between the two proteins.

cDNA constructs of the major timothy grass pollen, *Phl p 1*, have been obtained.⁸² The investigators used lambda Zap with expression in *E. coli* to obtain the allergens. Activity of the recombinant proteins was identified by monoclonal antibodies and serum from grass pollen allergic individuals. The IgE reactive region was found to be on the 23 amino acid C-terminal portion of the molecule. There was a high degree of sequence homology with *Lol p 1*. *Phl p 1* contains one glycosylation site as deduced by its cDNA sequence. Deglycosylation, however, had little effect on the IgE binding activity of the protein.⁷⁹

Others have isolated Bermuda grass group I allergens, *Cyn d 1*,^{17, 66} and Johnson grass allergens, *Sor h 1*.⁴ Using conventional chromatogra-

phy techniques, Matthiesen et al⁶⁶ purified *Cyn d 1* and obtained its N-terminal amino acid sequence. Both *Cyn d 1* and *Sor h 1* show a high degree of homology with *Lol p 1*. Chang et al¹⁷ found 11 isoforms of *Cyn d 1*. The *Cyn d 1* isoforms varied from lot to lot of Bermuda grass extracts. It has been shown that *Cyn d 1* comprises approximately 15% weight/weight of the whole pollen extract.⁶⁶

The T-cell epitopes of the group I allergens have been studied extensively. Perez et al⁷⁷ found that the amino acid residues 191–210 may be the immunodominant T-cell epitope of *Lol p 1*. Further investigations have found extensive T-cell epitope cross-reactivity between *Lol p 1* and *Lol p 3*, although less cross-reactivity with *Lol p 2*.¹⁰ Schenk et al⁹⁶ established T-cell clones to timothy grass pollen, cocksfoot, rye grass, and cereal rye. They evaluated the T-cell epitopes among group I allergens. It was found that *Phl p 1* has multiple T-cell epitopes. Within the group I allergens there are extensive cross-reactive T-cell epitopes. The T-cell epitope recognition is not correlated with HLA haplotype nor is a restricted T-cell receptor V β gene used.

Groups II, III, and IV Grass Pollen Allergens

Sensitivity to the group II, III, and IV allergens is less extensive than that for the group I and V allergens. Both group II and III allergens are nonglycosylated 11-kD proteins. Group IV allergens are 57-kD basic proteins. These allergens have been isolated using conventional techniques. The amino acid sequences of group II and III allergens of rye grass are similar to each other and to *Lol p 1*. A cDNA from orchard (cocksfoot) grass has been obtained, which encodes for *Dac g 2*.⁸⁶ This allergen showed 90% amino acid sequence homology to *Lol p 2* and considerable homology with *Lol p 1* and 3. Approximately one third of non-immunotherapy treated patients have IgE antibodies to this recombinant allergen.⁸⁶

Group V Grass Pollen Allergens

Along with group I, the group V grass pollen allergens are recognized as major allergens in grass pollen sensitivity. The proteins are a heterogeneous group of allergens that have a molecular weight of approximately 30 kD and are nonglycosylated basic proteins. The allergens of rye grass, *Lol p 5*, are localized to starch granules of the pollen. Considerable confusion in the literature has risen because of the fact that several of these allergens previously were isolated and described under different names, for example, *Lol p 1* variant,^{56, 101} and were also known as group IX allergens.^{68, 72, 99, 127, 128}

Knox et al⁵⁶ were among the first to clone these allergens. Using lambda gt11 vector and expressing in *E. coli*, the clones were screened with a monoclonal antibody that subsequently was found to be directed at group V allergens.⁵⁶ The same group of investigators¹⁰¹ obtained the

amino acid sequences of this protein that showed IgE binding to the C-terminal domain.

Other investigators^{55, 87} purified the group V allergens from a number of grass pollen species including rye grass, orchard, fescue, timothy, and Kentucky blue grass. These group V allergens showed the characteristic feature of high content of alanine and hydroxyproline. All had similar sequences to each other and to a group V allergen from timothy. The group V allergens did not show similarity to group I allergens by sequence analysis.

