

THE MOLECULAR BIOLOGY OF FOOD ALLERGY

Susan L. Hefle, PhD

The use of molecular techniques such as amino acid sequencing, DNA sequencing, molecular cloning, use of synthetic peptides, and monoclonal antibodies in the study of allergenic proteins has resulted in a large amount of information about a wide variety of allergens.¹¹ The striking increase in knowledge of allergen structure since the early 1980s can be attributed directly to recombinant DNA technologies. Before the advent of recombinant technologies, progress on the molecular/biochemical characterization of allergens was slow due to several factors, including the large numbers of allergenic molecules, the quantities of allergens present in source material, and the availability of suitable methods for allergen identification.¹¹ Through the use of recombinant techniques, it is possible to create a reliable, reproducible, immortal allergen source available in stable, large quantities.¹³³ Molecular cloning has had a major bearing on the study of gene structure and also on the characterization of the polypeptides expressed by those genes. DNA sequencing techniques are so efficient and rapid that one can obtain the whole primary sequence of an allergen in a few days' time.⁴⁰ The use of recombinant DNA techniques for the study of allergenicity of proteins is in many ways a preferred method to the traditional procedures of protein purification, digestion, and analysis of peptides for both allergenicity and amino acid sequence.⁴⁰ Recombinant allergens can be produced at very high purity (an advantage over extracts from natural sources that may contain a number of other allergens and nonallergenic material), and provide recombinant allergens to use in place of natural

From the Food Allergy Research and Resource Program, University of Nebraska, Lincoln, Nebraska

allergens that are present in limited quantities in a given source. In addition, recombinant allergens may improve significantly diagnosis with some allergens that are labile and degrade rapidly (e.g., apple). Recombinant allergens provide better tools for allergen standardization, structural investigation, and T- and B-cell epitope identification. A further advantage of recombinant allergens is that they can be added to natural allergenic extracts to improve the quality of current diagnostic extracts, or a few (two to four) recombinant allergens cloned from a given source can be pooled for diagnostic purposes.¹⁸

A major objective for producing a recombinant allergen is that it be comparable to its natural counterpart, including IgE-binding. Recombinant allergens used *in vivo* should be pure (as defined in the World Health Organization (WHO) document on recombinant proteins).⁸⁶ Recombinant allergen samples must be demonstrated to lack toxicity using animal model systems and should be stable to the conditions under which routine allergen standardization is accomplished. Because a number of allergens possess inherent biologic activity, such as enzymes or lectins, their recombinant counterpart may possess these activities as well. If there exist undesired traits, such as toxicity, these traits may have to be removed. The safety and efficacy of recombinant allergens are major concerns and are issues that must be considered prior to the use of such recombinant allergens in human beings.

The advantage of molecular cloning could be realized fully in the possibilities of manipulation of allergenic sequences synthesized according to amino acid sequences of cloned allergens.¹³² The molecular structure could be modified to reduce IgE-binding capacity, but retain T-cell receptor activity. The modification of some epitopes may, therefore, lead to suppression of the entire immune response to an allergen. Opportunities such as this may assist greatly in advancing the embryonic science of immunotherapy for food allergies. In addition, recombinant food allergens will help define the immunologic and pathophysiologic mechanisms regarding sensitivity.²⁷

Molecular biologic advances in allergy, in their truest definition, refer to production of recombinant allergens. In the area of food allergy, molecular biologic advances are modest compared with those in the area of inhalant allergies. In a limited number of cases, recombinant allergens have been produced, but most of the molecular-level knowledge of food allergens has been gained using conventional molecular techniques such as amino acid sequencing and use of synthetic peptides. This article, therefore, reviews the molecular biology of allergenic food proteins under a larger umbrella than solely recombinant techniques.

ALLERGEN NOMENCLATURE

In response to the heightened progress in recombinant technology with regard to allergens, a nomenclature system has been adopted. This

system has been described recently in detail.⁸⁷ Allergens are designated according to the accepted taxonomic name of their source. The first three letters of the genus are used, followed by the first letter of the species and then an Arabic number. The numbers are assigned to these allergens according to the order of their identification, and generally the same number is used to designate homologous allergens of related species. For example, the first allergen described in brown shrimp, *Penaeus aztecus*, is designated *Pen a* 1 and the homologous molecule from Indian shrimp *Penaeus indicus* is named *Pen i* 1.

Members of an allergen group that have more than 67% amino acid sequence homology are designated as *isoallergens*.⁸⁷ Each isoallergen may have multiple forms of very similar sequences that are designated as *variants*. Furthermore, the nomenclature system provides rules for describing allergen genes, mRNAs, cDNAs, and recombinant and synthetic peptides of allergenic interest.⁸⁷

Researchers customarily describe allergens as "major" or "minor." Major allergens generally are defined as proteins for which 50% or more of the allergic patients studied have specific IgE.^{87, 88} Examples of major allergens are *Ara h* 1 from peanuts³⁰; ovalbumin, ovomucoid and ovotransferrin from eggs⁹³; and *Pen a* 1 from shrimp.³⁷ The significance of minor allergens, which are those that fall below the 50% definition, has been debated. Minor allergens may be the result of experimental artifact or may contain similarities in structure to major allergens that allow for IgE-binding, but which do not have the conformation necessary to elicit histamine release. For example, research has shown that although peanut-allergic patients possess IgE that can bind to proteins from many other legumes, resulting in positive skin-test and RAST results, the clinical manifestations of such cross-reactivity are apparently rare, as the patients are only documented to be allergic to peanut and perhaps one other leguminous food.¹⁶ On the other hand, given that the most popular technique used for identifying allergens is a process that causes proteins to be broken down into subunits (sodium dodecyl sulfate-polyacrylamide gel electrophoresis: SDS-PAGE), minor allergens actually could be parts of larger major allergens, and, therefore, may have the potential to cause significant reactions in some individuals. The major allergens often are found in abundance in a food. *Ara h* 1 apparently is part of a storage protein of the peanut.²⁷ This is not always the case, however; for example, the major codfish allergen, *Gad c* 1, comprises a small fraction of the total protein of the codfish, yet is the major allergen.⁴⁹ On the other hand, shrimp tropomyosin is the major shrimp allergen,³⁷ whereas beef and pork tropomyosins are apparently not allergenic.

MOLECULAR BIOLOGY OF SPECIFIC FOOD ALLERGENS

A summary of identified food allergens to date is given in Table 1.

Table 1. IDENTIFIED FOOD ALLERGENS

Allergen	Food	Amino Acid Sequence (Ref)	cDNA Sequence (Ref)	Known Homology or Type of Protein
<i>Ara h 1</i>	peanut		C (27)	Vicilin
<i>Ara h 2</i>	peanut		P (28)	
<i>Ber e 1</i>	Brazil nut	C (5)	C (5)	
<i>Bra j 1</i>	oriental mustard	C (111)		Parvalbumin Ovomucoid Ovalbumin Ovotransferrin Lysozyme OBAP α -Amylase inhibitor Tropomyosin Tropomyosin Tropomyosin
<i>Fag e 1</i>	buckwheat	P (146)		
<i>Gad c 1</i>	codfish	C (49)		
<i>Gal d 1</i>	eggwhite	C (83)	C (35)	
<i>Gal d 2</i>	eggwhite	C (116)	mRNA (104)	
<i>Gal d 3</i>	eggwhite	C (158)	mRNA (73)	
<i>Gal d 4</i>	eggwhite	C (33)	mRNA (80)	
<i>Gly m 1</i>	soybean	P (123)		
<i>Hor v 1</i>	barley		C (105)	
<i>Met e 1</i>	shrimp		C (96)	
<i>Pen a 1</i>	shrimp	P (37)		Trypsin inhibitor Amylase inhibitor α -subunit of β -conglycinin
<i>Pen i 1</i>	shrimp	P (136)		
<i>Sin a 1</i>	yellow mustard	C (107)	C (65)	
α -Lactalbumin	milk	C (21)	C (71)	
Bovine serum albumin	milk	C (66)		
β -Lactoglobulin	milk	C (20)		
Casein	milk	C (22, 109, 128)	C (4)	
KSTI	soybean	C (89)		
Rice	rice		C (72)	
Wheat	wheat	C (61, 62, 98)		
68 k soybean			C (134, 135)	

C = complete sequence; P = partial sequence; OBAP = oil-body-associated protein.

Cow's Milk

A number of milk proteins have been identified as allergenic or immunogenic in humans. Caseins and β -lactoglobulin appear to be the major allergens in cow's milk,^{68, 131} and to a lesser degree, α -lactalbumin and bovine serum albumin have been implicated.⁶³

Caseins

The frequency of IgE-reactivity to individual casein proteins has not been evaluated systematically. The caseins are a family of proteins that share certain similarities, such as the clustering of nonpolar and polar residues to form domains and presence of phosphoseryl groups. The structural homologies of the α - and β -caseins suggest that they may have evolved from a common ancestral gene.¹⁴⁴ The flexible, highly solvated polar domain of α - and β -caseins should be the most susceptible portion for proteolysis. κ -casein does not contain the cluster of phosphoseryl residues, and hence, does not bind as much calcium as the α - and β -caseins do.¹⁴⁴

α -_{s1}-casein has at least five genetic variants. The amino acid sequence of the protein (B variant has 199 residues and a molecular weight of approximately 23,614 D) has been established.¹¹⁰ Residues 41–80 encompass eight sites of post-translational phosphorylation, whereas the remaining C-terminal section is fairly hydrophobic. Based on the predicted structure, hydrophobic and hydrophilic domains connected by a segment of α -helix have been identified.¹⁵⁶ The amino acid sequences of the α -_{s2}-caseins have been established.²² These caseins have varying degrees of post-translational phosphorylation, and are the most hydrophilic of the casein family. The amino acid sequence has been obtained (207 amino acid residues, molecular weight of approximately 25,230 D).²² Four genetic variants have been identified.¹⁵⁶

