

Food Allergens

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Robert K. Bush¹ and Susan L. Hefle²

¹William S. Middleton V.A. Hospital and Department of Medicine and Food Research Institute, University of Wisconsin, Madison, Wisconsin; ²Food Allergy Research and Resource Program, University of Nebraska, Lincoln, Nebraska

Susan L. Hefle is formerly of the William S. Middleton V.A. Hospital and Department of Medicine and Food Research Institute, University of Wisconsin, Madison, Wisconsin.

I. INTRODUCTION

This article reviews the biochemistry of common and less commonly allergenic foods and the allergenic proteins that have been identified. The techniques utilized to document the allergenicity of various foods and allergenic proteins are presented. Often, particularly in the case of anaphylaxis, oral challenges have not been conducted. Rather, the allergenic material in question has been implicated presumptively based on the demonstration of a positive skin test or an *in vitro* assay for specific immunoglobulin E (IgE) antibodies.

A. Allergen Nomenclature

In response to the heightened progress in recombinant technology, an allergen nomenclature system has been adopted. This system was described recently in detail by King et al.¹ 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 usually 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 mol-

ecule from Indian shrimp, *Penaeus indicus*, is *Pen i 1*.

Members of an allergen group that have more than 67% amino acid sequence homology are called isoallergens.¹ Each isoallergen may have multiple forms of closely similar sequences that are designated as variants. The system can describe polymorphic forms that differ slightly from each other in their amino acid sequences or degree of glycosylation. Furthermore, the nomenclature system provides rules for describing allergen genes, messenger RNAs (mRNAs), cDNAs, and recombinant and synthetic peptides of allergenic interest.¹ Examples of the use of this nomenclature system for describing several of the major food allergens are presented in Table 1.

In addition, researchers customarily describe allergens as *major* or *minor*. Major allergens are generally defined as proteins for which 50% or more of the allergic patients studied have specific IgE.^{1,2} Examples of major allergens are *Ara h 1* from peanuts,³ ovalbumin, ovomucoid, and ovotransferrin from eggs,⁴ and *Pen a 1* from shrimp.⁵ The significance of minor allergens 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 do not have the conformation necessary to elicit histamine release. For example, research has shown that peanut-allergic patients possess IgE that can bind to proteins from many other legumes, resulting in positive

TABLE 1
Major Food Allergens Identified

Allergen source	Allergens (systematic and original names)	Mol wt (kDa)	Sequence data ^a	Ref. ^b
<i>Arachis hypogea</i> (peanut)	<i>Ara h 1</i>	63.5	C	138
<i>Bertholletia excelsa</i> (Brazil nut)	<i>Ber e 1</i> ; 2S albumin	12	C	194
<i>Brassica juncea</i> (oriental mustard)	<i>Bra j 1</i> ; 2S albumin	14	C	349
<i>Gadus callarias</i> (cod)	<i>Gad c 1</i> ; allergen M	12	C	69
<i>Gallus domesticus</i> (chicken — eggs)	<i>Gal d 1</i> ; ovomucoid	28	C	31
	<i>Gal d 2</i> ; ovalbumin	44	C	35
	<i>Gal d 3</i> ; ovotransferrin	78	C	37
	<i>Gal d 4</i> ; lysozyme	14	C	42
<i>Glycine max</i> (soybean)	<i>Gly m 1</i>	34	P	174
<i>Penaeus aztecus</i> (brown shrimp)	<i>Pen a 1</i> ; tropomyosin	36	P	5
<i>Penaeus indicus</i> (indian shrimp)	<i>Pen i 1</i> ; tropomyosin	34	P	92
<i>Metapenaeus enis</i> (greasyback shrimp)	<i>Met e 1</i> ; tropomyosin	34	C	94
<i>Sinapis alba</i> (yellow mustard)	<i>Sin a 1</i> ; 2S albumin	14	C	268

^a Amino acid sequence obtained directly or deduced from cDNA sequence.

^b References refer to those where partial (P) or complete (C) sequence data are available.

skin test and RAST results. However, the clinical manifestations of such cross-reactivity are rare, as the patients are only documented to be allergic to peanut and perhaps one other leguminous food.⁶ 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 (SDS-PAGE, see "Principles and Characteristics of Food Allergens" by Steve L. Taylor and Sam B. Lehrer in this issue), minor allergens could actually be parts of larger major allergens. Therefore, they may have the potential to cause significant reactions in some individuals.

The major allergens are often found in abundance in a food. *Ara h 1* is part of a peanut storage protein.⁷ 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.⁸

II. COMMONLY ALLERGENIC FOODS OF ANIMAL ORIGIN

A. Cow's Milk

Throughout the world, cow's milk (*Bos taurus*) is used for the majority of human milk consumption. IgE-mediated sensitivity to cow's milk is one of the most common food allergies. The overall prevalence of cow's milk allergy in infants and children worldwide is estimated to be between approximately 2.5% in the first 3 years of life.⁹⁻¹¹ Symptoms often begin at age 3 months or younger, but many children lose sensitivity by age 3. Cow's milk allergy in adults is rather unusual. It is often manifested in children by vomiting and diarrhea, with approximately one-third to one-half of those affected experiencing skin manifestations such as atopic

dermatitis (AD), urticaria, and angioedema, or erythematous rashes.

Cow's milk comprises a number of proteins. Traditionally, two major groups of cow's milk proteins have been identified: caseins, which comprise 80% of the total protein, and whey proteins, which contribute 20% of the total. Caseins are phosphoproteins that precipitate from raw skim milk after acidification to pH 4.6 at 20°C, whereas whey proteins remain in the fluid ("serum") after the precipitation of caseins.¹² The nomenclature of specific milk proteins utilizes a Greek letter with or without a subscript preceding the class name to identify the family of proteins. The genetic variant of the protein is indicated by an upper case Arabic letter with or without a numerical superscript following the class name. Post-translational modifications are added in sequence. Many milk proteins are heterogeneous.¹² Characteristics of the major milk proteins are found in Table 2.

1. Major Allergens

A number of milk proteins have been identified as allergenic or immunogenic in humans. Many patients are allergic to more than one milk

protein as identified by skin-test reactivity or by oral challenge. Individuals allergic to cow's milk will often exhibit the presence of IgE antibodies in their serum to goat or sheep's milk.^{13,14} Caseins and β -lactoglobulin are the major allergens in cow's milk.^{10,14,15} The percentage of persons allergic to individual cow's milk proteins or protein families will vary depending on the method used to define the response. By oral challenge, β -lactoglobulin produces more positive responses than does casein.^{14,16} When skin tests were employed, casein was slightly more prevalent (63%) than β -lactoglobulin (62%).¹⁰ Although these individual studies show one group of proteins more reactive than the other by the methods employed, the general consensus is that caseins and β -lactoglobulin are most frequently the cause of allergic reactions to cow's milk.

Caseins — The caseins are a family of chemically related proteins. The frequency of reactivity to individual casein proteins has not been systematically evaluated. α_{s1} -casein has at least five genetic variants. It is a 23-kDa phosphoprotein with sequential mouse epitopes.¹⁷ The amino acid sequence of the protein has been established.^{12,18} Based on the predicated structure, hydrophobic and hydrophilic domains connected by a segment of α -helix have been identified.¹² α_{s2} -caseins have

TABLE 2
Major Milk Proteins

Protein	Concentration (g/l)	Percentage of total protein	Mol wt (kDa)
Caseins	24–28		
α -caseins	15–19		23.6–25.2
α_{s1}	12–15	34	
α_{s2}	3–4	8	
β -caseins	9–11	25	23.9
κ -caseins	3–4	9	19
γ -caseins	1–2		11.5–20.5
Whey proteins	5–7		
β -lactoglobulin	2–4	9	18.2
α -lactalbumin	1–1.5	4	14.1
Proteose-peptones	0.6–1.8	4	
Blood proteins			
Albumin	0.1–0.4	1	67
Ig	0.6–1.0	2	160–200

Adapted from Yunginger.¹⁹⁹

varying degrees of posttranslational phosphorylation. Four genetic variants have been identified.¹² β -caseins have one major component with seven genetic variants and eight minor components that are proteolytic fragments of the major component. The molecular weight of the major component is 23,980.^{12,18} Modeling studies indicated that the protein has hydrophobic side chains dispersed over the C-terminal end and center surface of the structure, with a hydrophilic N-terminus. κ -caseins comprise two genetic variants. This protein is cleaved at residue 105 to 106 by rennin (chymosin) into two domains. The hydrophobic domain (para- κ -casein) is not soluble, whereas the polar domain (macropeptide) is extremely soluble.¹²

β -lactoglobulin — Whey proteins comprise approximately 20% of milk proteins. The most abundant whey protein is β -lactoglobulin, an 18-kDa protein belonging to the lipocalin family. There are at least six genetic variants of this protein. The primary structure has been obtained.^{12,18} The complete DNA sequence encoding β -lactoglobulin has been reported and shows 91% sequence homology with ovine β -lactoglobulin.¹⁹

2. Minor Allergens

To a lesser degree, the whey proteins α -lactalbumin and bovine serum albumin (BSA) have been implicated in allergic reactions to cow's milk.¹⁶

α -lactalbumin — α -Lactalbumin consists of two genetic variants and its molecular weight is approximately 14 kDa. The protein has been cloned and the nucleotide sequence identified.²⁰ The primary amino acid structure has been determined.^{12,18} The two genetic variants differ in only one amino acid sequence. Sequence analysis indicates homology to lysozyme. This protein apparently promotes transfer of galactose to glucose to form lactose, the major milk sugar. α -Lactalbumin produces both positive skin tests and positive oral challenges in a significant number of cow's milk-allergic individuals.¹⁴

BSA — BSA can be detected in milk and is identical in characteristics to bovine blood serum albumin. BSA has produced both positive skin tests and oral challenges in some cow's milk-allergic

individuals.¹⁶ BSA is a heterogeneous protein with a molecular weight of 67 kDa comprising approximately 1% of the total milk protein.

Other proteins — Other milk components may occasionally be allergenic. These include bovine immunoglobulins (Ig), which comprise less than 2% of the total milk protein, β_2 -microglobulin, transferrin, lactoferrin,¹⁴ lactoperoxidase, alkaline phosphatase, and catalase.²¹ Maillard reaction adducts, which are lactose-protein conjugates, may occasionally act as allergens. A β -lactoglobulin lactose conjugate was shown in one study to be 10 to 100 times more potent in intradermal skin tests than native β -lactoglobulin.¹⁴

3. Structure-Epitopes

Although caseins are significant allergens, no data are available regarding their human T- or B-cell epitopes. For α_{s1} -casein, the mouse T- and B-cell epitopes have been identified as sequential epitopes.¹⁷ Because α_{s1} -casein is the casein subunit that most often interacts with IgE from infants and children with clinically allergic symptoms,¹⁵ it would be helpful to have information regarding its epitopes in human systems. Baldo²¹ found that the glycomacropeptide from κ -casein (amino acid residues 106 to 167) and the polypeptide fragment encompassing amino acid residues 99 to 167 reacted with most of the serum in his IgE-blotting study; the latter peptide proved more reactive, and might indicate the presence of an IgE epitope in amino acid sequence 99 to 105.