Petersen et al⁷⁸ found that *Phl p 5* comprised about 6% of the weight volume of the whole timothy extract. At least eight isoforms of the protein were isolated. IgE binding activity differed between the isoforms, although both C- and N-terminal ends of the molecule had binding sites. For some isoallergens, the C-terminal seemed to be more potent. The proteins may have RNase activity.¹¹

Mohapatra et al⁶⁸ were the first to clone group V allergens from Kentucky Bluegrass, which were originally designated as group IX allergens. The cloned allergen was recognized by human IgE antibodies but not by specific monoclonal antibodies. Using nucleotide probes based on a partial sequence, the same group of investigators⁹⁹ obtained several isoforms of this group of allergens. Northern blotting analysis revealed that the allergens were confined to the pollen itself. Further investigations¹²⁷ found these allergens in a wide variety of grass pollen species including Bermuda, canary, orchard, red top, smooth brome, tall oat, perennial rye, timothy, quack, Johnson, colonial bent, and reed canary grass pollen extracts.

Evidence that these allergens belong to the group V family of grass pollen allergens was provided.⁷² The Kentucky Bluegrass allergens showed epitopes that cross-reacted with acid group V allergens of timothy grass but not to *Lol p 1*. Epitope mapping studies for IgE binding showed that there are at least 10 antibody binding epitopes on the recombinant Kentucky Bluegrass group V allergen.¹²⁸ Again the immunodominant portion of the molecule appeared to be at the C-terminal end.

Group VI Grass Pollen Allergens

Timothy grass pollen group VI allergens have been obtained by conventional purification methods and cloned molecularly. Three isoforms of the *Phl p 6* allergen were obtained by two-dimensional SDS polyacrylamide gel electrophoresis. These proteins had a molecular weight of 13 kD and isoelectric points ranging from 5.2 to 5.5. Microsequencing of the amino acids indicated N-terminal sequence homology with *Phl p 5*. In addition, IgE cross-reactivity to *Phl p 5* was observed.⁸⁰ The same group of investigators also performed cDNA cloning of *Phl p 6*.⁸¹ Based on the deduced amino acid sequence, there was a high degree of homology between the N- and C-terminal ends of *Phl p 5* and *Phl p 6*, which suggests a common precursor gene.

Group X Grass Pollen Allergens

The group X allergens from grass pollen have been isolated from Bermuda and Kentucky Bluegrass (*Cyn d* 10 and *Poa p* 10, respectively). These have been identified as cytochrome c. These allergens are considered to be minor allergens.^{104, 108}

Other Grass Pollen Allergens

A cDNA library was constructed using messenger RNA from cocksfoot grass and screened with polyclonal rabbit antisera and human IgG antibodies. A 24-kD protein was cloned. This showed IgE binding in approximately 75% of cocksfoot allergic patients. Its sequence, however, was not reported, and its relationship to other grass allergens is not known.¹²⁰

Proflin has also been identified as a grass pollen allergen.^{112, 116} This ubiquitous protein is widely distributed in the plant and animal kingdoms. Its overall contribution to grass pollen allergy currently is not known.

Recombinant Grass Pollen Allergens for Diagnostic Purposes

Using recombinant *Phl p* 1 and *Phl p* 5, 66% of the total IgE directed against native grass pollen allergens were absorbed by the recombinant proteins.⁶¹ Using a plaque lift technique to blot for cloned *Phl p* 1, *Phl p* 5, and proflin, positive binding was detected in 97 of 98 sera of grass pollen allergic individuals.¹¹⁹ These data suggest that a combination of recombinant group I, group V, and proflin allergens would be useful in immunodiagnostic techniques for grass pollen allergy.

RAGWEED AND OTHER WEED POLLEN ALLERGENS

Ragweed pollen is one of the most important allergen sources in the United States. Major allergens described for ragweed (*Ambrosia artemisiifolia*) include *Amb a* 1 and 2 and the minor allergens *Amb a* 4, 5, 6, 7, and cystatin.

Amb a 1 Family of Allergens

The native protein of *Amb a* 1 is a 38-kD acidic two-chain polypeptide that is nonglycosylated. Four families of *Amb a* 1 have been identi-

fied and are numbered *Amb a 1.1* to *1.4*. cDNA constructs have been prepared from libraries that were screened with monoclonal antibodies to *Amb a 1*.^{44, 85} It has been proposed that there is a 26-residue leader peptide and that the mature allergen contains 370 amino acid residues. Of three groups of cloned *Amb a 1* allergens, there is 80% amino acid sequence homology. Ninety-five percent of patients allergic to ragweed have antibodies to *Amb a 1*. The epitopes of *Amb a 1* are conformational. Some sera only recognize *Amb a 1.1*; whereas most react with all recombinant forms. Further, all recombinant members of the *Amb a 1* family have been shown to stimulate T-cell lines.¹²

Amb a 2

The *Amb a 2* family of allergens are 40-kD proteins that share approximately 68% to 70% sequence identity to *Amb a 1*. They also have been shown to cross-react with *Amb a 1* at the B- and T-cell level.^{44, 92} Both *Amb a 1* and *Amb a 2* have homology with tomato pollen protein (45%) and pectate lysate (19%).⁴⁴ Although direct pectate lysate activity has not been demonstrated, it has been theorized that this enzymatic property may be important in the digestion of pectin in plant walls during formation of the pollen tube.