β -caseins are a group of proteins that have one major component with seven genetic variants, and eight minor components, which are proteolytic fragments of the major component. The molecular weight of the major component has been established (A² variant has 209 amino acid residues, molecular weight of 23,983 D).¹²⁸ β -caseins are the most hydrophobic of the caseins, and modeling studies indicate that the protein has hydrophobic side chains dispersed over the C-terminal end and center surface of the structure, with a hydrophilic N-terminus.¹⁵⁶

The κ -casein molecule has a molecular weight of 19,007 D and is composed of 168 amino acid residues¹⁰⁹ (B variant). κ -caseins have two genetic variants. This protein is cleaved between residues 105 and 106 by rennin (chymosin) into two domains. The hydrophobic domain (*para*- κ -casein) is not soluble, whereas the polar domain (macropeptide) is extremely soluble.¹⁴⁴

Whey Proteins

Whey proteins comprise approximately 20% of milk proteins. The most abundant whey protein is β -lactoglobulin, a protein of molecular weight 18,277 D (162 amino acid residues)²⁰ and with at least six genetic variants. The complete DNA sequence encoding β -lactoglobulin has been determined, and shows 91% sequence homology with ovine β -lactoglobulin.⁴

α -Lactalbumin, molecular weight of 14,174 D (123 amino acid residues),²¹ has two genetic variants. The two genetic variants differ in only one amino acid sequence. The cDNA sequence of α -lactalbumin has been reported (703 nucleotides).⁷¹ The cDNA library was made from size-selected (>400 bp) double-stranded cDNA in *Escherichia coli* NM522, and screened using a rat α -lactalbumin cDNA probe. A sequence similarity of 43% has been reported for lysozyme and α -lactalbumin.⁸⁵

Bovine serum albumin (BSA) is identical in characteristics to bovine blood serum albumin. BSA is a heterogeneous protein with a molecular weight of 66,430 D (583 amino acid residues),⁶⁶ and comprises approximately 1% of the total milk protein. The molecule is visualized as having three major domains, each consisting of two large double loops and a small double loop, and appears to be in the shape of an ellipsoid.²³

Cows' Milk Epitopes

Although caseins are significant allergens, no data are available regarding their human T-cell or B-cell epitopes. Because α_{s1} -casein is the casein that most often interacts with IgE from infants and children with milk-allergic symptoms,¹³¹ it would be useful to have information regarding the human epitopes of this protein. For α_{s1} -casein, some mouse T- and B-cell epitopes have been elucidated and identified as sequential epitopes.¹⁴⁰

Bernard et al.,¹⁵ using β -casein peptides created by proteolytic processes, found that of 13 serum samples from subjects highly allergic to β -casein, one third of the sera reacted mainly with the region 1–52 (region 1–28 being the most reactive). Another one third reacted to region 53–105, and one third to 106–209, mainly to the 106–139 portion. Therefore, it appears that human B-cell epitopes for β -casein are spread over the entire molecule.

Baldo¹⁰ found that the glycomacropeptide from κ -casein (amino acid residues 106–167) and the polypeptide fragment encompassing amino acid residues 99–167 bound with IgE in most of the milk-allergic serum in an immunoblotting study. The 99–167 peptide proved more reactive, and might indicate the presence of an IgE epitope in amino acid sequence 99–105 (P-H-P-H-L-S-F).

Although some preliminary work has been done on rat B-cell epitopes of β -lactoglobulin,⁷⁰ Ball et al.,¹² using serum from milk-allergic children, reported that the synthetic peptide T-D-Y-K-K-Y-L-L-F-C-M-E of β -lactoglobulin ("A" variant) represented a major sequential human

allergenic epitope. β -lactoglobulin peptide 124–134 (R-T-P-E-V-D-D-E-A-L-E-A) and α -lactalbumin peptide 5–18 (K-C-E-V-F-R-E-L-K-D-L-K-G-Y-A) bound IgE. Synthetic β -lactoglobulin peptides 41–52, 91–100, and 130–130, however, did not bind IgE.³

Egg

Eggwhite appears to be more allergenic than egg yolk. Eggwhite proteins have been studied extensively; most have been purified and their amino acid sequences have been determined. The major eggwhite proteins are ovalbumin, ovotransferrin, ovomucoid, ovomucin, and lysozyme. Although the eggwhite proteins have been studied extensively and some molecular biology studies have been done, most researchers have used biochemical methods to elucidate epitope information.

Egg yolk can be separated into two fractions using ultracentrifugation: a granule (sedimented) fraction containing 60% protein and 35% lipid, and a plasma (supernatant) solution, containing 18% protein and 80% lipid.¹²⁵ The granule fraction contains α - and β -lipovitellins (high-density lipoprotein), phosvitin, and low-density lipoprotein.³¹

Ovomucoid

Ovomucoid has been documented in several studies as a major allergen of egg and has been named *Gal d 1*. It is a glycoprotein with a molecular weight of 28 kD and an isoelectric point (pI) of 4.1, is a trypsin inhibitor, and makes up 10% of the protein in eggwhite. Ovomucoid has been cloned,¹⁴² and the mRNA sequence has been determined from cDNA.³⁵ The amino acid sequence of ovomucoid, which was obtained by conventional methods in 1987, contains 186 residues.⁸³ There are three tandem domains, each homologous to pancreatic secretory trypsin inhibitor; however, in the domestic chicken, only one domain has inhibitory capabilities.¹⁴³

Ovalbumin

Ovalbumin (*Gal d 2*), the most abundant protein in eggwhite, is a monomeric phosphoglycoprotein with a molecular weight of 42,699 D and a pI of 4.5. The 385-residue amino acid sequence was established by deduction from cyanogen-bromide cleavage fragments and other proteolytic fragments.¹¹⁶ Purified ovalbumin has three variants, A₁, A₂, and A₃, which contain two, one, and no phosphate groups per molecule, respectively.¹²⁵ The mRNA nucleotide sequence of ovalbumin was obtained from a duplex DNA copy of the mRNA and its subsequent cloning in pMB9.¹⁰⁴

Ovotransferrin (Conalbumin)

Ovotransferrin (*Gal d 3*) has a molecular weight of 80 kD (686 amino acid residues) and a pI of 6.0. Jeltsch and Chambon⁷³ determined the

nucleotide sequence of an almost full-length, double-stranded cDNA clone.¹⁵⁸ Through the use of the sequence of the 5' end of the ovotransferrin gene,³⁶ a complete sequence of chicken ovotransferrin mRNA was acquired.¹⁵⁸ In addition, the amino acid sequence was obtained using conventional methods.⁷³ Ovotransferrin has antimicrobial activity and iron-binding properties by way of its two homologous iron-binding domains. There is approximately 40% homology in the sequences of the two domains, which are believed to have arisen from gene duplication 500 million years ago.¹⁵⁷

Lysozyme

Lysozyme (*Gal d 4*) is a 14.3-kD protein with a pI of 10.7. Of all the proteins of eggwhite, lysozyme has been investigated most thoroughly at the molecular level. Its 129 amino acids have been sequenced by use of proteolytic fragments and Edman degradation.³³ It is a single polypeptide chain cross-linked by four disulfide bridges. The chain is folded upon itself so that the first 40 residues from the N-terminal end form a compact globular domain.¹⁷ There is a second hydrophilic domain (residues 40–85), which forms one site of the active site cleft. Its mRNA with exons and flanking introns also have been identified.⁸⁰ Recombinant portions of lysozyme used in the study of mouse T-cell epitopes have been produced.¹²⁴ A consensus on the role of lysozyme in egg allergy, however, has not been established.

Egg Epitopes

Some progress has been made in determining the T- and B-cell epitopes of ovalbumin. A synthetic peptide prepared from ovalbumin demonstrates that sequences that are recognized by human IgE antibodies also may stimulate rabbit T cells.⁷⁶ This peptide comprises amino acid sequences 323–339. Renz et al¹²⁷ found this same sequence to be important in the generation of immediate hypersensitivity responses in BALB/c mice exposed via the respiratory route. Shimojo et al¹³⁹ also found that an ovalbumin-specific T-cell line from an egg-allergic patient recognized peptide 323–339. The 323–339 sequence is K-G-A-E-N-I-E-A-H-A-A-H-V-A-Q-S-I.⁷⁶

B-cell epitopes for ovalbumin have been established more clearly. Johnsen and Elsayed⁷⁶ demonstrated that IgE-binding occurs with amino acid sequence 323–339, the same sequence noted previously in two studies to stimulate T cells in humans and mice. Four linear and two conformational epitopes on ovalbumin using rabbit polyclonal and human IgE with synthesized peptides of ovalbumin found region 1–10 to be a haptenic epitope. In addition, region 11–70 encompassed a linear determinant and was thought to be a fragment of a conformational determinant located in residues 20–70. Residues 71–285 gave two linear epitopes (105–122 and 323–339) and parts of them (85–92, 132–148, 214–232, and 368–385) were felt to be likely involved in a conformational

epitope.⁴⁵ In another study, this group found that region 11–70 does not encompass continuous epitopes, but probably conformational one(s), and whereas peptides 34–46 and 47–55 were not reactive, the allergenicity was distributed over the whole 11–70 region, with 33–70 the strongest. The activity of region 11–19 was weak, but was capable of specific IgE inhibition. Kahlert et al,⁸¹ using cyanogen bromide cleavage of a commercial ovalbumin preparation, demonstrated IgE-binding to peptide sequences 41–172 and 301–385.

Ovomucoid has prominent carbohydrate-containing domains.⁹⁷ Ovomucoid residues 1–64 encompass domain I, residues 65–130 are domain II, and residues 131–186, domain III. It appears that human IgE binds to the glycosylated domains but not to the nonglycosylated domains, although it is questioned if the carbohydrate moiety acts as an IgE-binding epitope.⁸⁵ Konishi et al⁹¹ found in a mouse model that domain II contained 40% of the allergenicity of ovomucoid, but full allergenic potential could only be realized in combination of domain II with either domain I or domain III. In humans, T-cell proliferative responses for three egg-allergic subjects were studied with highly purified ovomucoid domains.⁴⁴ T cells from two of the subjects proliferated to domain II, although T cells from the third patient did not proliferate to any of the purified domains.