4. Dose Response

The exact quantity of milk protein required to produce sensitization (IgE antibody production) or clinical symptoms is not known. Trace quantities of milk proteins in processed foods may produce symptoms in sensitive individuals.²² Some infants may develop cow's milk allergy despite breastfeeding. β -Lactoglobulin levels in women's breast milk increased from a basal level of 0.0 to 3.5 $\mu\text{g/l}$ (after abstinence from cow's milk ingestion for at least 24 h) to as much as 0.01 to

2.34 µg/l after administration of 400 ml of cow's milk.²³ This may explain the source of sensitization in some situations. Because β -lactoglobulin could not be detected in all samples, it may not be the only explanation. Intact proteins, such as BSA, have also been measured in peripheral blood from individuals who have eaten these proteins.²⁴

B. Egg

Eggs from chickens (*Gallus domesticus*) are widely used for human consumption. Egg allergy is one of the most frequently implicated causes of immediate food-allergic reactions in children in the U.S. and Europe.²⁵ Frequently, egg sensitivity disappears by the fourth or fifth year of life or no later than the first decade of life; however, one third of individuals have clinical sensitivity that lasts over 6 years.²⁵ Although there is extensive cross-reactivity among the proteins from various birds, hen eggs tend to be slightly more allergenic than duck eggs.²⁶ The egg white (albumin) is more allergenic than the yolk. Egg white proteins have been studied extensively, and most have been purified and their amino acid sequences determined.

Eggs are composed of 56 to 61% egg white and 27 to 32% egg yolk. The egg white is approximately 87 to 89% water and 9 to 11% protein, whereas the egg yolk contains 50% water, 32 to 35% lipid, and 16% protein.²⁷ The predominant protein in the egg white is ovalbumin, comprising 54% of the protein present. Other major proteins in the egg white are ovotransferrin (12%), ovomucoid (11%), ovomucin (3.5%), and lysozyme (3.4%). In addition to these proteins, a variety of other proteins have been identified. Ovomucin is a complex glycoprotein with two subunits of 180 and 400 kDa. Ovoid inhibitor is a 44-kDa protein whose amino acid sequence is known and whose gene sequence has been obtained by cDNA cloning.²⁸ Ovoflavoprotein has been found in both egg white and egg yolk, and is known to be a riboflavin-binding protein. Avidin composes 0.5% of egg white protein. It is a heterogenous tetrameric glycoprotein with a molecular weight between 66 and 69 kDa, and acts as a biotin-binding protein. Other proteins include ovomacroglobulin, the G₂, G₃ globulins (which are not well characterized),

and cystatin, an inhibitor of cysteine proteases. The characteristics of egg white proteins are summarized in Table 3.

Egg yolks can be separated into two fractions using ultracentrifugation. The sedimented fraction is called the granule fraction, containing 60% protein and 35% lipid. The other fraction, the clear supernatant solution, is called the plasma fraction, containing 18% protein and 80% lipid.²⁷ The granule fraction contains lipovitellin (high-density lipoprotein), phosvitin, and low-density lipoprotein. The low-density lipoprotein in the egg yolk exhibits a much higher molecular weight (3 to 10×10^6 Da) than that present in the egg white (160 to 190 kDa). Phosvitin comprises 16% of total egg yolk protein and is the iron-carrying molecule.^{29,30} The characteristics of egg yolk proteins are summarized in Table 4.

1. Major Allergens

Ovomucoid — Ovomucoid (*Gal d 1*) is a glycoprotein with a molecular weight of 28 kDa, a pI of 4.1, and exhibits trypsin inhibitor activity. It contains no tryptophan, which is found in most other egg white proteins. Its amino acid sequence contains 186 residues.³¹ There are three tandem domains, each of which is homologous to pancreatic secretory trypsin inhibitor, and each acts as a native globular protein. Its predicted secondary structure indicates some α -helical and β -structural elements, and polymorphism of the protein exists. Ovomucoid has also been documented in several studies as a major allergen. For example, in one study, 48 of 68 sera samples from egg-sensitive patients had positive crossed radioimmuno-electrophoresis (CRIE) to ovomucoid,³² whereas in a similar study, 21 of 34 sera samples from egg-allergic patients had positive RAST and CRIE to ovomucoid.³³ However, Bernhisel-Broadbent et al.³⁴ suggested that the use of commercially purified ovalbumin has led to the erroneous concept of ovalbumin as a major egg allergen, because ovomucoid was found to have contaminated commercial ovalbumin preparations (less than 1%). In this study, ovomucoid was found to be a more potent allergen than purified ovalbumin by skin testing (ST) and RAST in 18 children with egg allergy.

TABLE 3
Characteristics of Egg White Proteins

Protein	Percentage total protein	Mol wt (kDa)	pI	Carbohydrate moiety
Allergens				
Ovalbumin	54	45	4.5	Y
Ovotransferrin	12–13	77.7	6.0	Y
Ovomucoid	11	28	4.1	Y
Ovomucin	1.5–3.5	$0.23\text{--}8.3 \times 10^6$	4.5–5.0	Y
Lysozyme	3.4–3.5	14.3	10.7	N
Unproven allergenicity				
G2 Globulin	4.0	49	5.5	Y
G3 Globulin	4.0	49	5.8	Y
Ovoinhibitor	0.1–1.5	49	5.1	Y
Ovoglycoprotein	0.5–1.0	24.4		
Ovoflavoprotein	0.8	32	4.0	Y
Ovomacroglobulin	0.5	$0.76\text{--}0.9 \times 10^6$	4.5–4.7	Y
Cystatin	0.05	12.7	5.1	N
Avidin	0.05	68.3	10	Y

Adapted from Li-Chan and Naka.⁵¹

TABLE 4
Characteristics of Major Egg Yolk Proteins

	Percentage of total protein		
Protein	Granule	Plasma	Mol wt (kDa)
Granule			
Lipovitellin	70		400
Phosvitin	16		160–190
Low-density lipoprotein	12		—
Plasma			
Low-density lipoprotein		64	3–10 × 10 ⁶
Livetin		14	45–150

Adapted from Yunginger.¹⁹⁹

Ovalbumin — Ovalbumin (*Gal d 2*) is a monomeric phosphoglycoprotein with a molecular weight of 43 to 45 kDa and a pI of 4.5. The 385 amino acid sequence has been established.³⁵ Purified ovalbumin has three variants, A₁, A₂, and A₃, which contain two, one, and no phosphate groups per molecule, respectively. The susceptibility to denaturation increases in order of decreasing degree of phosphorylation. The mRNA nucleotide sequence of ovalbumin has been reported.³⁶

Ovalbumin has been documented in several studies as a major allergen. For example, 68 of 68 sera from egg-sensitive patients were positive in CRIE to ovalbumin in one study,³² whereas in a similar study,³³ 34 of 34 sera from egg-allergic patients were positive in RAST and CRIE to ovalbumin.

Ovotransferrin (conalbumin) — Ovotransferrin (*Gal d 3*) has a molecular weight of 77 kDa, and a pI of 6.0. Its 686 amino acid residues and sequence have been identified directly³⁷ and de-

duced indirectly by mRNA sequencing.³⁸ It has antimicrobial activity and iron-binding properties. Ovotransferrin has also been documented in several studies as a major egg allergen. For example, in one study, 35 of 68 sera from egg-sensitive patients had positive CRIE to ovotransferrin,³² whereas in a similar study, 20 of 34 sera from egg-allergic patients had positive RAST and CRIE to ovotransferrin.³³

Apovitellins — Apoproteins derived from the low-density lipoprotein fraction of the egg yolk are major allergens for some egg-sensitive individuals. Apovitellin I has been shown to be a major allergen in RAST studies using sera from egg-sensitive individuals.^{39,40} Apovitellin VI was found to be a major allergen in RAST analysis in a study by Walsh et al.⁴⁰ Anet et al.³⁹ discovered that the apovitellins III, V, and VI were minor allergens for the individuals in their study.

2. Minor Allergens

Lysozyme — Lysozyme (*Gal d 4*) is a 14.3-kDa protein with a pI of 10.7. Its 129 amino acids have been sequenced.⁴¹ 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.⁴² There is a second hydrophilic domain (residues 40 to 85) that forms one site of the active site cleft. Its mRNA with exons and flanking introns have also been identified.⁴³ The role of lysozyme in egg allergy has not been established. Miller and Campbell⁴⁴ found lysozyme to be a major allergen by ST, but Langeland³² found that 0 of 68 sera from egg-allergic patients had a positive CRIE to lysozyme. Anet et al.³⁹ found that 4 of 9 sera from egg-sensitive patients were positive for lysozyme using RAST.

Ovomucin — Walsh et al.⁴⁰ found that ovomucin was a minor allergen in RAST studies using sera from egg-allergic subjects.

3. Structure — Epitopes

Phosvitin — Walsh et al.⁴⁰ also concluded that phosvitin was a minor allergen in RAST analyses using sera from egg-allergic subjects.

Some progress has been made in determining the T- and B-cell epitopes of ovalbumin. Shinoda et al.⁴⁵ examined the T-cell proliferative response to ovalbumin in children with AD who were sensitive to egg protein. The proliferating cell population appears to be CD4⁺ CD45 RA⁺ T cells. The epitopes responsible for this proliferative response were not examined. However, a synthetic peptide prepared from ovalbumin demonstrates that sequences that are recognized by human IgE antibodies may also stimulate rabbit T cells.⁴⁶ This peptide comprises amino acid sequences 323 to 339. Renz et al.⁴⁷ found this same sequence to be important in the generation of immediate hypersensitivity responses in Balb/c mice exposed via the respiratory route. T-cell clones for ovalbumin have been established.⁴⁸ These cell lines appear to be CD4⁺ cell lines that secrete interleukin 4 (IL-4), suggesting that these cells are of the T-helper 2 cell type (TH2).

B-cell epitopes for ovalbumin have been more clearly established. Johnsen and Elsayed⁴⁶ demonstrated that IgE binding occurs with a peptide of amino acid sequences 323 to 339. Kahlert et al.,⁴⁹ using cyanogen bromide cleavage of a commercial ovalbumin preparation, demonstrated IgE binding to peptide sequences 41 to 172 and 301 to 385. Data obtained from studies of ovotransferrin (*Gal d 3*) show seven continuous epitopes.⁵⁰ Ovomucoid has prominent carbohydrate-containing domains.⁵¹ 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.⁵² The lysozyme IgG-binding epitope has been studied, and three discontinuous epitopes have been identified⁵³ (Table 5). It is of note that lysozyme and bovine lactalbumin have approximately 43% sequence identity.⁵²

Homology in the DNA sequence of egg white proteins and egg yolk apovitellin II has been reported.⁵⁴ It is possible that the yolk and white could have some IgE-epitope homology. Interestingly, Anet et al.³⁹ found some cross-reactivity between egg yolk and egg white in RAST inhibition studies.

Little is known about the dose of egg proteins required to elicit an immune response. Ovalbumin is not easily degraded. It is hypothesized to

TABLE 5
Amino Acid Sequences of Three Discontinuous
Epitopes of Lysozyme

Epitope D1.3		
Residues	18-27	D-N-Y-R-G-Y-S-L-G-N
	116-129	K-G-T-D-V-Q-a-w-l-r-g-c-r-L
Epitope HEL-5		
Residues	41-53	Q-a-T-N-R-T-D-G-s-t-d-Y
	67-70	G-R-T-P
	84	L
Epitope HEL-10		
Residues	15-21	H-G-l-d-n-Y-R
	63	W
	73-75	R-w-L
	89-102	T-a-s-v-N-c-a-K-K-l-v-S-D-G

Note: Residues denoted by lower case letters are not in contact with the antibody combining site.