Amb a 3* and *Amb a 4

Amb a 3 is an 11-kD glycoprotein. Its amino acid sequence has been established by conventional methodology. It appears to be related to copper proteins and may be involved in electron transport.¹⁰⁴ *Amb a 4* is a 22.8-kD basic protein that contains approximately 189 amino acids. Its function is not known. Both *Amb a 1* and *Amb a 3* are considered minor allergens.¹⁰⁴

***Amb a 5* and Homologs**

The amino acid sequence of a group of ragweed allergens from short ragweed, giant ragweed (*A. trifida*), and Western ragweed (*A. psilostachya*) have been obtained by conventional sequencing method and also have been cloned. These proteins with 40 to 50 amino acids have about 50% sequence homology, although individual allergens appear to be polymorphic. Ghosh et al³⁸ used PCR and degenerate primers to obtain overlapping cDNA clones. By using PCR and anchored PCR, they were able to determine the complete nucleotide sequence for *Amb t 5* from giant ragweed pollen. They had difficulty obtaining positive clones when screening the cDNA library with human serum and oligonucleotide probes. Later, the same group of investigators was able to express *Amb a 5* and *Amb t 5* in *E. coli* using pGEX-2T vector.⁸⁴ The recombinant *Amb t 5* was indistinguishable from the native protein by

spectroscopy and by antibody binding studies. Recombinant *Amb a 5* had approximately 50% of the antibody binding activity of the native proteins, although both were equally effective in stimulating *Amb a 5* specific T-cell lines. These studies demonstrated the limitation of expression of this protein in *E. coli*. Further investigations³⁹ lead to the molecular cloning of *Amb p 5* from Western ragweed. Two groups of genes were isolated based on the cDNA nucleotide sequence. One isoallergen termed *Amb p 5A* showed 90% identity to *Amb a 5* whereas as *Amb p 5B* had 65% amino acid homology with *Amb a 5*. The purified recombinant allergen *Amb p 5* was capable of inducing histamine release from the basophils of sensitive individuals.

Studies of T-cell epitopes on the *Amb a 5* group of allergens and its homologs have been conducted. *Amb a 5* is topographically similar to *Amb t 5*, but significant differences exist in the packing of the side chains in the hydrophobic core of molecules as determined by nuclear magnetic resonance spectroscopy.⁶⁷ T-cell epitope mapping studies of *Amb a 5* and *Amb t 5* revealed that free sulfhydryl groups play a major role in T-cell recognition and in cross-reactivity in T-cell epitopes within these allergens.¹²⁹ A dominant T-cell epitope has been found on amino acid residues 25–39.¹⁰⁴ The T-cell response to *Amb a 5* allergens in homologs is controlled by DR2.2 MHC class II antigens.⁶⁴

***Amb a 6*, *Amb a 7*, and Cystatin**

Amb a 6 has been cloned molecularly. It is a polymorphic, 8-kD, nonglycosylated protein. Sequence homology with plant lipid transferase enzymes has been shown.⁶³ The IgE response in Caucasians allergic to ragweed has been linked to HLA-DR5 molecules.⁶⁵

Roebber et al⁹⁰ used conventional techniques to isolate *Amb a 7*. This is a 12-kD protein that is similar to plastocyanins (blue copper protein). The relationship of this allergen to *Amb a 4* has not been established.

Rogers et al⁹³ used cDNA library screen with human IgE antibodies and were able to isolate a cystin proteinase inhibitor, cystatin. The importance of this allergen has yet to be established.

Mugwort and Pellitory Weed Allergens

The weed wall pellitory (*Parietaria officinalis* and *P. judaica*) is an important allergen in Southern Europe and in the Mediterranean area. Sensitivity to these weeds accounts for approximately 80% of the cases of pollinosis in some areas. A purified allergen from *P. officinalis*, *Par o 1*, was isolated.⁷³ This is a 14-kD glycoprotein. Its 12 N-terminal amino acid residues have been determined; the amino acid sequence is similar to *Par j 1*. This allergen was found to account for positive skin tests in pellitory sensitive individuals and for 85% of the IgE activity in serum of allergic individuals as determined by RAST inhibition studies.