Codfish

Gad c 1

The most comprehensive analysis of a food allergen has been the elegant work by Aas and Elsayed and colleagues, which resulted in the purification and characterization of the major codfish allergen, *Gad c 1* (originally designated Allergen M). *Gad c 1* belongs to a group of muscle tissue proteins known as *parvalbumins*,⁴⁹ which control the flow of calcium in and out of cells and are only found in the muscles of amphibians and fish. *Gad c 1* is an acidic protein (pI 4.75) with a molecular weight of 12,328 D. It is composed of 113 amino acids, which encompass three domains named AB, CD, and EF.⁴⁶ The CD and EF domains coordinate one Ca^{+2} -binding site each, whereas the AB domain does not have this property.

Epitopes

Gad c 1 contains at least five IgE-binding sites.⁴⁸ Two tryptically derived allergenic fragments, TM1 and TM2, were equally active in allergenicity studies.⁵¹ TM1 is composed of amino acid residues 1–75 and envelops the AB and CD domains, whereas TM2 is composed of amino acid residues 76–113 and envelopes the EF domain.

Whereas tryptic hydrolysis studies of TM1 showed that region 33–44 was important for allergenicity,⁵⁰ studies using synthetic peptides

established that region 49–64 encircles two repetitive sequences (Asp-Glu-Asp-Lys and Asp-Glu-Leu-Lys) that appear to be mutually important for IgE-binding.⁵⁴ It was shown that region 41–64 contained three homologous tetrapeptides, repeated in three sites, interspaced by six amino acids in a segment of 24 residues. IgE-binding capability is apparently independent of both the constitution and sequence of the spacer amino acids.⁵⁴

The AB domain shares more than 30% amino acid sequence homology with the CD and EF domains and is composed of residues 13–32. Synthetic peptides of this region demonstrated that it can bind IgE divalently.⁵³

Tryptic hydrolysis studies of TM2 initially showed that region 88–96 is partially responsible for the allergenicity.⁴⁷ In addition, however, region 88–103 in the EF domain was shown to have 37.5% amino acid sequence homology with the CD domain peptide and is proposed to have a monovalent binding function.⁵²

Shrimp

Pen a 1 and *Pen i 1*

Daul et al³⁷ isolated the major shrimp allergen, named *Pen a 1*, from boiled brown shrimp (*P. aztecus*) using conventional methods. *Pen a 1* has a molecular weight of 36 kD, and is readily isolated from the boiling water⁹⁵ and meat of cooked shrimp. *Pen a 1* is composed of 312 amino acid residues and 2.9% carbohydrate and has a pI of 5.2. It is referred to as *Pen i 1* if isolated from a different species of shrimp, *P. indicus*.¹³⁶ Endoproteinase studies of *Pen a 1* resulted in amino acid sequencing of a 21-residue peptide that demonstrated significant homology (60%–85%) with tropomyosin from various species, consistent with the conclusion that *Pen a 1* was shrimp tropomyosin.³⁷ The greatest homology occurred with fruit fly (*Drosophila*) tropomyosin (72%–87%); lesser (60%–62%) homologies were observed with tropomyosin from various mammalian species. The higher homology seen with *Drosophila* tropomyosin can be construed as being indicative of the phylogenic connection between shrimp and insects.³⁷

Met e 1

Leung et al⁹⁶ produced a recombinant shrimp allergen from a cDNA library of the greasyback shrimp, *Metapenaeus enis*. Shrimp mRNA and phage λ gt11 were used to construct the library, with which *E. coli* Y1090 was then infected. Sera from shrimp-allergic patients was used to screen the library. A 2-kb cDNA fragment was cloned into the plasmid expression vector pGEX, and screening was done with sera from shrimp-allergic patients. Five positive IgE-binding clones cross-hybridized with each other and bound IgE in sera from all eight shrimp-allergic patients

in the study. A 60-kD fusion protein was produced, which bound IgE from all eight serum samples. After consideration for the glutathione *S*-transferase, the deduced molecular weight of the recombinant protein was 34 kD. The allergen has 281 amino acid residues, and is similar in amino acid composition to *Pen a* 1 and *Pen i* 1. In addition, *Met e* 1 showed 76% similarity and 65% identity with *Drosophila* tropomyosin.

tRNA

A minor allergenic tRNA moiety from boiled shrimp (*P. indicus*) has been described.¹¹³ The tRNA allergen possessed 11% of its dry weight as amino acids. After enzyme treatment, 84% of the amino acids were lost, but allergenicity was retained. The tRNA was not sequenced. It is possible that the allergenicity was caused by RNA-associated proteins/peptides, as the RNA was not totally barren of amino acid residues. This remains the only documented example of a nucleic acid from food implicated in inducing an IgE response.

Epitopes

Shanti et al¹³⁶ reported that two tryptically derived peptide sequences from *Pen i* 1 bound shrimp-specific IgE. These were region 50–66 and region 153–161: 50–66 is M-Q-Q-L-E-N-D-L-D-Q-V-Q-E-S-L-L-K and 153–161 is F-L-A-E-E-A-D-R-K. Both peptides inhibited binding of specific IgE to shrimp tropomyosin. Corresponding regions of tropomyosins from different vertebrates showed little cross-reactivity in region 50–66, but demonstrated significant allergenic cross-reactivity with tropomyosins from mammalian species in region 153–160: seven of nine amino acids for chicken, rabbit, and human, and six of nine for rat tropomyosin. Many tropomyosins have homology in the 155–161 region; Shanti et al suggested that lack of homology in residues 153 (Leu) and 154 (Ala) between other tropomyosins and shrimp tropomyosin implies that they may be crucial for IgE-binding.

Leung et al⁹⁶ found that the recombinant shrimp allergen *Met e* 1 also possessed an IgE-binding sequence identical to the 50–66 region of Shanti et al,¹³⁶ and another small IgE-binding sequence of F-L-A-E-E-A-D-R-K, similar to the 153–161 region.

Peanut (*Arachis hypogaeae*)

Peanut proteins have been classified as albumins, arachin, and conarachin, or nonarachin.^{6, 38} Most of the storage proteins of the peanut are globulins, which make up 87% of the total protein.⁷⁵ The globulins are made up of two major proteins, arachin and conarachin, which correspond to legumin and vicilin, respectively. Under different conditions of ionic strength and pH, they associate and dissociate, making exact classification of individual components difficult.^{77, 78, 114} Arachin and con-

arachin have similar amino acid compositions and comparable electrophoretic mobilities, suggesting structural similarities. In its native state, arachin exists as a molecule of at least 600 kD and it readily dissociates into a 340- to 360-kD dimer and 170- to 180-kD monomer.¹⁵⁹ Johnson and Naismith⁷⁷ showed by ultracentrifugation that conarachin could be further divided into two fractions, one 2S and one 8.4S. Later, these were designated conarachin I and conarachin II (or α -conarachin), respectively. Conarachin I (molecular weight, 142 kD) constitutes nearly 30% of total peanut protein.¹²⁶ Conarachin II has a molecular weight of 290 kD³⁹ and represents 15% to 25% of the total peanut protein.¹²⁶ Conarachin II contains no carbohydrate and has a pI of 3.9.¹³⁷

The allergenicity of the peanut was found to be spread throughout the arachin and conarachin fractions,^{14, 145} and no single protein has been found to be solely responsible for all of the allergenicity of the peanut.²⁹

Ara h 1

Burks et al³⁰ identified a 63.5-kD (pI of 4.55) molecular weight glycoprotein peanut allergen using immunoblotting and ELISA methods with sera from peanut-sensitive atopic dermatitis patients. This allergen was found to be a major allergen and was named *Ara h 1*. This group went on to clone this important peanut allergen.²⁷ A peanut cDNA library was made from peanut (Florunner strain) mRNA, and was screened with IgE from peanut-allergic subjects. Of the resulting IgE-positive clones, a clone encoding *Ara h 1* was selected using *Ara h 1*-specific oligonucleotides and polymerase chain reaction (PCR) technology. Using the oligonucleotide GA (TC) AA (AG) GA (TC) AA (TC) GTNAT (TCA) GA (TC) CA derived from the amino acid sequence of an *Ara h 1* peptide (I-F-L-A-G-D-K-D-N-V-I-D-Q-I-E-K) as one primer and an oligo-dT 27-nucleotide-long stretch as the second primer, a portion of the mRNA that encodes this protein was amplified from peanut cDNA. A ³²P-labeled insert from this clone was used as a hybridization probe in a Northern blot containing peanut poly (A) + RNA. This insert hybridized to a single-sized mRNA of approximately 2.3 kb. The insert contained 1360 bases, not including the poly(A) tail. The sequence beginning at position 985 and extending through to position 1032 encoded an amino acid sequence identical to that determined from *Ara h 1* peptide I. DNA sequencing¹³⁰ of the cloned insert revealed that *Ara h 1* has significant homology to the vicilin seed storage protein family found in most higher plants. There was 64% homology over more than 1000 bases when the clone sequence was compared with broad bean and pea vicilins. Studies of IgE-binding from sera from 11 peanut-allergic subjects showed that eight bound to recombinant *Ara h 1* (produced in *E. coli* JM109 using vector pBluescript [Stratagene, La Jolla, CA]).