Adapted from Davies et al.⁵³

persist in the body and may stimulate memory B cells, possibly due to follicular dendritic cells retaining antigen-antibody complexes,³⁴ although this theory has not been proven.

C. Fish

The consumption of fish and inhalation of cooking vapors are causes of IgE-mediated reactions. There have been no published reports on the prevalence of IgE-mediated reactions to a particular species of fish, as most studies refer only to cod or to "fish" in general. However, fish is one among the most commonly implicated allergenic foods, and has been incriminated in fatal anaphylactic reactions.⁵⁵ Although the true prevalence of fish allergy is unknown, incidence of fish hypersensitivity is observed to be higher in countries where fish consumption is above average. For example, codfish allergy may be the most common food allergy in Scandinavian countries.⁵⁶

Most edible fishes belong to class Osteichthyes. Sharks are not in this class, as they are cartilaginous fish (order Squaliformes). The most commonly consumed fishes in the U.S. belong to only a few orders: Clupeiformes (salmons, trouts, whitefishes, smelts, pikes, herrings, sardines, anchovies, shad, menhadens, and alewives),

Perciformes (basses, perches, dolphins, snappers, groupers, orange roughy, redfishes, rockfishes, mackerels, swordfishes, and tunas), Gadiformes (codfishes, pollocks, haddocks, and hakes), Pleuronectiformes (flounders, halibuts, and soles), and Cypriniformes (carps and catfishes).⁵⁷

1. Major Allergens

Gad c 1 — The most comprehensive analysis of a food allergen was done 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). Several studies have documented that *Gad c 1* is the major codfish allergen,⁵⁸⁻⁶⁰ belonging to a group of muscle tissue proteins known as parvalbumins.⁸ Parvalbumins control the flow of calcium in and out of cells and are only found in the muscles of amphibians and fish. The existence of structurally related parvalbumins in different fish species may explain cross-reactivity in fish-allergic individuals, because *Gad c 1* shares approximately 34% homology with similar proteins from hake, carp, pike, and whiting.⁸ The proportion of *Gad c 1* in fresh white cod muscle tissue is 0.05 to 0.1%.

2. Minor Allergens

Ag-17-cod — CRIE studies indicate that there are other minor codfish allergens distinct from *Gad c 1*,^{58,61} but these have not been characterized. One of these minor allergens was designated Ag-17-cod, and two of eight serum samples from cod-allergic subjects demonstrated IgE binding to this allergen.⁵⁸ It has also been reported that approximately 10% of cod-allergic individuals react to a distinct protein found in cod blood serum, but none appear to react exclusively to it.⁶²

Protamine sulfate — A low-molecular-weight protein widely used as a heparin antagonist, is a sperm protein of salmon and related fish species belonging to the families Salmonidae and Clupeidae (herring, sardines, trout).⁶³ One study revealed the presence of antiprotamine sulfate IgE in the serum of one fish-allergic subject. This subject also exhibited a positive skin test against protamine sulfate.⁶⁴ However, another study found no cross-reactivity between IgE to salmon and protamine sulfate in two fish-allergic subjects.⁶⁵ Although protamine sulfate has been identified as an allergen in some reports of individuals with fish hypersensitivity,⁶⁶ one study showed that protamine administration did not cause any adverse reaction in 16 fish-allergic subjects.⁶⁷ Therefore, it can be concluded that protamine sulfate is rarely allergenic for individuals with fish hypersensitivity.

Surimi 63-kDa protein — Mata et al.⁶⁸ found that surimi, a collection of one or many different varieties of small fish that are minced and washed extensively, gave a single protein band of 63.5 kDa in SDS-PAGE. Using RAST, they showed that six of six sera from fish-allergic patients showed slightly positive responses to surimi, although skin-prick tests were only positive in two of six patients. No further conclusions can be reached on the significance of this allergen until more research is completed.

3. Structure-Epitopes

a. *Gad c 1*

Gad c 1 is an acidic protein (pI 4.75) with a molecular weight 12,328 Da, and is composed of 113 amino acids and one glucose molecule.⁶⁹ As a parvalbumin, the tertiary structure of *Gad c 1* comprises three domains: AB, CD, and EF. The CD and EF domains coordinate one Ca^{+2} -binding site each, whereas the AB domain does not have this property (Figure 1).

Gad c 1 contains at least five IgE-binding sites.⁷⁰ The single arginine at position 75 in *Gad c 1* plays a major role in the tertiary structure of the allergen, but modification of the arginine residue resulted in no difference in IgE reactivity.⁷¹ Tryptic cleavage at the arginine residue resulted in two allergenic fragments, TM1 and TM2, which were equally active in skin-prick tests, Prausnitz-

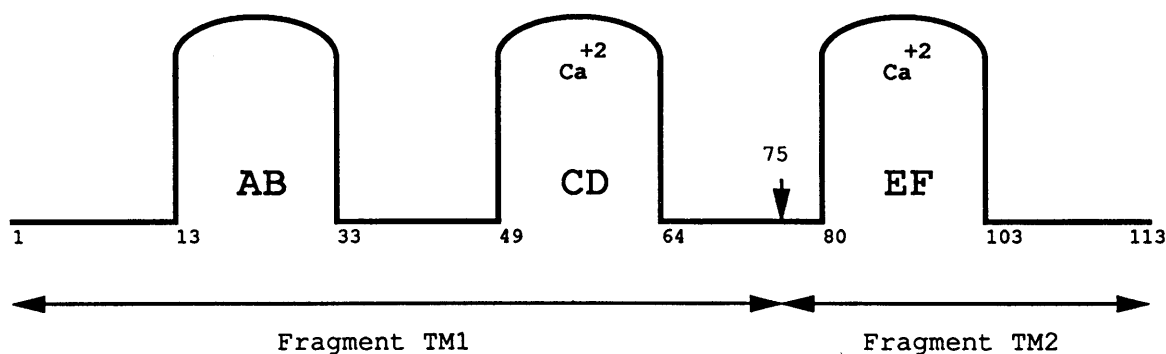


FIGURE 1. The *Gad c 1* molecule. Calcium is bound by loops CD and EF, while loop AB lacks this property. Fragment TM1 contains amino acid residues 1 through 75; fragment TM2 contains amino acids 76 through 113. (Adapted from Elsayed and Apold.⁷⁰)

Kustner (passive sensitization) tests, and RAST inhibition studies.⁷² TM1 comprises amino acid residues 1 to 75 and envelopes the AB and CD domains. The acidic residues 59 to 62 are associated with the Ca²⁺ binding of the CD domain.⁸ The single glucose molecule present in *Gad c 1* is located at the Cys-18 position.⁷³ This carbohydrate moiety does not appear to be involved in allergenicity, as the allergenic activity of TM1 without the carbohydrate was equal to that of TM2.⁷² Fragment TM2 comprises amino acid residues 76 to 113 and envelopes the EF domain; residues 90 to 101 constitute the Ca²⁺-binding region of the EF domain.⁷⁴ Fragment TM2 also contains one residue of tryptophan, which seems to have no bearing on allergenicity.⁷⁵ Further tryptic hydrolysis studies of fragment TM1 followed by skin tests and Prausnitz-Kustner tests showed that region 33 to 44 was important for allergenicity.⁷⁶ Tryptic hydrolysis studies of TM2 showed that region 88 to 96 is partially responsible for the allergenicity.⁷⁷

Studies using synthetic peptides established that region 49 to 64 encircled two repetitive sequences (Asp-Glu-Asp-Lys and Asp-Glu-Leu-Lys). These two tetrapeptides are mutually important for antibody binding, as region 49 to 64 showed relatively high RAST inhibition (39%) compared with *Gad c 1* (68%), and produced positive Prausnitz-Kustner tests. Region 57 to 64 did not show any allergenic activity.⁷⁸ In a subsequent article, it was shown that region 41 to 64 contained three homologous tetrapeptides, repeated in three sites, interspaced by six amino acids in a segment of 24 residues. A series of synthetic peptides of this region showed that at least two of the tetrapeptides were necessary for interaction with antibody, as all peptides encompassing a minimum of two of the tetrapeptides produced positive RAST inhibition and Prausnitz-Kustner tests.⁷⁹ IgE-binding capability is independent of both the constitution and sequence of the spacer amino acids.

Studies of synthetic peptides also showed that residues 88 to 103 in the EF domain have 37.5% amino acid sequence homology with the CD domain peptide, but lack the essential terminally located tetrapeptides responsible for antibody binding in region 41 to 64. However, this residue bound specifically to IgE both *in vivo* (Prausnitz-Kustner tests) and *in vitro* (RAST inhibition tests).⁸⁰ Therefore, region 88 to 103 was proposed to have a monovalent binding function that can consequently block, but not elicit allergic reactions.

The AB domain, which does not bind calcium, nonetheless shares more than 30% amino acid sequence homology with the CD and EF domains, and comprises residues 13 to 32. Synthetic peptides of this region showed that the AB domain is functionally divalent in Prausnitz-Kustner and RAST inhibition analyses. It reacts in RAST inhibition in equimolar concentrations at a ratio of 6:1 in comparison with *Gad c 1*.⁸¹

Regions 13 to 32 and 49 to 64 possess the divalent determinants necessary to elicit an allergic reaction. Repeating amino acid sequences are abundant in *Gad c 1*.⁸ In regions 35 to 41 and 67 to 73, five of the seven residues are identical (Leu-X-Ala-Phe-X-Ala-Asp), suggesting repeating IgE-binding sites. Region 65 to 83 is found between the CD and EF domains. A similar region joins the AB and CD domains and embraces section 33 to 44. These two regions possess a high degree of homology.⁷¹ This, plus the high degree of immunologic cross-reactivity between TM1 and TM2, all point to IgE-binding sites distributed in a repetitive manner along the polypeptide chain.

b. Protamine Sulfate

Salmine AI, a protamine from salmon, has the amino acid sequence



Iridine Ia, a protamine from rainbow trout, differs from salmine AI only in that there is an insertion of an additional Arg at position 19 (underlined).

Although there is not much information available on the amount of fish required to produce an allergic response in fish-allergic individuals, it was reported that cod-allergic patients reacted to less than 1 mg of purified *Gad c 1* disguised in a 50-g meatball.⁵⁹ O'Neil et al.⁸² found that 1 g or less of catfish and 4 g or less of other species tested (codfish or snapper) elicited allergic reactions in fish-sensitive individuals in double-blind placebo-controlled food challenges (DBPCFC). Positive reactions were obtained in DBPCFC using 2 oz (uncooked weight) of fish.⁸³ Another study reported that 8 g dehydrated fish administered over 1 h could elicit reactions.⁸⁴ However, these last two studies were not done in subjects with a history of anaphylaxis to fish.

D. Crustacea

At least 30 edible species of crustacea are commonly consumed in the U.S. The crustacean family (phylum Arthropoda, class Crustacea) includes shrimp, prawns, crabs, lobsters, and crayfish, and is a common cause of food hypersensitivity.⁸⁵ Although the prevalence is not known, researchers have estimated that more than 250,000 people in the U.S. have potential for developing allergic reactions to shellfish.⁸⁶ Like fish, a higher incidence of allergy to shellfish would be expected in geographic areas where more is consumed on a regular basis.