Sallusto et al⁹⁴ obtained CD4+ positive T-cell clones from patients

allergic to *P. judaica*. They found no preferential V β usage. Clones were either HLA-DR or HLA-DQ restricted. Five of twelve clones were Th type in that they produced IL-4 and IL-5 in response to antigen stimulation whereas 12 were Th0 type.

Mugwort (*Artemisia vulgaris*) is a common pollen allergen in Europe. Two allergens have been isolated and purified by immunochemical techniques. These are designated *Art v* 1 and *Art v* 2. *Art v* 1 is a 60-kD protein and *Art v* 2 is a 30- to 38-kD protein. The functional and biological activity of these proteins is unknown.¹⁰⁴

ANIMAL ALLERGENS

Cat Allergen

Allergy to domestic cats is the major cause of allergic disease and is associated with an increased risk for the development of asthma. The major cat (*Felis domesticus*) allergen, *Fel d* 1, has been studied extensively by conventional as well as molecular biology techniques. Approximately 80% of patients allergic to cats have antibodies to IgE antibodies to *Fel d* 1. Using cDNA cloning and genomic DNA analysis, the structure of *Fel d* 1 has been well established. It is a 36-kD heterodimer that contains two polypeptide chains. Both chains are glycoproteins, and different genes encode for each of them. Chain 1 is a 5- to 6-kD protein that shows homology to uteroglobin.^{42, 69} Chain 2 is a 16-kD protein that has two dominant forms. Chain Ch2l is preferentially expressed in salivary glands while chain Ch2s is preferentially expressed in skin, particularly in the sebaceous glands. Differences in IgE binding activity between fluid phase and solid phase binding studies have been demonstrated.¹⁰² IgE, however, has been shown to bind to both chains of the mature protein.^{91, 102} B-cell epitope studies indicate that IgE binding to the epitopes is conformational. IgE binding is lost by reduction alkylation and pretreatment of the protein in alkaline conditions. T-cell epitopes are on both chains.⁹¹ Immunotherapy trials are underway using T-cell epitope-derived peptides. These will be discussed in a subsequent article in this issue.

Mouse and Rat Allergens

Exposure to urinary proteins from mice and rats is an occupational hazard in laboratory animal workers as well as individuals keeping these rodents as pets. A major urinary protein from the mouse, *Mus m* 1, has been purified. Multiple genes encode for this 22-kD acidic protein. The proteins are synthesized primarily in the liver and excreted in the urine. The genes encoding protein expression are controlled by antigenic hormone stimulation.¹⁰⁴

A rat urinary specific protein, *Rat n* 1, is a 20-kD pre-albumin acid glycoprotein. A second rat protein, *Rat n* 2, is α_2 u-globulin. This protein also has multigene control. *Rat n* 2 is found in increased concentrations

in the urine of post-pubertal male rats. This protein belongs to the major urinary protein (MUP) family similar to that of *Mus m 1*. These lipocalin-like proteins appear to bind pheromones and thus play a role in the sexual behavior of the animals.¹⁰⁴

Other Animal Allergens

A major allergen termed *Can f 1* from dogs has been isolated by conventional methods. The amino acid sequence of this protein has not been established but monoclonal antibodies have been raised against it for use in assessing exposure levels. Serum albumin from cats and dogs share similar amino acid sequences and have been identified as allergenic in some individuals sensitive to cats and dogs. Recently, a bovine allergen has been cloned.⁷⁶ Using cow epithelial RNA and a uniZap RX vector, the protein was expressed in *E. coli*. cDNA libraries were identified by screening with human allergic serum. The protein expressed is an 11-kD allergen that has sequence homology with the oligomycin-sensitivity-conferring protein of the mitochondrial adenosine triphosphate synthase complex. This allergen appears to be a minor one in that only 20% of serum from patients allergic to cattle showed IgE binding activity.