To begin the process of determining the IgE epitopes on recombinant *Ara h 1*, its sequence was digested with Exo III for varying lengths of time to produce a sub-library of clones with progressively shorter inserts.¹⁴¹ Clones produced in this manner were sequenced around dele-

tion point 1 to ensure that the shortened, re-ligated clones were in frame and would produce the appropriate peanut peptide. These clones then were expressed, and their products were tested in IgE-binding studies. All peptides produced by the shortened clones retained some IgE-binding, indicating that there are multiple *Ara h* 1 epitopes involved in peanut allergy.¹⁴¹ Computer-modeling predicted several antigenic sites along the molecule, but a predominance of sites near the amino terminus. There are at least three IgE-binding epitopes on recombinant *Ara h* 1.²⁵

Ara h 2

Burks et al²⁹ have identified and purified another peanut allergen, named *Ara h* 2. The allergen consists of two proteins whose molecular weights are 16.5 and 17.5 kD in SDS-PAGE (pI of 5.2). N-terminal sequence analysis was used to determine the first 35 amino acids; the proteins differed slightly in amino acid sequence. A set of oligonucleotide probes representing all of the possible coding sequences from the first (common) seven amino acids then was constructed. This set of probes and a poly-dT sequence were used as primers in the PCR to amplify *Ara h* 2 from first-strand cDNA synthesized from peanut mRNA, which resulted in two bands of 475 and 525 bp in length, respectively. Northern blot analysis using total peanut mRNA and α -³²P dCTP-labeled PCR products as a probe revealed that the amplified DNAs represented nearly full-length mRNAs.⁶⁰ There are at least two IgE-binding epitopes on recombinant *Ara h* 2.²⁶

Soybean (*Glycine max*)

Soybeans contain multiple allergens. Globulins are the major proteins of soybean, and when subjected to ultracentrifugation, separate into 2S, 7S, 11S, and 15S fractions. The 2S, 7S, and 11S fractions demonstrate specific IgE-reactivity and considerable cross-reactivity.^{24, 120, 138} Variable IgE-binding patterns on immunoblots suggest that no one fraction binds more IgE. Only in one study has the 11S fraction found to be less allergenic than the other fractions, even though it comprises much of the total storage protein in the soybean.¹²⁰ Because of the wide range of different analytical methods used in the study of these proteins, Catsimpoolas et al³⁴ have proposed the following nomenclature: the 2S component that is different from soybean trypsin inhibitor is referred to as α -conglycinin and the 7S component isolated by the method of Robert and Briggs¹²⁹ is referred to as β -conglycinin (vicilin). The 7S component isolated by the method of Koshiyama and Iguchi⁹² is referred to as γ -conglycinin, and the 11S component is referred to as glycinin. The 15S fraction consists mainly of polymers of glycinin.¹¹⁵ Because of the economic importance of soybeans, these proteins have been the focus of

much research effort, and many of them have been cloned and sequenced.^{58, 115}

Gly m 1

Gly m 1 is described by Ogawa et al¹²⁰ as a 30-kD molecular weight protein, a part of the 7S globulin fraction. Sixty-five percent of the subjects in this study had specific IgE for *Gly m 1*. In a later paper, this same research group¹²³ found that the native allergen had a molecular weight of more than 300 kD. The monomeric form had a molecular weight of 32 kD and a pI of 4.5. *Gly m 1* has an N-terminal 15 amino acid sequence and amino acid composition identical to that of the soybean seed 34-kD oil-body-associated protein (also called the soybean vacuolar protein P34). It is interesting to note that the 34-kD protein possesses 30% sequence homology to *Der p 1*, the major dust mite allergen, which is also a thiol protease.¹²³ The N-terminal sequence for *Gly m 1* is K-K-M-K-K-E-Q-Y-S-C-D-H-P-P-A. In addition, the 34-kD oil-body-associated protein bound strongly to IgE from soybean-allergic sera and monoclonal antibodies against *Gly m 1* in immunoblotting studies and demonstrated close homology to papain-like thiol proteinases.⁸² The 34-kD oil-body-associated protein has been assumed to comprise approximately 5% of the total seed cotyledon protein in the Miyagisiro variety of soybean, although amounts may vary depending on the content of lipid in soybean seeds.⁹⁰

68-kD Allergen

Ogawa et al¹²¹ found that 25% of IgE from sera of soybean-allergic individuals with atopic dermatitis recognized a 68-kD protein of the 7S globulin fraction in immunoblotting studies. The protein was shown to be the α subunit of β -conglycinin, with a pI of 5.0 to 5.2. The sera recognized the α subunit but did not recognize the α' or β subunits, even though they have a high degree of homology with the α subunit (the α and α' subunits share more than 90% homology).¹³⁴ The IgE-binding site in the 68-kD minor allergen of soybeans was judged to be located in the residue sequence 232–383. The predicted IgE-binding region on the α subunit, residues 232–383, corresponds to residues 258–417 on the α' subunit.¹³⁵

Kunitz Soybean Trypsin Inhibitor (KSTI)

KSTI has been described as an allergen in one soy-allergic subject working with KSTI in an occupational setting.¹¹² KSTI has been sequenced.^{84, 89} It is composed of 181 amino acid residues and has a molecular weight 20 kD. Brandon et al¹⁹ found that KSTI has at least two distinct antigenic sites, one of which is retained under denaturing conditions, and may be linear. KSTI was shown to elicit specific IgE antibodies in BALB/c mice.¹²²

Brazil Nut (*Bertholletia excelsa* H.B.K.)

The major allergen from Brazil nuts, *Ber e 1*, is a 2S high-methionine protein¹¹⁹ that apparently is composed of two subunits. The 9-kD subunit of the protein was found to contain 77 amino acids. A 3-kD subunit also has been reported. The cDNA sequence for *Ber e 1* has been established.⁵ Poly(A) RNA was produced from Brazil nut embryos and cloned in dimer-vector pARC7. Resulting clones were screened by colony hybridization using a 5'-labeled probe consisting of a mixture of six synthetic oligodeoxynucleotides complementary to the six possible RNA sequences that could encode a methionine-rich region found in the partial amino acid sequence of the 9-kD subunit. Sequencing generally was done by Sanger's method,¹³⁰ although some regions required sequencing by Maxam and Gilbert's method.¹⁰³ Characterization of the cDNA clones was done by hybrid-selected translation.⁹⁹ *Ber e 1* has 44% homology with the castor bean high-methionine protein and 21% homology with the rapeseed high-methionine protein.

This 2S high-methionine protein was used in studies to attempt to correct the inherent deficient methionine levels of soybeans. Although successful, the genetic transfer of *Ber e 1* to soybeans imparted Brazil nut allergenicity to the soybean,¹¹⁸ and hence, commercial development of this transgenic crop was abandoned.

Mustard

Yellow mustard (*Sinapis alba*) and oriental mustard (*Brassica juncea*) are members of the Brassicaceae family. *Sin a 1* is a 2S albumin from yellow mustard seed, has a molecular weight of 15 kD,¹⁰⁸ and is the major yellow mustard allergen.¹⁰⁷ It is a seed storage protein consisting of two disulfide-linked polypeptide chains, with 39 and 88 amino acid residues, respectively.¹⁰⁷ The amino acid sequences of both chains have been established. In addition, this protein has been isolated from rapeseed, castor bean, and Brazil nut.¹⁰⁷ Gonzalez de la Pena et al⁶⁵ described the cloning and expression of *Sin a 1*, the major allergen from yellow mustard seed. Cloning was carried out by means of PCR using nondegenerate oligo primers and coding for the N- and C-terminal regions of the mature protein. Sequence analysis showed the presence of two nucleotide sequences, indicating polymorphism and suggesting existence of multiple isoforms of the allergen. This 2S protein is a member of the Napins family, which is encoded by genes without introns, is synthesized as polypeptide precursors, and which in turn must be processed by specific maturation proteases to render the two chains of the mature protein.⁶⁵

Further studies have suggested that IgE-binding to *Sin a 1* is conformational, as reduction and carboxyamidomethylation of both polypeptide chains produced a substantial decrease in IgE-binding. Specific IgE-binding also was decreased when the only tyrosine residue of the protein

underwent nitration. A murine monoclonal antibody, which also binds to this tyrosine site, decreased IgE-binding by 50%.¹⁰⁶

The major allergen of oriental mustard seed, *Bra j* 1, has been isolated,⁶⁴ and its amino acid sequence has been determined.¹¹¹ The *Bra j* 1 allergen showed microheterogeneity, was classified as an albumin, and was immunologically cross-reactive with the major yellow mustard allergen, *Sin a* 1. In addition, it showed 89% sequence identity with *Sin a* 1.¹¹¹ Its molecular weight ranges from 16 to 16.4 kD. Monslave et al.¹¹¹ found that *Bra j* 1 and *Sin a* 1 share a common murine monoclonal epitope: a histidine residue seems to be essential for recognition by the antibody. The synthetic peptide Q-P-H-V-I-S-R-I-Y-Q-T-A-T, which corresponds to *Sin a* 1 residues 55–68, was recognized by the monoclonal antibody, and sera from 7 of 11 mustard-allergic patients showed IgE-binding to it. Both of these proteins appear to have an α -helical structure and are storage proteins with a high glutamine content.¹⁰⁸

Rice (*Oryza sativa*)

The major rice allergens have been found to consist of microheterogeneous albumin proteins with molecular weights ranging from 14 to 16 kD and with pIs of 6 to 8.¹⁰¹ They are encoded in a multigene family and belong to the α -amylase/trypsin inhibitor family in cereal seeds.^{2, 102} The nucleotide sequence of cDNA coding for the major rice allergen has been determined.⁷² The nucleotide sequence of 486 bp has an open reading frame that encodes for a 162 amino acid residue and has a deduced molecular weight of 14,764 D. A cDNA library was constructed from mRNA from maturing rice seeds and phage λ gt11. The clones were screened using a nucleotide sequence that contained the complementary sequence to the N-terminal amino acid sequence of a 16-kD major rice allergen. cDNA inserts were subcloned into pUC118 or 119, and sequencing was done by the method of Sanger et al.¹³⁰ In another study, four rice seed proteins encoded by cDNAs belonging to the α -amylase/trypsin inhibitor gene family were overexpressed as TrpE-fusion proteins by vector pATH3 in *E. coli* HB101. The molecular weight of the fusion protein was approximately 52 kD, and taking into consideration that TrpE is 37 kD, the remainder of the clones had deduced molecular weights of 14 to 15 kD. Although rabbit polyclonal and murine monoclonal antibodies specific for this recombinant rice allergen have been studied, no human IgE-binding studies have been done.⁷

The deduced amino acid sequence of the major rice allergen has been shown to have homology to barley trypsin inhibitor (20%) and wheat α -amylase inhibitor (40%).⁷² Because a single protein seems to account for much of the allergenic reactivity, attempts have been made to reduce rice allergenicity by selecting strains induced by chemical mutation to produce hypoallergenic cultivars.¹¹⁷ A second approach has been to introduce chimeric genes that encode antisense RNAs of rice allergic protein into rice with a hydromycin phosphotransferase gene by

electroporation to reduce the amount of allergenic protein formed in the grain.¹⁰⁰ This approach has had minor success.