1. Shrimp: Major Allergens

a. Antigens I and II

Shrimp is the most studied of the crustacea allergens. Hoffman et al.⁸⁷ were the first to partially characterize allergens from shrimp. Two allergenic proteins were found in the body and shell extracts of raw shrimp, and were called antigen I and II. In a study of 11 shrimp-sensitive

subjects, 7 of 11 serum samples bound to antigen I. As only a trace of antigen I was found in raw shrimp and shell extracts, it was thought to be a heat-labile protein composed of two noncovalently bound polypeptide chains with a molecular weight of 21 kDa. Purification by gel filtration of antigen I resulted in a molecular weight of 45 kDa, suggesting it was a dimer. Antigen I had an pI of 4.75 to 5 and contained 189 amino acid residues and 0.5% carbohydrate.

Antigen II, isolated easily from boiled shrimp, was an acidic, heat-stable glycoprotein with a molecular weight of 38 kDa and a pI of 5.4 to 5.8, composed of 341 amino acid residues and 4% carbohydrate. It appeared to be the major allergen for the subjects in this study, as it bound IgE in all of the 11 shrimp-allergic serum samples. Antigen II gave a correlation coefficient of 0.98 with cooked shrimp in RAST inhibition studies. The allergens were not evaluated using skin tests, so divalent binding ability was not assessed. Antigens I and II were considered to be unrelated, based on amino acid composition and immunologic studies.

b. SA-I and SA-II

Nagpal et al.⁸⁸ described two allergenic polypeptides isolated from boiled shrimp. Allergen SA-I had a molecular weight of 8.2 kDa and was not analyzed further. The second allergen, SA-II, was composed of 301 amino acid residues, had a molecular weight of 34 kDa, and appeared to be similar to antigen I isolated by Hoffman and colleagues,⁸⁷ but was reported not to possess any carbohydrate.

Nagpal et al. stated that approximately 54% of the allergenic epitopes of SA-I and SA-II were shared, suggesting that SA-I was a fragment of SA-II. Taking this into consideration, SA-I contributed approximately 33% and SA-II approximately 56% of the total IgE-binding activity of crude boiled shrimp extract. The authors suggested that the remaining IgE-binding activity (11%) was contained in the shrimp tRNA allergen discussed below. These allergens were not evaluated using skin-test methods.

c. *Pen a 1* and *Pen i 1*

Daul et al.^{5,89,90} isolated a major shrimp allergen, *Pen a 1*, from boiled brown shrimp (*P. aztecus*) and reported that its sequence was similar to fruit fly tropomyosin. *Pen a 1* has a molecular weight of 36 kDa, is readily isolated from the boiling water⁹¹ and meat of cooked shrimp, and is similar to SA-II.⁸⁸ It constitutes 20% of the soluble protein in crude cooked shrimp extract and inhibited the RAST reactivity of pooled shrimp-sensitive subjects' serum to whole body shrimp meat extract by 75%. The allergen bound IgE in 28 of 34 (82%) sera from shrimp-sensitive individuals.

Pen a 1 comprises 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*.⁹²

Endoproteinase Lys-C studies of *Pen a 1* resulted in protein sequencing of a 21-residue peptide that demonstrated significant homology (60 to 85%) with tropomyosin from various species, consistent with the conclusion that *Pen a 1* was a shrimp tropomyosin.⁵ The greatest homology occurred in region 129 to 149; 72 to 87% with fruit fly tropomyosin, and 60 to 62% with tropomyosin from various mammalian species. The higher homology seen with *Drosophila* tropomyosin can be construed as being indicative of the phylogenetic connection between shrimp and insects. The amino acid sequence of the 21 residue peptide is

V-L-E-N-R-S-L-S-D-E-E-R-M-D-A-L-E-N-Q-L-K

Shanti et al.⁹² also reported that sequenced tryptic digests of *Pen i 1* were similar to fruitfly tropomyosin, and that two tryptically derived peptide sequences from shrimp tropomyosin bound shrimp-specific IgE. These were regions 50 to 66 and region 153 to 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 50 to 66 and 153 to 161 inhibited binding of SA-II-specific IgE to shrimp tropomyosin and 50% inhibition was attained at 100 pmol/ml for both peptides. Other tryptically derived peptides (some less than 2 kDa in molecular weight) inhibited IgE binding to a

lesser extent, but these peptides may not have been free of minor IgE-binding components.

Corresponding regions of tropomyosins from different vertebrates showed little cross-reactivity in region 50 to 66, but demonstrated significant allergenic cross-reactivity with tropomyosins from mammalian species in region 153 to 160: seven of nine amino acids for chicken, rabbit, and humans, and six of nine for rat tropomyosin. Fruit fly tropomyosin was identical to the SA-II allergen in region 153 to 161. Many tropomyosins have homology in the 155 to 161 region; the authors (Shanti et al.⁹⁵) 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.

The amino acid composition of shrimp allergens *Pen a 1*, antigen II, and SA-II is similar (Table 6). This further indicates that these three allergens are the same protein, shrimp tropomyosin,⁹³ although both antigen II and *Pen a 1* have associated carbohydrate moieties.

TABLE 6
Amino Acid Composition of Shrimp Allergens *Pen a 1*, Antigen II, and Sa-II

	<i>Pen a 1</i>	Antigen II	SA-II
Mol wt (kDa)	36	38	34
Alanine	33	31	21
Arginine	26	19	30
Aspartic acid	40	58	39
Cysteine	ND	2	3
Glutamic acid	80	61	75
Glycine	10	20	6
Histidine	1	4	3
Isoleucine	35	30	30
Leucine	6	12	6
Lysine	26	27	27
Methionine	8	9	6
Phenylalanine	4	9	6
Proline	2	6	3
Serine	14	15	12
Threonine	12	12	9
Tryptophan	ND	ND	4
Tyrosine	4	7	6
Valine	13	19	15
Total	312	341	301

Adapted from Daul et al.⁵

d. Met e 1

Leung et al.⁹⁴ produced a recombinant shrimp allergen from a cDNA library of the greasyback shrimp, *Metapenaeus enis*. The allergen has 281 amino acid residues, is similar in amino acid composition to *Pen a 1* and *Pen i 1*, and has a molecular weight of 34 kDa in SDS-PAGE. In immunoblotting studies, the recombinant allergen bound IgE in serum samples from all eight individuals in the study with histories of anaphylactic reactions to shrimp. Leung et al.⁹⁴ confirmed the observations of other groups identifying the 34-kDa allergen as shrimp tropomyosin. They also found that the recombinant shrimp allergen *Met e 1* possessed an IgE-binding sequence identical to the 50 to 66 region of Shanti et al.⁹² shown above, and another small IgE-binding sequence of F-L-A-E-E-A-D-R-K, similar to the 153 to 161 region.

2. Shrimp: Minor allergens

a. Transfer RNA

A minor allergenic tRNA moiety from boiled shrimp (*P. indicus*) has been described.⁹⁵ The "purified" RNA allergen possessed 11% of its dry weight as amino acids. After enzyme treatment, 84% of the amino acids were lost, but allergenicity was retained. Approximately 1 µg shrimp RNA caused 89% inhibition of a solid-phase shrimp RNA RAST. However, Nagpal et al.⁹⁵ only used one patient's sera in their studies, and so their results may not reflect normal clinical reactivity. It is possible that the allergenicity was due to RNA-associated proteins/peptides, as the RNA was not totally barren of amino acid residues. The RNA allergen was not analyzed in skin tests, so its divalent binding ability was not assessed. This remains the only documented example of a nucleic acid from food implicated in inducing an IgE response.

b. Dose Response

Daul et al.⁹⁶ found that six subjects reacted positively in a total of seven double blind chal-

lenges in 30 subjects with shrimp hypersensitivity. Four positive reactions were to a dose of four shrimp equivalents (one shrimp equivalent is approximately 8 mg, or the amount of protein extract obtained from a standard 4-g medium-sized shrimp) and three positive reactions occurred to a dose of 16 shrimp equivalents. It appears that only 1 to 2 g of shrimp is needed to elicit an anaphylactic reaction in sensitive individuals.⁹⁷

Oropharyngeal pruritus and occasional subjective throat-pharyngeal swelling was related by most of the individuals experiencing positive challenges at a lower dose of shrimp than that inducing their objective positive symptoms.⁹⁶ In a group of subjects having a history consistent with immediate type I hypersensitivity reactions to shrimp, only atopic patients reported anaphylaxis after ingestion; 30 nonatopic patients reported generalized pruritus as their only symptom.⁹⁸

3. Crab

Snow crab has been shown to cause allergic sensitization in occupational settings.^{99,100} Heat-labile and -stable allergens have been found in snow crab extracts, and snow crab-specific IgE bound more to boiled snow crab than to raw crab.¹⁰⁰

The most prominent bands in SDS-PAGE gels were 37-42 kDa in crab cooking water and in extracts of cooked crab meat. Immunoblotting of these SDS-PAGE-separated proteins showed that the majority of snow crab-allergic serum samples displayed IgE binding to the 37- to 42-kDa bands, but a majority also demonstrated heavy radio-staining to bands at or near 14 kDa (Hefle, Bush, Cartier, Malo, Lehrer, personal communication).

4. Lobster

The IgE-binding ability of distinct spiny lobster precipitins resolved in crossed immunoelectrophoresis (CIE) was demonstrated in CRIE using 14 crustacea-sensitive sera.¹⁰¹ Thirteen crustacea-allergic serum samples reacted in CRIE to these precipitins. Spiny lobster extract contained four IgE-binding precipitins; antigens 8 (positive in ten sera) and 13 (positive in five sera)

are the major allergens, giving the most radiostaining. Antigens 3 and 6 gave weak radiostaining in eight and two sera, respectively.

5. *Crawfish (Crayfish)*

In the study discussed above,¹⁰¹ crawfish precipitins were also evaluated for their ability to bind crustacea-specific IgE. Six crawfish antigens produced positive radiostaining using CRIE. Antigen 11 was the main allergenic component (positive in nine sera); antigen 12 (also positive in nine sera) may also be a major allergen, but it was situated under the antigen 11 arc and, therefore, could have been an artifact of coprecipitation. Antigens 6 (positive in one sera), 8 (positive in six), 10 (positive in two), and 13 (positive in seven) exhibited radiostaining to varying degrees.

III. COMMON ALLERGENIC FOODS OF PLANT ORIGIN

A. Peanuts

The peanut is an annual plant belonging to the family Leguminosae and is native to South America. In the U.S., several types are grown, although the three most popular are the Virginia, Spanish, and runner varieties. Virginia peanuts are used primarily for whole kernel consumption and confections. Runner types are used most frequently for oil production and peanut butter.¹⁰² Most of the peanut crop in the U.S. (63%) is used for production of peanut butter.¹⁰³ Children are often exposed to peanuts at an early age, usually in the form of peanut butter. Although a popular food, the peanut may be the most common allergenic food known. Allergic reactions to peanuts are often acute and severe. Peanut allergy is seldom outgrown.