FUNGAL ALLERGENS

Aspergillus

Two groups of investigators^{7, 70} have molecularly cloned a major allergen from *Aspergillus fumigatus*, *Asp f 1*. This is an 18-kD protein that was found to have sequence homology with a cytotoxin, mitogillin.^{7, 70} The protein is produced only during the growth phase of the organism.⁵ The gene encoding for this protein is found in *A. fumigatus* and *A. restrictus* but has not been found in *A. flavus*, *A. niger*, *A. terreus*, or *A. nidulans*.⁵ The recombinant allergen has been shown to bind IgE in vitro and produce positive skin tests in *Aspergillus* sensitive individuals.⁷⁰ Further investigations have found that patients with allergic asthma and *Aspergillus* sensitivity have positive skin tests in IgE antibodies to the recombinant *Asp f 1* whereas patients with atopic dermatitis and *Aspergillus* sensitivity do not.²⁸

Alternaria

A major allergen, *Alt a 1*, has been purified by immunochemical techniques from *Alternaria alternata*.²⁴ The protein is a 30-kD dimer that is linked by disulfide bonds. The 20 amino acid sequence of the N-terminal portion of the molecule has been obtained.²⁴ A 20-mer peptide

representing the N-terminal of *Alt a 1* was synthesized and found to have IgE binding, although this was somewhat weak.¹²⁶

Other *Alternaria* allergens have been cloned, and these have been tentatively termed *Alt a 6*, *Alt a 7*, and *Alt a 10*.¹ These allergens may be relatively minor, although their full allergenic potential has not been explored. They do, however, share interesting homologies with other proteins from fungi. *Alt a 6* is a 53-kD protein that has sequence homology similar to a P2 ribosomal protein from *Cladosporium herbarum*. *Alt a 7* is a 22-kD protein that has sequence homology with *ycp4* yeast protein. Lastly, *Alt a 10* is an 11-kD protein that is an alcohol dehydrogenase.¹

Cladosporium

Zhang et al¹²⁵ produced a cDNA clone coding for the allergen *Cla h 3* from *Cladosporium herbarum*. The allergen was produced by using RNA and a lambda Zap 11 vector. Clones were identified by screening with human IgE antibodies. The encoded protein has a molecular mass of 11 kD and an isoelectric point of 3.94. It was found to have homology to fungal ribosomal p2 proteins. Previously, *Cla h 1* and *Cla h 2* had been purified by immunochemical techniques,¹⁰⁴ although their amino acid sequences have not been determined. Achatz et al¹ have cloned additional *Cladosporium* allergens. One is a 53-kD alcohol dehydroxygenase that has sequence homology to that of the *Alternaria* allergens described previously. Likewise, another *Cladosporium* allergen, which has a molecular weight of 22 kD, also showed sequence homology with *ycp4* yeast proteins. Lastly, a 48-kD protein was cloned, which has sequence homology to enolase. The full extent of the allergenicity of these proteins is not known.

Candida albicans

Shen et al⁹⁸ cloned a 40-kD allergen from *Candida albicans*. This protein had 77% amino acid homology with an alcohol dehydrogenase from *Saccharomyces cerevisiae*. This allergen had IgE binding activity and may be similar to allergens from *Cladosporium* and *Alternaria*.¹

Psilocybe cubensis

An allergen termed *Psi c 2* from the Basidiomycete *Psilocybe cubensis* has been cloned.⁵¹ This was expressed in *E. coli* as a 23-kD fusion protein. It was found to inhibit IgE binding to a 16-kD allergen from *Psilocybe* in immunoprint assays. This protein has 78% identity with a cyclophilin.

INSECT AEROALLERGENS

Cockroaches

Sensitivity to cockroach allergens has been recognized as an important contributor to asthma, particularly in inner-city inhabitants. A number of cockroach allergens have been identified by conventional methods. The German cockroach allergen, *Bla g 1*, from *Blattella germanica* shows skin test reactivity in approximately 30% of cockroach allergic individuals. Recently, *Bla g 2*, a major allergen that shows skin test reactivity in approximately 58% of cockroach allergic individuals, has been cloned.⁸ This is a 36-kD allergen that has sequence homology to aspartic proteases. It has been detected in the digestive organs such as the esophagus and gut of *B. germanicus* but not of American cockroaches (*Periplaneta americana*). Thus, the protein appears to be a digestive enzyme of the cockroach.

Arruda et al⁸ have cloned three additional cockroach allergens, *Bla g 4*, *Bla g 5*, and *Bla g 6*. *Bla g 4* is a 21-kD calycine family member that binds pheromones. *Bla g 5* has a molecular weight of 25 kD and has been identified as a glutathione transferase. *Bla g 6* is a troponin with a molecular weight of 25 kD.

An additional cockroach allergen that has been cloned has yet to be named.⁴⁷ The recombinant protein has a molecular weight of greater than 80 kD and shown to bind IgE in more than 50% of sera from cockroach allergic individuals.

An allergen from the American cockroach, *Per a 3*, has been cloned molecularly.¹²¹ This allergen has a molecular weight of 72 kD; its function is unknown.