Wheat and Barley

Although the vast majority of complaints of hypersensitivity to wheat and barley manifest themselves in the form of baker's asthma, an occupationally acquired syndrome, a certain number of individuals are allergic to ingested wheat. Proteins of wheat, rye, and barley flour are the most commonly implicated sources of allergens for baker's asthma. The major allergens in bakers' asthma have been identified as a group of α -amylase inhibitor (AI) proteins.⁵⁶ It is interesting to note that the deduced amino acid sequence of the major rice allergen has been shown to have homology to barley trypsin inhibitor (20%) and wheat AI (40%).⁷² One could theorize that some of the relevant allergenic proteins involved in wheat ingestion allergy could be AI proteins. This has not been shown to date, however. In one study, approximately 25% of wheat-allergic children were found to react to another cereal grain (barley, oat, or rye).⁷⁹ This phenomenon may be caused by cross-reaction with AIs or other allergenic proteins common to these cereals.

α -Amylase inhibitors from barley and wheat share an amino acid sequence homology of 37%.⁶² A monoclonal antibody directed against a 14 kD-wheat AI also recognizes a similar component in rye flour,⁵⁷ suggesting shared epitopes. A number of proteins from these cereal grain flours have been purified,⁹ and allergens from wheat^{60, 61, 94} and barley¹⁰⁵ have been cloned. Consequently, several trypsin/AI proteins from wheat species (*Triticum durum* Desf cv Agathe,⁶² *T. aestivum* L. genomes AABBDD cv Chinese Spring,⁶¹ *T. turgidum* L. genomes AABB cv Senatoree-Caplelli,⁶¹ *T. aestivum* cv Timgalen⁹⁸) have been cloned molecularly and their DNA sequences reported. Five continuous IgE-binding epitopes have been identified on the wheat AI.¹⁵⁴

Mena et al¹⁰⁵ have cloned the major barley allergen, which is a 14.5-kD barley endosperm protein. This is a glycosylated monomeric member of a multigene family of inhibitors of α -amylase/trypsin from cereal grains. Its deduced amino acid sequence contains 132 residues. A cDNA library constructed from mRNA from immature barley endosperms (*Hordeum vulgare* L. cv. Abissynian 2231) and vector λ NM1149 was created and screened with a 23-nucleotide degenerate synthetic probe derived from the N-terminal sequence of the purified allergen (corresponding to residues 4–11 from the N-terminal sequence).¹³ The complete sequence was obtained using the method of Sanger et al.¹³⁰ The resulting cDNA encoded for the known 20 N-terminal amino acid residues.¹³ Sequence analysis showed that the recombinant barley monomeric AI had 57% homology to the barley homodimeric AI, and 59% with the wheat monomeric AI. Computer modeling has predicted three antigenic sites for the barley monomeric AI, whereas wheat monomeric AI and barley homodimeric AI have two each.

Buckwheat (*Fagopyrum esculentum* Moench)

A major allergen of buckwheat seed, a protein with a molecular weight of 24-kD, was described by Urisu et al.¹⁴⁶ All of the buckwheat-allergic patient sera in the study showed IgE-binding to this protein. The N-terminal amino acid sequence showed 37.9% to 48.3% identity with the light chain of an 11S globulin from dicot species (pea, soybean, cotton, mouse-ear cress, rape, pumpkin, cucurbit, sunflower); 51.7% identity with a similar protein in monocots (rice, oat); and 31.0% identity with a protein from the gymnosperm of the Douglas fir pine.¹⁴⁶ These proteins correspond to B subunit oligomers of globulin storage proteins. No further analysis has been done on this allergen.

Pollen Allergies and Foods

It is well-documented that individuals with tree pollen allergies often suffer intolerance to nuts, fruits, and vegetables.^{32, 55} Apples^{8, 32} and hazelnuts^{8, 32} are the most common offenders, although reactions to botanically unrelated fruits also occur.^{59, 153} Individuals who suffer from grass or weed pollen allergies often show sensitivity to carrot, celery, potato, and some spices.^{1, 151}

Bet v 1

Bet v 1 is the major allergen of birch pollen and the most important allergen for birch pollen-food cross-reactions. It is a 17-kD cytosolic protein whose cDNA sequence is highly conserved in dicotyledonous plants.⁷⁴ It has homology to a family of mRNAs that are induced in somatic tissues of some higher plants by infection with pathogens.¹⁵⁵ No biologic properties of *Bet v 1* are yet known. Birch pollen cDNA was made using mRNA of birch pollen and λ gt11. The library was screened for IgE-binding proteins with serum from birch pollen-allergic individuals. The cDNA was subcloned into pKK223-3, then transformed into *E. coli* JM105. Serum from birch pollen-allergic individuals was used to probe the expressed proteins.¹⁴⁷

Ebner et al⁴² found immunologic cross-reactivity and nucleotide sequence similarity between *Bet v 1* from birch pollen and an apple allergen with a molecular weight of 17 to 18 kD. *Bet v 1* cDNA was ligated into the plasmid SK⁺ Bluescript, and a complete *Bet v 1* coding region was excised. This region hybridized to apple RNA in Northern blots, showing the presence of highly homologous transcripts in birch pollen and apple. Vieths et al¹⁵² have conducted amino acid sequencing of the 26 N-terminal residues of the 18-kD apple allergen and found significant sequence homology (62%) between this protein and that of the major birch pollen allergen *Bet v 1*. Hsieh et al⁶⁹ found that the amino-terminal amino acid sequences of the apple IgE-binding 18-kD and 31-kD proteins share about 50% sequence identity with *Bet v 1* and

other disease resistance proteins of various plants.⁶⁹ In addition, binding of IgE from patients with hazelnut allergy to an 18-kD hazelnut allergen could be blocked by preincubation of sera with recombinant *Bet v 1*.⁶⁷ *Bet v 1* is considered to be a B-cell epitope in toto, as only cDNAs encoding the entire open reading frame could be isolated by screening expression libraries with serum from birch pollen-allergic patients.¹³²

Profilin (Bet v 2)

Profilin is involved in birch pollen-fruit sensitivity, but also plays a wider role in cross-reactivity with other foods.¹⁴⁹ In contrast to other major allergens that occur in botanically related species, profilins are thought by some to represent important cross-sensitizing allergens responsible for allergic symptoms,⁷⁴ as approximately 20% of pollen-allergic patients show IgE-binding to profilin.¹⁴⁸ *Bet v 2*, a birch pollen protein identified as a profilin with a molecular weight of 14 kD, also has been determined to be a cross-reactive allergen in a variety of fruits and vegetables.¹⁵⁰ Recombinant *Bet v 2* was produced in the same manner discussed previously for *Bet v 1*, but screening was done with serum from birch pollen profilin-allergic individuals.

Ebner et al⁴³ found in immunoblotting studies that preincubation of serum from birch pollen-allergic patients with recombinant *Bet v 2* reduced cross-reactive IgE-binding to pear, celery, carrot, and potato proteins. In addition, IgE from three *Bet v 2*-allergic subjects bound to the purified 15-kD celery allergen, and this binding could be prevented by preincubation with recombinant *Bet v 2*.¹⁴⁹

SUMMARY

Recombinant food allergens will significantly improve diagnosis by allowing standardization of food allergen extracts. In addition, the quality of extracts could be improved through the use of recombinant analogs of scarce or labile allergens. Treatment in the form of immunotherapy would see the most potential in use of molecular biology techniques in the manipulation of food allergen genetic sequences. Genetic modification of some allergenic epitopes and subsequent use in immunotherapy may lead to repression of the entire immune response to an allergen. Advances such as this may assist the growth and success of the embryonic science of immunotherapy for food allergies. In addition, recombinant food allergens will help delineate the immunologic and pathophysiologic mechanisms regarding sensitivity.

At present, the inventory of recombinant food allergens is small. The list of cloned food allergens certainly will increase with time and will provide invaluable information and the keys to facilitating new therapies for the treatment of food allergy.