Peanut proteins have been customarily classified as albumins (water soluble) or globulins (saline soluble). Most of the storage proteins are globulins, which make up 87% of the total protein.¹⁰⁴ Over the years, peanut proteins have been further fractionated, and classified as albumins, arachin, and conarachin or nonarachin.¹⁰⁵⁻¹⁰⁹ The globulins are made up of two major proteins, arachin and conarachin, which correspond to

legumin and vicilin, respectively. The former are primarily composed of large molecular weight globulins known as α -arachin and α -conarachin.¹⁰⁶

Arachin and conarachin readily associate and dissociate under different conditions of ionic strength and pH, making exact classification of individual components difficult.¹¹⁰⁻¹¹² In addition, arachin and conarachin have similar amino acid compositions and comparable electrophoretic mobilities, suggesting structural similarities. Both arachin and conarachin components are glycoproteins containing neutral and amino sugars.^{108,113} Immunochemical and electrophoretic analysis of polymorphism in peanuts show varietal differences,^{114,115} but most wild varieties contain much less arachin than do cultivated strains. A 45-kDa polypeptide is found in almost all genotypes,¹¹⁶ whereas an arachin subunit of 36 kDa is only found in certain cultivars.¹¹⁷

Arachin — In its native state, arachin exists as a molecule of at least 600 kDa and readily dissociates into a 340- to 360-kDa dimer and a monomer of approximately 170 to 180 kDa.^{118,119} Arachin contains approximately six subunits ranging in size from 19 to 42 kDa in SDS-PAGE,^{119,120} with pI ranging from 5.8 to 8.3.¹¹⁹ The globulin ranges from 0.6 to 0.3% in carbohydrates.^{108,113,120} A subunit of arachin has been isolated that comprises 201 amino acid residues.¹²¹ Although basic amino acids dominate the structure, there is no distinct basic linear region.

Conarachin — Johnson and Naismith¹¹¹ showed by ultracentrifugation that conarachin could be further divided into two fractions, 2S and 8.4S. Later, these were designated conarachin I and conarachin II (or α -conarachin), respectively. Although conarachin I (molecular weight 142 kDa) constitutes nearly 30% of total peanut protein,¹²² it has not been examined to any great extent. Conarachin II has a molecular weight of 290 kDa¹⁰⁹ and represents 15 to 25% of the total peanut protein.¹²² Various studies have reported from six to eight subunits within conarachin II.^{109,111,123,124} Basha and Cherry,¹²⁵ using SDS-PAGE, found that conarachin II dissociates into seven subunits, with molecular weights of 84, 46, 34, 31, 26, and 23 kDa. Shetty and Rao¹²⁴ found only one major subunit at 64 kDa, and minor ones of 60, 32, and 21 kDa. Conarachin II contains no carbohydrate, and has a pI of 3.9.¹²⁴

1. Peanut Allergens

A multiplicity of peanut allergens have been reported.^{3,126-132} These have yet to be fully identified and characterized. Part of the problem lies in the large number of allergenic peanut proteins; investigators have reported more than 20.^{126,129,130} Barnett et al.¹²⁶ found 16 IgE-binding protein bands in raw peanut extracts and 7 in roasted peanut extracts. Bush et al.,¹²⁹ using two-dimensional PAGE, found 11 IgE-binding proteins in extracts from raw peanuts. Studies using RAST inhibition^{126,127,131} and IgE enzyme-linked immunosorbent assay (ELISA)¹²⁸ have shown that no single protein is solely responsible for all of the peanut allergenicity. The peanut constituents relevant to allergy are either proteins or glycoproteins. ST and DBPCFC studies have shown that peanut oil is not allergenic.¹³³

Barnett et al.¹²⁶ examined the allergenicity of various peanut constituents by RAST and CRIE using sera from peanut-sensitive patients. They found that the allergenicity of the peanut is spread throughout the arachin and conarachin fractions. Taylor et al.¹³² also found that the arachin and conarachin fractions are allergenic in RAST inhibition assays.

Using the RAST technique, Heiner and Neucere¹³⁴ tested the allergenicity of different peanut preparations, including extracts of cotyledons, hypocotyls, and other parts of the peanut kernel. They found that the cotyledons, the hearts (axial germ tissue), and the skins were allergenic. The cotyledons (kernels) are probably the major source of allergen for most individuals, as the skins and hearts are often removed during processing. This is because the hearts contain saponins that impart a bitter flavor, and the skin contains catechol tannins and related compounds, which give finished products an undesirable color.¹³⁵

a. Major Allergens

Peanut-1 — Sachs et al.¹³¹ isolated and partially purified a peanut allergen, named "Peanut-1", from raw peanuts. From SDS-PAGE analysis, Peanut-1 was determined to contain two major bands, with molecular weights of 20 and 30 kDa. There were also several minor bands whose

molecular weights were reported to be above and below these two, but were not identified. Thin-layer isoelectric focusing gave a pI of 5.25 to 5.75 for Peanut-1. The authors concluded that Peanut-1 was a major acidic glycoprotein with non-identical subunits, but was not the only allergenic moiety present in peanuts.

Concanavalin A-reactive glycoprotein (CARG) — Gleeson and Jermyn¹³⁶ first described the isolation of a CARG from raw peanuts. The isolated protein had a molecular weight of 69 kDa and contained 12% carbohydrate. Barnett and Howden¹²⁷ later identified and purified a 65-kDa CARG peanut allergen. Based on results obtained with RAST inhibition studies, the authors characterized CARG as a major allergen, because approximately 50% of serum samples from peanut-sensitive patients in this study demonstrated IgE binding to this protein. CARG constitutes approximately 1% of the total peanut protein,¹³⁶ has pI of 4.6, contains 2.4% carbohydrate, and is stable at and above 100°C and over the pH range of 2.8 to 10.0. Removal of the carbohydrate moiety of the CARG slightly decreased, but did not completely eliminate the allergenic activity.¹²⁷ Meier-Davis et al.¹³⁰ using SDS-PAGE and immunoblotting of crude roasted peanut extract, identified three IgE-binding bands at 15, 20, and 66 kDa molecular weight that appeared to be major allergens, but did no further characterization of these bands.

Ara h 1 — Burks et al.³ identified a 63.5-kDa molecular weight glycoprotein peanut allergen using immunoblotting and ELISA methods with sera from peanut-sensitive AD patients. This allergen, *Ara h 1*, was found to have a pI of 4.55. Although it appears that CARG and *Ara h 1* are perhaps the same protein, *Ara h 1* does not bind to concanavalin A.

Ara h 2 — In a later report, Burks et al.¹²⁸ identified and purified another peanut allergen, *Ara h 2*, with a molecular weight of 17 kDa in SDS-PAGE and pI of 5.2. The physicochemical characteristics of the above peanut allergens are listed in Table 7.

2. Structure-Epitopes

Ara h 1 has been cloned and its amino acid sequence deduced.¹³⁷ It contains multiple IgE-

TABLE 7
Characteristics of Isolated Peanut Allergens

Name	Mol wt (kDa)	pI	Carbohydrate moiety	Ref.
<i>Ara h 1</i>	63.5	4.55	Yes	3
<i>Ara h 2</i>	17	5.2	Yes	128
CARG	65	4.6	Yes	127
Peanut-1	20 30	5.25–5.75	Yes	131

binding epitopes¹³⁸ and has significant sequence homology with the vicilin seed storage proteins.⁷

3. Dose Response

A research team at the Mayo Clinic reported four cases of death due to peanut-induced anaphylactic shock in a period of 16 months.⁵⁵ In this and other reports,^{139,140} victims unknowingly ingested food containing peanut protein. Yunginger et al.⁵⁵ point out that in the cases they examined, the amount of peanut protein ingested was probably in the range of milligram to gram quantities. Using DBPCFC, 50 to 100 mg of peanut protein has elicited allergic symptoms in some children.¹⁴¹ However, acutely sensitive patients are not orally challenged, due to the threat of anaphylaxis. In a study using rush immunotherapy for treating peanut allergy, Oppenheimer et al.¹⁴² reported that 30 mg to 8 mg of peanut (mean approximately 4 g) administered in a double-blind fashion elicited reactions in peanut-sensitive subjects.

B. Soybeans

Soybean globulins are the major proteins. By adjusting the pH of the saline-soluble soybean protein fraction to 4.5, the globulins precipitate, leaving a resulting whey fraction (constituting 6 to 8% of the protein).¹⁴³ The whey fraction contains hemagglutinin, trypsin inhibitors, and urease,¹⁴⁴ ranging from 1S to 6S.¹⁴⁵

When subjected to ultracentrifugation, the globulins separate into 2S, 7S, 11S, and 15S fractions, which have been used to classify the various soybean protein components.¹⁴⁶ Because of the wide range of different analytical methods

used in the study of these proteins, Catsimpoolas et al.¹⁴⁷ proposed the following system: the 2S component different from soybean trypsin inhibitor is referred to as α -conglycinin and the 7S component isolated by the method of Robert and Briggs¹⁴⁸ is referred to as β -conglycinin (vicilin). Glycinin and β -conglycinin comprise 70 to 80% of the protein fraction of soybeans.^{149,150} The 7S component isolated by the method of Koshiyama and Iguchi¹⁵¹ is referred to as γ -conglycinin and the 11S component is referred to as glycinin. The 15S fraction consists mainly of polymers of glycinin.¹⁵²

α -Conglycinin (2S) — α -Conglycinin is one of the major fractions of soybean. The 2S fraction possesses heat-stable components of 18.2 and 32.6 kDa, but also contains trypsin inhibitor and cytochrome c activity.¹⁴⁴ Vaintraub and Shutov¹⁵³ found that the 2S fraction could be resolved into two components, 2.8S and 2.3S. The 2.8S component gave a 36-kDa band in PAGE and had a pI of 4.4. All of the trypsin inhibitor activity of the soybean was thought to be contained in the 2S fraction, including the Bowman-Birk- (6 to 10 kDa) and Kunitz-type trypsin inhibitors (KSTI) (20 to 25 kDa).¹⁵⁴ However, it was later found that the activity was due to coprecipitation of the inhibitors during the isolation of the globulins.¹⁵⁵

β -Conglycinin (7S) — This glycoprotein exists as a trimer and/or hexamer in solution, and probably in seed.¹⁵⁴ The monomeric form is 150 to 170 kDa, and the dimer approximately 370 kDa; the pI has been determined to be 4.9. β -Conglycinin is made up of three subunits denoted α , α' , and β , and at least seven different forms can exist (B_0 to B_6) as a result of different combinations of subunits.^{156,157} The α and α' subunits have a molecular weight of 54 kDa, and the β subunit has a mol wt of 42 kDa.¹⁵⁴ All three subunits contain

4.5% carbohydrate.¹⁵⁶ Sato et al.¹⁵⁸ described a "basic" 7S globulin (pI = 9.1 to 9.3) that had two kinds of subunits of mol wt 16 and 26 kDa; the native molecule gave a 42-kDa band in the absence of reduction in SDS-PAGE. The pIs for the 16- and 26-kDa polypeptides were 6.5 to 7.0 and 7.7 to 7.9, respectively. Coates et al.¹⁵⁹ investigated the electrophoretic profiles of the predominant subunits after cyanogen bromide treatment. The α' subunit was composed of major bands at 47, 19.5, and 15.5 kDa. The 47- and 19.5-kDa bands were glycoproteins. The α subunit gave major bands at 54 and 19.5 kDa, and both were glycosylated. The β -subunit had no methionine residues, and therefore, no resulting bands were observed; the β' -subunit gave four fragments, although their molecular weights were not described. This study also described the derived amino acid sequence from the sequence of a cDNA of an α -subunit clone.