Stinging Insect Venoms

Stinging insect reactions are an important cause of systemic anaphylactic reactions. Stings from honey bees, hornets, wasps, yellow jackets, fire ants, and the Australian jumper ant have been implicated.

Honey Bee Venom

Phospholipase A2, which is an important constituent in bee venom, is a major allergen. A cDNA clone from honey bee venom glands has been obtained that encodes for phospholipase A2.⁵⁸ This allergen showed homology to phospholipase from mammalian pancreas and snake venom. Recombinant phospholipase A2 showed positive skin test reactivity, although correct refolding of the recombinant allergen is a prerequisite while glycosylation is less important.⁷¹ In mice, enzymatically active recombinant phospholipase A2 was necessary to induce an IgE antibody response.³¹ Both enzymatically active and inactive recombinant phospholipase A2, however, will release histamine from the basophils

of sensitized individuals.³⁷ Glycosylation of the protein has been demonstrated to be a requirement for some T-cell recognition.³¹ Two immunodominant epitopes that induce T-cell responses have been identified on the phospholipase A2 molecule. These include amino acids 50–69 and 83–97. T-cell lines reactive to phospholipase A2 produce IL-4 when stimulated by these peptides.²⁶

Wasp, Hornet, and Yellow Jacket Venom

A group of allergens from vespids has been studied by molecular techniques. *Dol m 5* was cloned from white-faced hornet venom. It has two isoforms that differ by approximately 23% in their amino acid sequences but are antigenically similar. The allergen was found to be similar to the pathogenesis-related protein of the tobacco leaf. Hornet, wasp, and yellow jacket venoms (*Dol a 5*, *Dol m 5*, *Ves m 5*, *Ves v 5*, *Pol a 5*, and *Pol e 5*³⁶) are similar major allergens. A variable degree of cross-reactivity among these venoms has been identified.⁶²

Ant Allergens

Using conventional methods, Hoffman⁵⁰ isolated several fire ant (*Solenopsis invicta*) allergens. *Sol i 1* is a phospholipase. Others have been identified as *Sol i 2*, *Sol i 3*, and *Sol i 4*. Molecular weights for these allergens are approximately 13 kD for *Sol i 2* and *Sol i 4*, and 24 kD for *Sol i 3*. The latter allergen has approximately 50% identity with *Dol m 5*.

Donovan et al³⁰ used molecular cloning to produce a recombinant allergen to the venom of the Australian jumper ant (*Myrmecia pilosula*). This allergen is known as *Myr p 1*. There was no known sequence homology with other allergens, and IgE binding to the C-terminal end of the molecule was demonstrated.

LATEX ALLERGENS

Latex sensitivity can arise from direct contact of the skin and can be manifested as a delayed hypersensitivity reaction rash or as an IgE-mediated contact urticaria. Systemic absorption of latex allergens through mucosal surfaces can result in systemic anaphylaxis. Airborne allergens can induce episodes of asthma in sensitive individuals. With the increased use of latex gloves in medical facilities, latex sensitivity has reached epidemic proportions.

A number of proteins from latex have been identified as potential allergens. The full gamut of latex allergens has yet to be identified. Two allergens have been purified; one termed *Hev b 1* (*Hevea brasiliensis*) is a rubber elongation factor. This is a 14-kD monomer that is linked noncovalently as a homotetramer. The full protein has a molecular weight of 58 kD.²⁵

Recently, a 27-kD protein was isolated from latex and shown to have sequence homology to fruit lysozymes.¹²² It is anticipated that additional latex allergens will be purified and characterized by both conventional and molecular biology methods.

SUMMARY

The application of molecular biology techniques to the study of allergens has led to an ever increasing list of well-characterized allergenic proteins. Studies of the cDNA and amino acid sequences of these proteins has enhanced our understanding of the biologic functions of these proteins. Further, this knowledge helps to explain cross-reactivity between various plant and animal species. For example, exposure to birch pollen may lead to sensitization to profilin, which is contained in hazel nut seeds and is manifested by anaphylaxis when individuals sensitive to birch pollen ingest hazel nuts.

Clinically, recombinant allergens have the potential to be used as standardized reagents in diagnostic tests for allergy. An understanding of the T- and B-cell epitopes of these proteins have potential therapeutic implications. T-cell reactive peptides can induce tolerance and may usher in a new mode of treatment for allergic disease. Molecular biology approaches have resulted in a new era of theoretical and practical applications to the diagnosis and treatment of allergic diseases.

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