References

1. Aalberse RC, Koshite V, Clemens JGJ: Immunoglobulin E antibodies that cross-react with vegetable foods, pollen and Hymenoptera venom. *J Allergy Clin Immunol* 68:356, 1981
2. Adachi T, Izumi H, Yamada T, et al: Gene structure and expression of rice seed allergenic proteins belonging to the α -amylase/trypsin inhibitor family. *Plant Mol Biol* 21:239, 1993
3. Adams S, Barnett D, Walsh BJ, et al: Human IgE-binding synthetic peptides of bovine β -lactoglobulin and α -lactalbumin: In vitro cross-reactivity of the allergens. *Immunol Cell Biol* 69:191, 1991
4. Alexander LJ, Hayes G, Pearse MJ, et al: Complete sequence of the bovine beta-lactoglobulin cDNA. *Nucleic Acids Res* 17:6739, 1989
5. Altenbach SB, Pearson KW, Leung FW, et al: Cloning and sequence analysis of a cDNA encoding a Brazil nut protein exceptionally rich in methionine. *Plant Mol Biol* 8:239, 1987
6. Altschul AM, Neucere NJ, Woodham AA, et al: A new classification of seed proteins: Application to the aleurins of *Arachis hypogaea*. *Nature* 203:501, 1964
7. Alvarez AM, Fukuhara E, Nakase M, et al: Four rice seed cDNA clones belonging to the α -amylase/trypsin inhibitor gene family encode potential rice allergens. *Bioscience, Biotechnology, and Biochemistry* 59:1304, 1995
8. Andersen KE, Lowenstein H: An investigation of the possible immunological relationship between allergen extracts from birch pollen, hazelnut, potato and apple. *Contact Dermatitis* 4:73, 1978
9. Armentia A, Sanchez-Monge R, Gomez L, et al: In vivo allergenic activities of eleven purified members of a major allergen family from wheat and barley flour. *Clin Exp Allergy* 23:410, 1993
10. Baldo BA: Milk allergies. *Australian Journal of Dairy Technology* 39:120, 1984
11. Baldo BA: Structural features of allergens large and small with emphasis on recombinant allergens. *Curr Opin Immunol* 3:841, 1991
12. Ball G, Shelton MJ, Walsh BJ, et al: A major continuous allergenic epitope of bovine β -lactoglobulin recognized by human IgE binding. *Clin Exp Allergy* 24:758, 1994
13. Barber D, Sanchéz-Monge R, Gomez L, et al: A barley flour inhibitor of insect α -amylase is a major allergen associated with baker's asthma. *FEBS Lett* 248:119, 1989
14. Barnett D, Baldo BA, Howden MEH: Multiplicity of allergens in peanuts. *J Allergy Clin Immunol* 72:61, 1983
15. Bernard H, Wal JM, Creminon C, et al: Allergenic epitopes on bovine β -casein. *J Allergy Clin Immunol* 93:210, 1994
16. Bernhisel-Broadbent J, Sampson HA: Cross-allergenicity in the legume botanical family in children with food hypersensitivity. *J Allergy Clin Immunol* 83:435, 1989
17. Blake CCF, Koenig DF, Mair GA, et al: Structure of hen egg-white lysozyme. *Nature* 206:757, 1965
18. Bousquet J, Valenta R: In vivo and in vitro use of recombinant allergens. *Allergy and Clinical Immunology News* 6:54, 1994
19. Brandon DL, Haque S, Friedman M: Antigenicity of native and modified Kunitz soybean trypsin inhibitors. *Adv Exp Med Biol* 199:449, 1986
20. Braunitzer G, Chen R, Schrank B, et al: Automatische sequenzanalyse eines proteins (β -lactoglobulin AB). *Hoppe-Seyler's Zeitschrift fur Physiologische Chemie* 353:832, 1972
21. Brew K, Castellino FJ, Vanaman TC, et al: The complete amino acid sequence of bovine α -lactalbumin. *J Biol Chem* 245:4570, 1970
22. Brignon G, Ribadeau-Dumas B, Mericer JC, et al: Complete amino acid sequence of bovine α_{s2} -casein. *FEBS Lett* 76:274, 1977
23. Brown JR: Structure and evolution of serum albumin. *Albumin structure biosynthesis: Function. Proceedings of the FEBS Meeting* 50:1, 1977
24. Burks AW, Brooks JR, Sampson HA: Allergenicity of major component proteins of soybean determined by enzyme-linked immunosorbent assay (ELISA) and immu-

- noblotting in children with atopic dermatitis and positive soy challenges. *J Allergy Clin Immunol* 81:1135, 1988
25. Burks AW, Cockrell G, Connaughton C, et al: Epitope specificity and immunoaffinity purification of the major peanut allergen, *Ara h I*. *J Allergy Clin Immunol* 93:743, 1994
 26. Burks AW, Cockrell G, Connaughton C, et al: Epitope specificity of the major peanut allergen, *Ara h II*. *J Allergy Clin Immunol* 95:607, 1995
 27. Burks AW, Cockrell G, Stanley JS, et al: Recombinant peanut allergen *Ara h 1*, expression and IgE-binding in patients with peanut hypersensitivity. *J Clin Invest* 96:1715, 1995
 28. Burks AW, Helm RM, Cockrell G, et al: Cloning of the *Ara h II* peanut allergen by polymerase chain reaction (PCR) amplification [abstract]. *J Allergy Clin Immunol* 91:341, 1993
 29. Burks AW, Williams LW, Connaughton C, et al: Identification and characterization of a second major peanut allergen, *Ara h II*, with use of the sera of patients with atopic dermatitis and positive peanut challenge. *J Allergy Clin Immunol* 90:962, 1992
 30. Burks AW, Williams LW, Helm RM, et al: Identification of a major peanut allergen, *Ara h I*, in patients with atopic dermatitis and positive peanut challenges. *J Allergy Clin Immunol* 88:172, 1991
 31. Burley KW, Cook WH: Isolation and composition of avian egg yolk granules and their constituents α - and β -lipovitellins. *Canadian Journal of Biochemistry and Physiology* 39:1295, 1961.
 32. Calkoven PG, Aalbers M, Koshte VL, et al: Cross-reactivity among birch pollen, vegetables and fruits as detected by IgE antibodies is due to at least three distinct cross-reactive structures. *Allergy* 42:382, 1987
 33. Canfield RE: The amino acid sequence of egg white lysozyme. *J Biol Chem* 238:2698, 1963
 34. Catsimpoolas N, Ekenstam C, Meyer EA: Separation of soybean whey proteins by isoelectric focusing. *Cereal Chemistry* 46:357, 1969
 35. Catterall JF, Stein JP, Kristo P, et al: Primary sequence of ovomucoid messenger RNA as determined from cloned complementary DNA. *J Cell Biol* 87:480, 1980
 36. Cochet M, Gannon F, Hen R, Maroteaux L: The chicken conalbumin gene: Studies of the organization of cloned DNAs. *Nature* 282:567, 1979
 37. Daul CB, Slattery M, Reese G, et al: Identification of the major brown shrimp (*Penaeus aztecus*) allergen (*Pen a I*) as the muscle protein tropomyosin. *International Archives of Allergy and Applied Immunology* 105:49, 1994
 38. Dawson R: Comparison of fractionation of groundnut proteins by two different methods. *Anal Biochem* 41:305, 1971
 39. Dechary JM, Talluto KF, Evans WJ, et al: Alpha-conarachin. *Nature* 190:1125, 1961
 40. Donovan R, Baldo BA: Recombinant DNA approaches to the study of allergens and allergenic determinants. In Baldo BA (ed): *Molecular Approaches to the Study of Allergens: Monographs in Allergy*, vol. 28. Basel, Karger, 1990, p 52
 41. Dreborg S: Food allergy in pollen-sensitive patients. *Ann Allergy* 61:41, 1988
 42. Ebner C, Birkner T, Valenta R, et al: Common epitopes of birch pollen and apples—Studies by western blot and northern blot. *J Allergy Clin Immunol* 88:588, 1991
 43. Ebner C, Hirschehr R, Bauer L, et al: Allergens, IgE, mediators, inflammatory mechanisms: Identification of allergens in fruits and vegetables: IgE cross-reactivities with the important birch pollen allergen *Bet v 1* and *Bet v 2* (birch pollen). *J Allergy Clin Immunol* 95:962, 1995
 44. Eigenmann PA, Huang SK, Ho DG, et al: Characterization of T cell clones and cell lines specific to ovomucoid. *J Allergy Clin Immunol* 95:366, 1995
 45. Elsayed S: Four linear and two conformational epitopes on the major allergenic molecule of egg (*Gal d I*), ovalbumin. In Kraft D, Sehon A (eds): *Molecular Biology and Immunology of Allergens*. Ann Arbor, MI, CRC Press, 1993, p 287
 46. Elsayed SM, Aas K: Characterization of a major allergen (cod): Chemical composition and immunological properties. *International Archives of Allergy* 38:536, 1970
 47. Elsayed S, Apold J: Allergenic structure of allergen M from cod. II: Allergenicity of

- the limited tryptic hydrolysis peptides of fragment TM2. *International Archives of Allergy and Applied Immunology* 54:171, 1975
48. Elsayed S, Apold J: Immunochemical analysis of cod fish allergen M: Locations of the immunoglobulin binding sites as demonstrated by the native and synthetic peptides. *Allergy* 38:449, 1983
 49. Elsayed S, Bennich H: The primary structure of allergen M from cod. *Scand J Immunol* 4:203, 1975
 50. Elsayad S, Apold J, Aas K, et al: The allergenic structure of allergen M from cod: Tryptic peptides of fragment TM1. *International Archives of Allergy and Applied Immunology* 52:59, 1976
 51. Elsayed S, Aas K, Slette K, et al: Tryptic cleavage of a homogenous codfish allergen and isolation of two active polypeptide fragments. *Immunochemistry* 9:647, 1972
 52. Elsayed S, Ragnarsson U, Apold J, et al: Allergenic synthetic peptide corresponding to the second calcium-binding loop of allergen M. *Scand J Immunol* 14:207, 1981
 53. Elsayed S, Ragnarsson U, Netteland B: Solid-phase synthesis of the non-calcium-binding loop of cod allergen M: Direct evidence for the reactivity of the amino-terminal segment. *Scand J Immunol* 17:291, 1983
 54. Elsayed S, Sornes S, Apold J, et al: The immunological reactivity of the three homologous repetitive tetrapeptides in the region 41-64 of allergen M from cod. *Scand J Immunol* 16:77, 1982
 55. Eriksson NE, Formgren H, Svenonius E, et al: Food hypersensitivity in patients with pollen allergy. *Allergy* 37:437, 1982
 56. Franken J, Stephan U, Meyer HE, et al: Identification of alpha-amylase inhibitor as a major allergen of wheat flour. *International Archives of Allergy and Applied Immunology* 104:171, 1994
 57. Franken J, Stephan U, Neuber K, et al: Characterization of allergenic components of rye and wheat flour (*Secale*, *Triticum vulgare*) by western blot with sera of bakers: Their effects on CD23 expression. *International Archives of Allergy and Applied Immunology* 96:76, 1991
 58. Fukushima D: Recent progress on biotechnology of soybean proteins and soybean protein food products. *Food Biotechnology* 8:83, 1994
 59. Gall H, Kalveran KJ, Forck G, et al: Kiwi fruit allergy: A new birch pollen and associated food allergy. *J Allergy Clin Immunol* 94:70, 1994
 60. Garcia-Maroto F, Carbonero P, Garcia-Olmedo F, et al: Site-directed mutagenesis and expression in *Escherichia coli* of WMAI-1, a wheat monomeric inhibitor of insect α -amylase. *Plant Mol Biol* 17:1005, 1991
 61. Garcia-Maroto F, Marana C, Garcia-Olmedo F, et al: Cloning of cDNA and chromosomal location of genes encoding the three types of subunits of the wheat tetrameric inhibitor of insect α -amylase. *Plant Mol Biol* 14:845, 1990
 62. Gautier MF, Alary R, Joudier P: Cloning and characterization of a cDNA encoding the wheat (*Triticum durum* Desf.) CM16 protein. *Plant Mol Biol* 14:313, 1990
 63. Goldman AS, Sellars WA, Halpern SR, et al: Milk allergy. II: Skin testing of allergic and normal children with purified milk proteins. *Pediatrics* 32:572, 1963
 64. Gonzalez de la Pena MA, Menendez-Arias L, Monsalve RI, et al: Isolation and characterization of a major allergen from oriental mustard seed, *Bra j* I. *International Archives of Allergy and Applied Immunology* 96:263, 1991
 65. Gonzalez de la Pena MA, Villalba M, Garcia-Lopez JL, et al: Cloning and expression of the major allergen from yellow mustard seeds, *Sin a* I. *Biochem Biophys Res Commun* 190:648, 1993
 66. Hirayama K, Akashi S, Furuya M, et al: Rapid confirmation and revision of the primary structure of bovine serum albumin by ESIMS and Frit-FAB LC/MS. *Biochem Biophys Res Commun* 173:639, 1990
 67. Hirschwehr R, Valenta R, Ebner C, et al: Identification of common allergenic structures in hazel pollen and hazelnuts: A possible explanation for sensitivity to hazelnuts in patients allergic to tree pollen. *J Allergy Clin Immunol* 90:927, 1992
 68. Host A, Halken S: A prospective study of cow milk allergy in Danish infants during the first 3 years of life. *Allergy* 45:587, 1990
 69. Hsieh L-S, Moos M, Lin Y: Characterization of apple 18 and 31 kd allergens by