γ -Conglycinin (7S) — γ -Conglycinin is a 7S fraction of soybean protein and is a glycoprotein with a molecular weight of 154 to 177 kDa.¹⁶⁰⁻¹⁶² The pI is 5.4.¹⁶⁰ One report indicates that γ -conglycinin has nine subunits of 22 kDa,¹⁶³ another describes the subunits as glycopeptides of 38 kDa and simple peptides of 32 kDa, respectively.¹⁶⁴ Yamauchi et al.¹⁶² described γ -conglycinin as having three subunits of 50 kDa each. In this study, cross-linked subunits subjected to SDS-urea-PAGE gave a 54.4-kDa monomer, a 109-kDa dimer, and a 154-kDa trimer.

Glycinin (11S) — Of the globulins, the 11S fraction, or glycinin (legumin), is the most studied. It has a molecular weight of 320 to 360 kDa and is composed of 12 subunits,¹⁶⁵ which range from 10 to 45 kDa. The acidic subunits range from 37 to 45 kDa and the basic subunits are 20 kDa in molecular weight,^{166,167} although Nielsen et al.¹⁶⁸ found subunits of 54.3 to 63.7 kDa. The acidic subunits, A₁ to A₄, and the basic subunits, B₁ to B₄, are present in equimolar amounts in the glycinin molecule.¹⁶⁹ The acidic subunits share some sequence homology and antigenic determinants, suggesting conserved sequences.¹⁷⁰ The basic subunits share considerable sequence homology with each other, but are distinct from the acidic subunits.¹⁷¹ In the native glycinin, the subunits are packed into two identical hexagons, one

on top of the other, forming a cylinder shape.¹⁶⁵ The amino acid sequence of one of the subunits of glycinin has been elucidated.¹⁶⁷

1. Soybean Allergens

Soybeans contain multiple allergens. Shibasaki et al.¹⁴⁴ studied soybean globulin fractions 11S, 7S, and 2S by RAST and RAST inhibition using sera from three AD and one asthmatic soy-allergic patients. Specific IgE reactivity and considerable cross-reactivity were found in all the fractions. The 2S fraction had the highest potency in inhibiting RASTs for all fractions; furthermore, 50 μ g of 2S could inhibit 90% of the IgE binding for all other fractions in RAST studies. When the fractions were heated to 80°C for 30 min, the inhibitory activity of the 2S fraction was enhanced, whereas the others decreased to 39 to 75% of that of the native globulin. However, the potency of the 2S fraction decreased at temperatures beyond 80°C.

In a study using sera from eight AD pediatric patients with positive DBPCFC to soy, significant levels of specific IgE for the 7S fraction and IgG for the 11S fraction were found.¹⁷² Most antigens bound IgE, but the 7S fraction seemed to be more allergenic. Variable IgE-binding patterns on immunoblots suggested that no one component of the soy fractions bound more IgE. Specific IgE antibody was found to both the 7S and 11S fractions in most serum samples. IgE binding was demonstrated to the α -, α' -, and β -subunits of the 7S fraction and the A and B subunits of the 11S fraction.

In another study by Ogawa et al.,¹⁷³ most IgE-binding bands were assigned to protein components of the 7S fraction, although binding was also observed in bands from the whey and 2S fractions. Sixteen soybean proteins were observed in SDS-PAGE, ranging from 14 to 70 kDa. After immunoblotting with serum from 10 AD subjects, major IgE-binding bands were observed in the 7S fraction. The 11S fraction was scarcely recognized. Therefore, in this study it was not important for soy-allergic individuals with AD, although their hypersensitivities to soy were not verified by DBPCFC. IgE-binding bands occurred in the 7S fraction at 40 to 70 kDa, with major

binding to a 30-kDa fraction, a minor component of the fraction. The 30-kDa band has been designated *Gly m 1*. This 30-kDa band could not be detected in the 2S, 11S, or whey fractions. Specific IgE binding was observed to an acidic subunit of the 11S fraction. In the 7S fraction, ten major IgE-binding bands were observed, including the α - and β -subunits of β -conglycinin. IgE also bound to the 2S fraction. Ogawa et al.¹⁷³ suggest that the 20-kDa band is KSTI, but IgE-reactive bands occur at 17 and 15 kDa in this fraction also. They said that the data show that allergenic cross-reactivity does not exist between the 7S and the other fractions; however, cross-reaction was found between the 20-kDa band in the 2S fraction and the 18- to 21-kDa bands in the whey fraction. In this study, in contrast to other reports, the 11S fraction was less allergenic than the other fractions, even though it comprises much of the total storage protein in the soybean.

a. Major Allergens

Gly m 1 — *Gly m 1* is described by Ogawa et al.¹⁷³ as a 30-kDa mol wt protein, a minor constituent of the 7S globulin fraction. Sixty-five percent of the subjects in this study had specific IgE for *Gly m 1*; however, these individuals were AD patients who did not experience severe or anaphylactic reactions to soy. In a later article, this same research group¹⁷⁴ found that the native allergen had a molecular weight of more than 300 kDa by gel permeation chromatography. The monomeric form had a molecular weight of 32 kDa, and pI of 4.5, in two-dimensional electrophoresis. The first 15 amino acid residues of *Gly m 1* are identical to those of soybean seed 34-kDa oil body-associated protein (also called the soybean vacuolar protein P34). In addition, the 34-kDa oil body-associated protein bound strongly to IgE from sera and monoclonal antibodies that were made against *Gly m 1* in immunoblotting studies. The 34-kDa 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.¹⁷⁵ The presence of *Gly m 1* as a minor component of the 7S fraction could have been the result of elimina-

tion of the majority of the allergen during preparation of the crude 7S globulin fractions from defatted soybean flakes.

b. Minor Allergens

68-kDa allergen — Ogawa et al.¹⁷⁶ found that 25% of IgE from sera of soybean-allergic individuals with AD recognized a 68-kDa protein of the 7S globulin fraction in immunoblotting studies. The protein was 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.

KSTI — A study of KSTI as an allergenic protein was prompted by a soy-allergic woman working with KSTI in an occupational setting.¹⁷⁷ The patient was skin test and RAST positive to KSTI and whole soybean; there was no IgE reactivity with other trypsin inhibitors or to peanut extract. The whole soybean RAST was inhibited completely by KSTI. Of the two other sera from soy-allergic individuals utilized in the study, both were negative by RAST for KSTI, and only one was positive for whole soybean RAST. KSTI could not inhibit the IgE binding to the whole soybean extract. Therefore, KSTI appears to be a relatively minor allergen. Certain soybean cultivars have been produced with reduced or zero levels of KSTI to reduce its antinutritional effects.¹⁷⁸ KSTI has been sequenced.^{179,180} The inhibitor is made up of 181 amino acid residues. This group also found sequence variants of the inhibitor, deviating in nine sites in the molecule, with only a single amino acid substitution at each site. The KSTI has a molecular weight of 20 kDa. Brandon et al.¹⁸¹ found that KSTI has at least two distinct antigenic sites, one of which is retained under denaturing conditions, and may be linear.

S-II (20-kDa protein) — Herian et al.¹⁸² described a 20-kDa IgE-binding protein from soybean, designating it S-II. Two serum samples from subjects allergic to soy showed IgE-binding to a 20 kDa band. No IgE binding was observed to pure KSTI. Roasting seemed to enhance IgE binding to the 20-kDa allergen. Preliminary work indicated that S-II is not a basic subunit of glycinin. One serum sample from a soy-allergic subject

showed binding only to a 14 kDa band. Serum samples from subjects who were both allergic to soybeans and peanuts showed IgE binding to several bands in the range of 50 to 70 kDa, apparently to β -conglycinin subunits. No varietal differences in IgE binding of raw soybeans were observed. The IgE-binding proteins were not characterized further.

2. Structure-Epitopes

Glycinin — ELISA studies^{183,184} of the antigenic and allergenic properties of the subunits of glycinin showed that all the acidic chains reacted similarly to a rabbit antiserum raised against glycinin. The basic subunits had no reactivity. Nielsen et al.¹⁶⁸ theorized that these subunits are situated on the inside of molecule. IgG-ELISA using serum from ten soy-allergic adults showed that six samples had IgG-binding activity to glycinin preferably, and none seemed specific for a single subunit. In IgE-binding studies, four sera showed the most IgE binding to subunit A4, whereas another four had the most IgE binding to native glycinin. One sample had no IgE binding to glycinin, but significant binding to the subunits. Specific IgG is directed toward the native glycinin molecule, whereas IgE is directed toward the subunits. The authors suggested that the IgE might be directed toward more fragmented fractions, reflecting the possibility that a certain measure of antigenic processing is required for IgE formation. However, only 80% of the IgE-binding inhibition could be attained by the subunits; therefore, not all of the allergenicity of glycinin is due to the subunits. Antigenicity of glycinin was more conformation dependent than that of β -conglycinin.¹⁸⁵

Gly m 1 — *Gly m 1* has an N-terminal sequence and an amino acid composition identical to that of soybean seed 34-kDa oil body-associated protein, and close homology to papain-like thiol proteinases.¹⁸⁶ It is interesting to note that *Gly m 1* possesses 30% sequence homology to *Der p 1*, the major dust mite allergen, which is also a thiol protease.¹⁷⁴ The N-terminal sequence for *Gly m 1* is

10

K-K-M-K-K-E-Q-Y-S-C-D-H-P-P-A

and is identical to the sequence of the first 15 residues from the N-terminus of the 34-kDa oil body-associated soybean protein.

68-kDa allergen — The IgE-binding site in the 68-kDa minor allergen of soybeans was judged to be located in the residue sequence 232 to 383. IgE in soy-allergic sera recognized the α subunit, but did not recognize the α' - or β -subunits of β -conglycinin, even though they have a high degree of homology with the α -subunit. The α - and α' -subunits share over 90% homology.¹⁸⁷ The predicted IgE-binding region on the α -subunit, residues 232 to 383, corresponds to residues 258 to 417 on the α' -subunit.¹⁸⁸ Therefore, further investigation is necessary to determine whether structural differences between the two subunits can account for the difference in allergenicity for this select population of soy-allergic individuals.

3. Dose Response

There is little information available on the threshold dose of soybean protein required to elicit an allergic reaction. Double-blind challenge studies have been done with children suffering from AD and are, therefore, not exquisitely sensitive to soybean. James et al.¹⁸⁹ found that 250 to 500 mg soy could elicit reactions in their population of AD patients. However, soybean is capable of severe reactions in some individuals, as evidenced by the death of one child from eating pizza containing soybean-fortified sausage.¹⁹⁰ As with all commonly allergenic foods, the smallest amount of food required to elicit an allergic reaction from sensitive individuals is unknown.

C. Tree Nuts

1. Almond

Bargman et al.¹⁹¹ used immunoblotting techniques to detect IgE-binding proteins in almond extracts, using sera from seven almond-allergic individuals. Two major allergens were identified. One was a 70-kDa heat-labile protein; the other was a 45-50 kDa heat-stable protein. An extensive number of proteins with molecular weights ranging from 38 to 70 kDa bound IgE.