- microsequencing and evaluation of their content during storage and ripening. *J Allergy Clin Immunol* 95:960, 1995
70. Huang Q, Coleman JW, Stanworth DR: Investigation of the allergenicity of β -lactoglobulin and its cleavage fragments. *International Archives of Allergy and Applied Immunology* 78:337, 1985
 71. Hurley WL, Schuler LA: Molecular cloning and nucleotide sequence of bovine α -lactalbumin cDNA. *Gene* 61:119, 1987
 72. Izumi H, Adachi T, Fuji N, et al: Nucleotide sequence of a cDNA clone encoding a major allergenic protein in rice seeds: Homology of the deduced amino acid sequence with members of α -amylase/trypsin inhibitor family. *FEBS Lett* 302:213, 1992
 73. Jeltsch JM, Chambon P: The complete sequence of the chicken ovotransferrin mRNA. *Eur J Biochem* 122:291, 1982
 74. Jilek A, Swoboda I, Breiteneder H, et al: Biological functions isoforms, and environmental control in the *Bet v* I gene family. In Kraft D, Sehon A (eds): *Molecular Biology and Immunology of Allergens*. Ann Arbor, MI, CRC Press, 1993, p 39
 75. Johns CO, Jones DB: The proteins of the peanut, *Arachis hypogaea*: I. The globulins arachin and conarachin. *J Biol Chem* 28:77, 1916
 76. Johnsen G, Elsayed S, et al: Antigenic and allergenic determinants of ovalbumin. III: MHC Ia-binding peptide (OA 323-229) interacts with human and rabbit specific antibodies. *Mol Immunol* 27:821, 1990
 77. Johnson P, Naismith WEF: High molecular weight systems: The physicochemical examination of the arachin fraction of the groundnut globulins (*Arachis hypogaea*). *Discussions of the Faraday Society* 13:98, 1953
 78. Johnson P, Shooter EM: The globulins of the groundnut. II: Electrophoretic examination of the arachin system. *Biochimica et Biophysica Acta* 5:376, 1950
 79. Jones SM, Magnolfi CF, Cooke SK, et al: Immunologic cross-reactivity among cereal grains and grasses in children with food hypersensitivity. *J Allergy Clin Immunol* 96:341, 1995
 80. Jung A, Sippel AE, Grez M, et al: Exons encode functional and structural units of chicken lysozyme. *Proc Natl Acad Sci USA* 77:5759, 1980
 81. Kahlert H, Petersen A, Becker WM, et al: Epitope analysis of the allergen ovalbumin (Gad d II) with monoclonal antibodies and patients' IgE. *Mol Immunol* 29:1191, 1992
 82. Kalinski A, Melroy DL, Dwivedi RS, et al: A soybean vacuolar protein (P34) related to thiol proteases is synthesized as a glycoprotein precursor during seed maturation. *J Biol Chem* 267:12068, 1992
 83. Kato I, Schrode J, Kohr WJ, et al: Chicken ovomucoid: Determination of its amino acid sequence, determination of the trypsin reactive site, and preparation of all three of its domains. *Biochemistry* 26:193, 1987
 84. Kim SH, Hara S, Hase S, et al: Comparative study on amino acid sequences of Kunitz-type soybean trypsin inhibitors, Ti^a , Ti^b , and Ti^c . *J Biochem* 98:435, 1985
 85. King TP: B-cell epitopes. *Proceedings of the Conference on Scientific Issues Related to Potential Allergenicity in Transgenic Food Crops*, FDA Docket no. 94N-0053. Washington, DC, Food and Drug Administration, 1994, p 273
 86. King TP, Hoffman D, Lowenstein H, et al: Allergen nomenclature. *Allergy Clin Immunol News* 6:38, 1994
 87. King TP, Hoffman D, Lowenstein H, et al: Allergen nomenclature. *International Archives of Allergy and Applied Immunology* 10:224, 1994
 88. King TP, Norman PS, Connell JT: Isolation and characterization of allergens from ragweed pollen. *Biochemistry* 3:458, 1964
 89. Koide T, Ikenaka T: Studies on soybean trypsin inhibitors. 3: An amino acid sequence of the carboxyl-terminal region and the complete amino acid sequence of soybean trypsin inhibitor (Kunitz). *Eur J Biochem* 32:417, 1973
 90. Komatsu S, Kayimura H, Hirano H, et al: Soybean seed 34-kDa oil-body-associated proteins separated by two-dimensional gel electrophoresis. *Plant Sci* 81:21, 1992
 91. Konishi Y, Kurisaki J, Kaminogawa S, et al: Localization of allergenic reactive sites on hen ovomucoid. *Agricultural and Biological Chemistry* 46:305, 1982
 92. Koshiyama I, Iguchi N: Studies on soybean protein. Part I: A ribonucleoprotein and ribonucleic acids in soybean casein fraction. *Agricultural and Biological Chemistry* 29:144, 1965

93. Langeland T, Harbitz O: A clinical and immunological study of allergy to hen's egg. V: Purification and identification of a major allergen (antigen 22) in hen's egg white. *Allergy* 38:131, 1983
94. Lazaro A, Sanchez-monge R, Salcedo G, et al: A dimeric inhibitor of insect α -amylase from barley: Cloning of the cDNA and identification of the protein. *FEBS Lett* 172:129, 1988
95. Lehrer SB, Ibanez MD, McCants ML, et al: Characterization of water soluble shrimp allergens released during boiling. *J Allergy Clin Immunol* 85:1005, 1990
96. Leung PSC, Chu KH, Chow WK, et al: Cloning, expression, and primary structure of *Metapenaeus ensis* tropomyosin, the major heat stable shrimp allergen. *J Allergy Clin Immunol* 94:882, 1994
97. Li-Chan E, Nakai S: Biochemical basis for the properties of egg white. *Critical Reviews in Poultry Science* 2:21, 1989
98. Lullien V, Alary R, Joudrier P, et al: Characterization of a cDNA clone encoding the *Triticum aestivum* L. CM 16 protein: homology with the *Triticum durum* Desf. sequence. *Plant Mol Biol* 16:373, 1991
99. Maniatis T, Fritsch EF, Sambrook J: *Molecular Cloning. A Laboratory Manual*. New York, Cold Spring Harbor Laboratory Press, 1982, p 331
100. Matsuda T, Alvarez AM, Tada Y, et al: Gene engineering for hypo-allergenic rice: Repression of allergenic protein synthesis in seeds of transgenic rice plants by anti-sense RNA. *Proceedings of the International Workshop on Life Science in Production and Food-Consumption of Agricultural Products, Japan*, 1993
101. Matsuda T, Nomura R, Sugiyama M, et al: Immunochemical studies on rice allergenic proteins. *Agricultural and Biological Chemistry* 55:509, 1991
102. Matsuda T, Sugiyama M, Nakamura RR, et al: Purification and properties of an allergenic protein in rice grain. *Agricultural and Biological Chemistry* 52:1465, 1988
103. Maxam AM, Gilbert W: A new method for DNA sequencing. *Proc Natl Acad Sci USA* 74:560, 1977
104. McReynolds L, O'Malley BW, Nisbet AD, et al: Sequence of chicken ovalbumin mRNA. *Nature* 273:723, 1978
105. Mena M, Sanchez-Monge R, Gomez L, et al: A major barley allergen associated with baker's asthma is a glycosylated monomeric inhibitor of insect α -amylase: cDNA cloning and chromosomal location of the gene. *Plant Mol Biol* 20:451, 1992
106. Menendez-Arias L, Domínguez J, Moneo I, et al: Epitope mapping of the major allergen from yellow mustard seeds, *Sin a* I. *Mol Immunol* 27:143, 1990
107. Menendez-Arias L, Moneo I, Domínguez J, et al: Primary structure of the major allergen of yellow mustard (*Sinapis alba* L.) seed, *Sin a* I. *Eur J Biochem* 177:159, 1988
108. Menendez-Arias L, Monsalve RI, Gavilanes JG: Molecular and spectroscopic characterization of a low molecular weight seed storage protein from yellow mustard (*Sinapis alba* L.). *Int J Biochem* 19:899, 1987
109. Mercier JC, Brignon G, Ribadeau-Dumas B: Structure primaire de la caseine κ B bovine. Sequence complete. *Eur J Biochem* 35:222, 1973
110. Mercier JC, Grosclaude F, Ribadeau-Dumas B: Structure primaire de la caseine α_{s1} -bovine. *Eur J Biochem* 23:41, 1971
111. Monslave RE, Gonzalez de la Pena MA, Menendez-Arias L, et al: Characterization of a new oriental-mustard (*Brassica juncea*) allergen, *Bra j* IE: Detection of an allergenic epitope. *Biochem J* 293:625, 1993
112. Moroz LA, Yang WH: Kunitz soybean trypsin inhibitor, a specific allergen in food anaphylaxis. *N Engl J Med* 302:1126, 1980
113. Nagpal S, Metcalfe DD, Rao PV: Identification of a shrimp-derived allergen as tRNA. *J Immunol* 138:4169, 1987
114. Naismith WEF, McDavid HMR: Fractionation studies on the proteins of the groundnut (*Arachis hypogaea*). *Journal of Applied Chemistry* 8:605, 1958
115. Nielsen NC: Structure of soy proteins. In: *Altschul AM, Wilcke HL (eds): New Protein Foods*, vol 5. Orlando, Academic Press, 1985, p 27
116. Nisbet AD, Saundry RH, Moir AJG, et al: The complete amino acid sequence of hen ovalbumin. *Eur J Biochem* 115:335, 1981

117. Nishio T, Iida S: Mutants having a low content of 16-kDa allergenic protein in rice (*Oryza sativa* L.). Theoretical and Applied Genetics 86:317, 1993
118. Nordlee JA, Taylor SL, Townsend JA, et al: High methionine Brazil nut protein binds human IgE [abstract]. J Allergy Clin Immunol 93:209, 1994
119. Nordlee JA, Taylor SL, Townsend JA, et al: Identification of a Brazil-nut allergen in transgenic soybeans. N Engl J Med 334:688-692, 1996
120. Ogawa T, Bando N, Tsuji H, et al: Investigation of the IgE-binding proteins in soybeans by immunoblotting with the sera of soybean-sensitive patients with atopic dermatitis. J Nutr Sci Vitaminol 37:555, 1991
121. Ogawa T, Bando N, Tsuji H, et al: α -Subunit of β -conglycinin, an allergenic protein recognized by IgE antibodies of soybean-sensitive patients with atopic dermatitis. Bioscience, Biotechnology, and Biochemistry 59:831, 1995
122. Ogawa T, Tsuji H, Bando H, et al: Identification of a soybean protein producing IgE antibodies in BALB/c mice. Bioscience, Biotechnology, and Biochemistry 56:978, 1992
123. Ogawa T, Tsuji H, Bando N, et al: Identification of the soybean allergenic protein, Gly m Bd 30K, with the soybean seed 34-kDa oil-body-associated protein. Bioscience, Biotechnology, and Biochemistry 57:1020, 1993
124. Pfeifer JD, Wick MJ, Russell DG, et al: Recombinant *Escherichia coli* express a defined, cytoplasmic epitope that is efficiently processed in macrophage phagolysosomes for class II MHC presentation to T lymphocytes. J Immunol 149:2576, 1992
125. Powrie WD, Nakai S: Characteristics of edible fluids of animal origin: Eggs. In Fennema O (ed): Food Chemistry. New York, Marcel Dekker, 1985, p 829
126. Prakash V, Rao MSN: Physicochemical properties of oilseeds. Crit Rev Biochem 20:264, 1987
127. Renz H, Bradley K, Larsen GL, et al: Comparison of the allergenicity of ovalbumin and ovalbumin peptide 323-339: Differential expression of V β -expressing T-cell populations. J Immunol 151:7206, 1993
128. Ribadeau-Dumas B, Brignon G, Grosclaude F, et al: Structure primaire de la casein β bovine. Eur J Biochem 25:505, 1972
129. Roberts RC, Briggs DR: Characteristics of the various soybean globulin components with respect to denaturation by ethanol. Cereal Chemistry 40:450, 1963
130. Sanger F, Nicklen S, Coulson AR: DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci USA 74:5463, 1977
131. Savilathi E: Cow's milk allergy. Allergy 36:73, 1981
132. Scheiner O, Kraft D: Basic and practical aspects of recombinant allergens. Allergy 50:384, 1995
133. Scheiner O, Breiteneder H, Dolecek C, et al: Molecular and functional characterization of allergens: Basic and practical aspects. Proceedings of the Paul Ehrlich Seminar, 1994, p 1
134. Schuler MA, Schmitt ES, Beachey RN: Closely-related families of genes code for the α and α' subunit of the soybean 7S storage protein complex. Nucleic Acids Res 10:8225, 1982
135. Sebastiani FL, Farrell LB, Schuler MA, et al: Complete sequence of a cDNA of α subunit of soybean β -conglycinin. Plant Mol Biol 15:197, 1990
136. Shanti KN, Martin BM, Nagpal S, et al: Identification of tropomyosin as the major shrimp allergen and characterization of its IgE-binding epitopes. J Immunol 151:5354, 1993
137. Shetty KJ, Rao MSN: Studies on groundnut proteins: Part VII. Physicochemical properties of conarachin II. Indian J Biochem Biophys 14:31, 1977
138. Shibasaki M, Suzukim S, Tajima S, et al: Allergenicity of major component proteins of soybeans. International Archives of Allergy and Applied Immunology 61:441, 1980
139. Shimojo N, Katsuki T, Coligan JE, et al: Identification of the disease-related T cell epitope of ovalbumin and epitope-targeted T cell inactivation in egg allergy. International Archives of Allergy and Applied Immunology 105:155, 1994
140. Shon DH, Enomoto A, Yamauchi K, et al: Antibodies raised against peptide fragments of bovine α s1-casein cross-react with the native protein, but recognize sites distinct from the determinant on the protein. Eur J Immunol 21:1475, 1991
141. Stanley JS, Burks AW, Helm RM, et al: *Ara h* 1, a major allergen involved in peanut

- hypersensitivity, has multiple IgE binding domains [abstract]. *J Allergy Clin Immunol* 95:333, 1995
142. Stein JP, Catterall JF, Woo SLC, et al: Molecular cloning of ovomucoid gene sequences from partially purified ovomucoid mRNA. *Biochemistry* 17:5763, 1978
 143. Stevens L: Egg white proteins. *Comp Biochem Physiol* 100B:1, 1991
 144. Swaisgood HE: Characterization of edible fluids of animal origin: Milk. In Fennema OR (ed): *Food Chemistry*, ed 2. New York, Marcel Dekker, 1985, p 791
 145. Taylor SL, Nordlee JA, Yunginger JW: Evidence for the existence of multiple allergens in peanuts [abstract]. *J Allergy Clin Immunol* 69:128, 1982
 146. Urisu A, Kondo Y, Morita Y, et al: Identification of a major allergen of buckwheat seeds by immunoblotting methods. *Allergy and Clinical Immunology News* 6:151, 1994
 147. Valenta R, Duchene M, Pettenburger K, et al: Identification of profilin as a novel pollen allergen: IgE autoreactivity in sensitized individuals. *Science* 253:557, 1991
 148. Valenta R, Duchene M, Vrtala S, et al: Profilin, a novel pan-allergen. *Int Arch Allergy Immunol* 99:271, 1992
 149. Vallier P, DeChamp C, Valenta R, et al: Purification and characterization of an allergen from celery immunochemically related to an allergen present in several other plant species: Identification as a profilin. *Clin Exp Allergy* 22:774, 1992
 150. van Ree R, Voitenko V, van Leeuwen WA, et al: Profilin is a cross-reactive allergen in pollen and vegetable foods. *Int Arch Allergy Immunol* 98:97, 1992
 151. van Toorenenbergen AW, Dieges DH: Demonstration of spice-specific IgE in patients with suspected food allergies. *J Allergy Clin Immunol* 79:108, 1987
 152. Vieths S, Schoning B, Petersen A: Characterization of the 18-kDa apple allergen by two-dimensional immunoblotting and microsequencing. *International Archives of Allergy and Applied Immunology* 104:399, 1994
 153. Vocks E, Borga A, Szliska C, et al: Common allergenic structures in hazelnut, rye grain sesame seeds, kiwi, and poppyseeds. *Allergy* 48:168, 1993
 154. Walsh BJ, Howden MEH, et al: A method for the detection of IgE binding sequences of allergens based on a modification of epitope mapping. *J Immunol Methods* 121:275, 1989
 155. Walter MH, Liu JW, Grand C, et al: Bean pathogenesis-related (PR) proteins deduced from elicitor-induced transcripts are members of a ubiquitous new class of conserved PR proteins including pollen allergens. *Mol Gen Genet* 222:353, 1990
 156. Whitney RM: Proteins of milk. In Wong NP, Jernes R, Keeney M, et al (eds): *Fundamentals of Dairy Chemistry*, ed. 3. New York, Van Nostrand Reinhold, 1988, p 81
 157. Williams J: The evolution of transferrin. *Trends Biochem Sci* 7:394, 1982
 158. Williams J, Elleman TC, Kingston IB, et al: The primary structure of hen ovomucoprotein. *Eur J Biochem* 122:297, 1982
 159. Yamada T, Aibara S, Morita Y: Isolation and some properties of arachin subunits. *Agricultural and Biological Chemistry* 43:2563, 1979

Address reprint requests to

Susan L. Hefle, PhD
Food Allergy Research and Resource Program
University of Nebraska
351 Food Industry Building
Lincoln, NE 68583-0919