2. Brazil Nuts

Brazil nuts cause systemic anaphylaxis in some individuals. Using immunoblotting to detect Brazil nut peanut allergens, Arshad et al.¹⁹² found several allergenic fractions in the serum from allergic individuals. The major allergen from Brazil nuts, *Ber e 1*, is a high-methionine, 2S protein¹⁹³ that is composed of two subunits. The 9-kDa subunit of the protein contained 77 amino acids, and a 3-kDa subunit has also been reported. The cDNA sequence for *Ber e 1* has been established.¹⁹⁴ *Ber e 1* has 44% and 21% homology with the castor bean and rapeseed high-methionine proteins, respectively.

3. Hazelnuts

Hazelnut allergy is prominent in Europe among individuals with tree pollen allergies (see Section VII.F). Hirschwehr et al.,¹⁹⁵ using sera from 25 birch pollen-allergic subjects who reported adverse reactions to hazelnuts, found IgE binding to the 17-kDa major hazel pollen allergen, *Cor a 1* (100%), and to the 14-kDa hazel pollen protein profilin (16%). In addition, IgE bound to proteins of comparable molecular weights in hazelnut extract (18 and 14 kDa), suggesting that proteins similar to *Cor a 1* and to the 14-kDa hazel profilin might also be expressed in hazelnuts. In contrast, only four sera (22%) of 18 subjects with tree pollen allergy, but without any history of intolerance to hazelnuts, showed IgE binding to the 18-kDa protein of hazelnut extract, and none exhibited IgE reactivity to hazelnut profilin. Immunoblotting inhibition studies revealed that the 18-kDa protein shares IgE-binding similarities with *Cor a 1*, the major of hazel pollen, *Bet v 1* (the major allergen of birch pollen),¹⁹⁵ and *Bet v 2* (birch pollen profilin).¹⁹⁶ However, this protein has not yet been sequenced.

4. Pistachio

Pistachio, which is a member of the cashew and mango family (Anacardiaceae), has a prominent IgE-binding protein of 34 kDa.¹⁹⁷ Other allergens ranged in molecular weight from 41 to

60 kDa. Some cross-reactivity was demonstrated against peanut, walnut, and sunflower seeds.

D. Wheat

In the U.S., wheat is a dietary staple. The most frequent allergic complaint with cereal grains is occupational asthma from exposure to dusty work surroundings¹⁹⁸ (see Section VI). The wheat proteins include the water-soluble albumins, the saline-soluble globulins, the 70% aqueous ethanol-soluble prolamins, and the acid- or alkali-soluble glutelins.¹⁹⁹

Using serum from one subject who suffered asthma after ingestion of wheat, Hoffman²⁰⁰ found that the wheat globulin and albumin fractions were most reactive in a RAST. Sutton et al.²⁰¹ also observed that the highest IgE-binding activity was associated with the globulin fraction in a RAST study using the serum of 20 children with high RAST scores to wheat.

In six individuals with food-dependent exercise-induced anaphylaxis (F-EIA), ingestion of wheat 30 min before exercise was associated with a subsequent anaphylactic event and all demonstrated immediate positive skin-test reactions to wheat extracts.²⁰² Many of these subjects also had skin-test reactivity to trypsin and pepsin digests of wheat. The authors hypothesized that "neoantigens" or novel allergens developed from digestion. However, because the individuals reacted to the native proteins, polypeptides removed from the intact proteins may be a more likely explanation.

IV. LESS COMMONLY ALLERGENIC FOODS OF ANIMAL ORIGIN

A. Mollusks

The phylum Mollusca is made up of class Pelecypoda (bivalves), mussels, clams, cockles, oysters, and scallops; the class Gastropoda, consisting of abalones, conches, limpets, snails, and whelks; and the class Cephalopoda, the octopuses and squids.⁵⁷ Mollusk allergens have not been well studied, although they are known to cause IgE-mediated reactions.²⁰³⁻²⁰⁶

1. Oyster (*Bivalve*)

Oysters have been frequently known to induce adverse reactions in sensitive subjects.²⁰⁷ Lehrer and McCants²⁰⁷ tested six oyster-sensitive subjects (who exhibited only GI manifestations after ingestion) and found that skin tests and RAST did not appear to correlate with oyster sensitivity.²⁰⁷

2. Squid (*Cephalopod*)

Squids precipitate IgE-mediated reactions in sensitive subjects after ingesting or inhaling cooking vapors. All seven squid-allergic patients in one study demonstrated strong positive skin-test reactions to boiled squid extracts and various commercial crustacea extracts. In addition, serum samples from squid-allergic individuals were positive in specific IgE immunoassays for boiled squid extract.²⁰⁵

3. Limpet/Abalone (*Gastropods*)

Anaphylactic reactions have been reported following ingestion of grand keyhole limpets and abalones. Sensitive subjects had positive skin tests and RASTs to extracts of the offending shellfish.^{204,208} In one study, limpet-allergic subjects demonstrated positive skin tests and basophil histamine release in response to a cooked limpet extract, but not to a raw extract.²⁰⁴ By immunoblotting, the major IgE-binding proteins of grand keyhole limpets appeared to have molecular weights of 38 and 80 kDa, but further characterization has not been done.²⁰⁹

4. Snail (*Gastropod*)

In a study of ten subjects allergic to snails, eight experienced bronchial symptoms, whereas six reported no skin or GI symptoms. All subjects could ingest cephalopods and bivalves without adverse reaction,²⁰⁶ and all ten had positive basophil histamine release and skin tests for snail extracts.

In another study,²⁰³ 61% of 70 atopic subjects were positive for boiled snail extract in skin tests

and 19% showed RAST reactivity for snail antigens. Asthma symptoms were reported after ingestion of snail by 15% of the subjects. Six different IgE-binding protein bands resulted from SDS-PAGE and immunoblotting of the boiled snail extract, ranging in molecular weight from 12 to over 66 kDa. One microgram of snail extract used in basophil histamine release studies in skin-test- and RAST-positive subjects was positive. Keyhole limpet hemocyanin did not provoke cross-reactive basophil histamine release or skin-test responses in these subjects. Specific IgE binding was exhibited to a 66-kDa band (two of ten sera), a 24-kDa band (nine of ten), a 15-kDa band (three of ten), and a 12-kDa band (six of ten); however, these bands were not characterized further.

V. LESS COMMONLY ALLERGENIC FOODS OF PLANT ORIGIN

A. Buckwheat

Buckwheat is a member of the Polygonaceae group of weeds and is not related to the cereals.²¹⁰ Ingestion of buckwheat has been associated with GI symptoms, urticaria, angioedema, and anaphylaxis.^{211,212} Occupational exposure to buckwheat has been documented to cause occupational allergic reactions. Immunoblotting using sera from a patient who suffered repeated episodes of anaphylaxis following buckwheat ingestion revealed four IgE-binding bands in the molecular weight range 9 to 40 kDa, all of which were glycoproteins.²¹³ Yano et al.²¹⁴ found three proteins of molecular weight of 8 to 9 kDa that bound IgE from serum of patients with high RAST scores to buckwheat. One of the proteins was a trypsin inhibitor.

B. Lupin (*Lupinus albus*)

Lupin is a member of the legume family. It is a pea-like plant cultivated worldwide, primarily for use as a feed or to be plowed under for its nutrients.²¹⁵ However, this legume has also been evaluated over the years for use in foods for human consumption.

Hefle and Bush²¹⁶ reported the adverse reaction of a peanut-allergic child to a lupin-fortified pasta product. They investigated the lupin proteins further using ST and *in vitro* analysis of IgE binding from six peanut-allergic adults. The IgE-binding proteins of lupin have molecular weights of 21 kDa and from 35 to 55 kDa in SDS-PAGE, and are heat stable. Three of the six sera bound only weakly to the 21-kDa band, whereas this band appeared to be a prominent IgE-binding protein for the other three individual sera. The subjects experiencing a positive skin-test reaction to lupin extract also reported a history of adverse reactions to green peas.²¹⁶

C. Peas

Although peas are part of the legume family, the frequency of allergic sensitivity to peas is considerably less than to peanuts or soybeans. However, this may be related to the degree of exposure to pea proteins in human diets. Addition of leguminous proteins, such as pea and lupin, into the diet in increasing amounts could increase the prevalence of pea allergy.

The globulin fraction accounts for 75 to 80% of the total seed protein, whereas the albumin fraction constitutes most of the remainder, depending on the cultivar and the isolation methods used.²¹⁷ The green pea (*Pisum sativum* L.) legumin is approximately 256 kDa and is composed of six pairs of 20- and 40-kDa subunits. Each subunit is made up of a 60-kDa polypeptide chain.²¹⁸ Green pea vicilin is a trimer composed of 50-kDa subunits,^{219,220} and a vicilin-like pea protein called convicilin consists of four monomers of 71-kDa.²²¹

Crude pea and pea albumin extracts produced positive skin tests. However, the major pea globulins legumin (11S) and vicilin (7S) did not produce positive reactions in skin tests in a study of ten green pea-sensitive subjects.²²² The albumin fraction retained all of its allergenic activity when heated or boiled.

A 53-kDa major pea albumin designated "PMA-L" had two subunits of approximately 25 kDa. Another component, "PMA-S," had a molecular weight of 48 kDa and possessed two

24-kDa subunits. Neither PMA-L nor PMA-S was significantly degraded during germination, indicating that they are probably not seed storage proteins.²²³ Later work on a pea albumin designated "Psa MA" (*P. sativum* major albumin), a homodimer with subunits of 24 or 25 kDa, led to the discovery and sequencing of "Psa LA," a low-molecular-weight albumin component (11 kDa) with 54 amino acid residues. The protein is most probably a dimer of two 6-kDa polypeptides,²²⁴ and does not possess protease inhibitor activity. Psa LA did not react to antibodies raised against the pea storage proteins, Psa MA, or pea lectin by Ochterlony or immunoblotting techniques. A green pea allergen with an approximate molecular weight of 1.8 kDa and a carbohydrate content of 30% in SDS-PAGE was purified from pea dialysate,²²⁵ but was not characterized further.

D. Psyllium

Psyllium mucilloid is obtained from the seed husk of plants in the *Plantago* genus. It has been used in bulk laxatives since the 1500s, and its allergenicity in occupational settings is well documented.^{226,227} Psyllium was added to cereal products after observations that it was useful in lowering serum cholesterol levels in hypercholesterolemic patients.²²⁸ Ingestion of these psyllium-fortified cereal products has caused significant anaphylactic reactions;²²⁹ most of the affected individuals had been sensitized through occupational exposure, but some patients were not sensitized via this route. In one study,²²⁹ 20 patients who were either inhalation- or ingestion-sensitized possessed IgE to six psyllium protein bands in the molecular weight range of 20 to 36 kDa.²²⁹ These IgE-binding proteins were not characterized further.

E. Rice

Rice (*Oryza sativa*) is a dietary staple for approximately one-half of the world population. In Japan, rice frequently aggravates AD through IgE-dependent mechanisms. There is one report of two protein fractions of rice, glutelin and globu-

lin, being reactive with specific IgE from rice-allergic individuals as demonstrated by RAST.²³⁰ The major rice allergens consist of micro-heterogeneous albumin proteins, with molecular weights ranging from 14 to 16 kDa with pI of 6 to 8,¹⁷ encoded in a multigene family.²³¹ The nucleotide sequence of cDNA coding for the major rice allergen has been determined,²³² a 486-nucleotide sequence with an open reading frame that encodes for a 162 amino acid residue. The mature protein has a molecular weight of approximately 14.7 kDa. The deduced amino acid sequence has homology to barley trypsin inhibitor (20%) and wheat α -amylase inhibitor (40%).²³² The allergenic rice protein is heat stable and resistant to proteolysis. Because a single protein accounts 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.²³³ A second approach is to use the nucleotide sequence of the gene to prepare an anti-sense gene to reduce the amount of allergenic protein formed in the grain.²³³ Watanabe²³⁴ employed a process that involved the use of a protease to reduce the allergenicity of the rice grains. This was somewhat successful, but the amount of enzyme required was large.

F. Apples

Fresh apples may produce localized oral allergy syndrome (OAS). This syndrome seems much more common in Europe than in other parts of the world (see "Allergenic Foods" by Susan L. Hefle et al. in this issue). In Europe, apple is a common allergenic food. Ebner et al.²³⁵ showed immunologic cross-reactivity between *Bet v 1* from birch pollen and an apple allergen with a molecular weight of 17 to 18 kDa. They were also able to hybridize RNA from birch pollen to apple-derived transcripts that approximated 800 base pairs. Vieths et al.²³⁶ linked allergenicity to the 18-kDa protein in apple by immunoblotting and also found allergens at 13 kDa and at 30 to 50 kDa. They compared various apple varieties as to the concentration of the 18-kDa protein and noted that Golden Delicious and Granny Smith apples contained higher concentrations of this protein

than other varieties. Gloster and Jamba varieties were low in the 18-kDa protein. Vieths et al.²³⁷ have conducted amino acid sequencing of 26 N-terminal residues of the 18 kDa protein and found significant sequence homology (62%) between this protein and that of the major birch pollen allergen *Bet v 1*. The 18-kDa allergen may be related to disease resistance. Hsieh et al.²³⁸ found that of 34 sera of patients with tree pollen allergies in immunoblotting studies, 37.5% demonstrated IgE binding to an 18-kDa protein and 75% to a 31-kDa protein. In addition, IgE binding was observed to proteins of 12, 14, 16, 38, and 50 kDa mol wt (in SDS-PAGE). The amino-terminal amino acid sequences of the 18- and 31-kDa proteins share about 50% sequence identity with *Bet v 1* and other disease resistance proteins of various plants.²³⁸ Although Vieths et al.²³⁶ found that storage increased the levels of the 18-kDa allergen, this study found no observed increase in 18-kDa allergen content resulting directly from ripening and maturation. Therefore, it was concluded that the increased levels of the allergenic protein might be produced by factors related to disease resistance.²³⁸

G. Cabbage

Cabbage (*Brassica oleracea*) is a member of the Brassicaceae (mustard family). Using gel filtration techniques, allergenic fractions with molecular weights ranging from 20 to 67 kDa have been identified.²³⁹

H. Celery

Celery has been reported to be a cause of OAS symptoms in individuals with mugwort and birch pollen allergy (see Section VII.F). Occasionally, celery can also cause more serious manifestations. Vallier et al.²⁴⁰ identified a 15-kDa profilin protein from celery. This was a heterogeneous protein that showed two bands on SDS-PAGE. It was shown to be cross-reactive with birch and mugwort pollens using IgE antibodies from subjects with these allergies.^{240,241} As with apple, celery allergy is quite common in Europe

(see "Allergenic Foods" by Susan L. Hefle et al. in this issue).

I. Chocolate

Chocolate is reported by many patients to cause allergic reactions, but such sensitivity is frequently not reproducible. There are a few documented cases of actual sensitivity by DBPCFC. Skin-test reactivity as assessed with intradermal testing, however, is frequently positive.^{242,243} Because ST with chocolate produces many false-positive reactions, the use of these reagents as diagnostic tests should be avoided. Because it is questionable whether chocolate acts as a true IgE-binding allergen, no work to characterize its constituents has been done.

J. Melons

Watermelon, cantaloupe, honeydew melon, and banana occasionally cause OAS in ragweed pollen-allergic individuals. Occasionally, more severe systemic reactions can occur. Enberg et al.²⁴⁴ separated a protein in a watermelon extract by isoelectrofocusing at a pH range of 4 to 6, transferred the protein to a nitrocellulose membrane, and probed the membranes for IgE binding using serum from watermelon-sensitive subjects. No consistent IgE binding was observed. However, by using SDS-PAGE and immunoblotting, Jordan-Wagner et al.²⁴⁵ found a 15-kDa protein in watermelon extracts that bound IgE from watermelon-sensitive subjects. The protein was cross-reactive with a similar protein found in celery, cucumber, and carrot, but has not been further characterized.

K. Papain

Papain is a proteolytic enzyme derived from papaya. It is used as a meat tenderizer, for clarifying beer, and as a reagent in the biochemical, immunochemical, and pharmaceutical industries. Several reports have implicated papain as a cause of occupational asthma.^{246,247} Some individuals have developed sensitivity as a result of ingestion

of papaya,²⁴⁸ or meat tenderizer,²⁴⁹ or from the injection of chymopapain used to treat herniated intervertebral discs.²⁴⁸ Positive skin and RAST tests and oral challenges have confirmed the presence of IgE-mediated sensitivity to this enzyme.

L. Peach

Peach can produce allergic reactions ranging from OAS to anaphylaxis.²⁵⁰ Several studies of peach proteins analyzed by immunoblotting have been reported with variable results. Wade et al.²⁵¹ detected a 30-kDa allergenic protein in peach extracts, but not in pear or apple extracts. Taylor et al.²⁵² found several labile allergenic proteins or glycoproteins with molecular weights of 41, 67, and 72 kDa in fresh peach pulp. Leonart et al.²⁵³ described a peach skin protein doublet with a molecular weight of 8 to 10 kDa that bound IgE from peach-sensitive subjects. Pastorello et al.²⁵⁰ identified a 13-kDa allergenic protein common to several Prunoideae (apricot, cherry, peach, and plum). They also found a 14- and a 30-kDa allergen in the peach and cherry, respectively. None of the peach IgE-binding proteins have been purified or sequenced to date.

M. Potato

Sensitivity to potato is fairly uncommon. Immunoblotting studies using serum from subjects with sensitivity to potato have shown IgE binding to raw potato proteins with molecular weights ranging from 16 to 65 kDa with pI of 4.5 to 5.2.^{254,255} Some individuals with birch pollen allergy report lip and mouth itching from ingestion of raw potatoes. Often these individuals tolerate cooked potato without difficulty. The putative allergen is theorized to be profilin (see Section VII.F).

N. Tomato

Tomato (*Lycopersicon esculentum*) is a member of the Solanaceae family. Bleumink et al.²⁵⁶ fractionated tomato proteins using ion-exchange chromatography. An allergenic glycoprotein frac-

tion was isolated. However, allergens were polydispersed throughout a number of fractions. Allergenicity also depended on the state of ripening of the fruit. Skin-test reactivity was highest in red-ripe fruit stored for 14 d at room temperature. It was hypothesized that the allergenic fractions were produced by nonenzymatic browning (Maillard) reactions between proteins and reducing sugars during the ripening process.

O. Miscellaneous Food Allergens

1. Cottonseed

Cottonseed (*Gossypium* species) can be used as a source of edible oil and protein. The presence of a toxic pigment called gossypol limited its use in human foodstuffs until traditional breeding techniques resulted in gossypol-free cultivars.²⁵⁷ This development led to the use of cottonseed protein and oil in food products. Anaphylactic reactions have been documented after consumption of food supplements, candy, and bread containing cottonseed protein or flour.²⁵⁸⁻²⁶⁰ The source of the allergen appears to be the 2S proteins, which are water-soluble albumins.

2. Sesame Seed

Sesame seed (*Sesam indicum*) is an East Indian herb of the Pedaliaceae family. Both the seed and oil obtained from sesame seeds can produce anaphylaxis.²⁶¹ Using ultracentrifugation and immunoblotting techniques, multiple allergenic components have been noted with molecular weights ranging from 8 to 84 kDa.^{199,262} Using immunoblotting techniques and RAST inhibition, several cross-reactive bands have been identified among a variety of foods, including sesame seed, hazelnut, rye grain, kiwi, and poppy seed.²⁶³

3. Poppy Seed

Poppy seed produces systemic reactions on occasion.²⁶⁴ Other than possessing cross-reactivity as mentioned above, little work on its allergenic components has been done.

4. Spices

Several members of the Apiacea family, which includes celery, anise seed, fennel, coriander, and cumin, elicit positive skin tests in celery-allergic individuals, especially those with mugwort pollen and birch pollen sensitivity²⁶⁵ (see Section VII.F). Cross-reactivity between mugwort pollen and coriander has also been demonstrated using RAST inhibition techniques.²⁶⁶ Helbling et al.²⁶⁷ found some cross-reactivity between raw carrots and the apiaceous spices, anise, cumin, and coriander. Immunoblotting studies by this group also demonstrated IgE binding to 17-, 21-, and 23-kDa proteins in anise. IgE binding could only be detected to a 17-kDa protein in cumin and coriander.

5. Mustard

Yellow mustard (*Sinapis alba*) and oriental mustard (*Brassica juncea*) are members of the Brassicaceae family. Allergens from both of these plants have been cloned. *Sin a 1* is a 2S albumin from yellow mustard seed and is the major yellow mustard allergen. It is a seed storage protein consisting of two disulfide-linked polypeptide chains, each with 39 and 88 amino acid residues, respectively.²⁶⁸ The amino acid sequences of both chains have been established. This protein has also been isolated from rapeseed, castor bean, and Brazil nut.²⁶⁸ Further studies have suggested that IgE binding to the protein is conformational, because reduction and carboxyamidomethylation of both polypeptide chains produced a substantial decrease in IgE binding. Specific IgE binding was also decreased when the only tyrosine residue of the protein underwent nitration. This tyrosine residue is located at the 60th position on the 88 amino acid chain. A murine monoclonal antibody which also binds to this site decreased IgE binding by 50%. This role for tyrosine is similar to the importance of tyrosine and IgE binding to the codfish allergen, *Gad c 1*.²⁶⁸

Further studies of the genetics of the *Sin a 1* allergen were conducted using polymerase chain reaction (PCR) technology using nondegenerative oligo primers encoding for both the N- and C-terminal regions. Two nucleotide sequences were identified indicating polymorphism of the

gene. The 2S protein is a member of the Napins family, which is encoded by genes without introns, synthesized as polypeptide precursors, and processed by specific maturation proteases to render the two chains of the mature protein.²⁶⁹

Isolation of the major allergen of the oriental mustard seed, *Bra j* 1, has been reported.²⁷⁰ The oriental mustard is more commonly used in the U.S. and Japan, the yellow mustard being used predominantly in Europe. Table mustard is often a mixture made from the flour obtained from the seeds of both species. The *Bra j* 1 allergen showed microheterogeneity and was immunologically cross-reactive with the major yellow mustard allergen *Sin a* 1. Its molecular weight ranges between 16 and 16.4 kDa. Both of these proteins appear to have an α -helical structure that is resistant to proteolytic and thermal degradation, and are storage proteins with a high glutamine content. Similar proteins have been identified in cabbage and turnip, both of which are members of the same family of plants.

VI. FOODS AS INHALANT ALLERGENS

The ingestion of food proteins with sensitization occurring through the GI tract is the principal cause of food allergic reactions. In some cases, however, individuals become sensitized through a respiratory route, particularly following occupational exposure in the food-processing industry or perhaps as a consequence of sensitization to various pollens that share cross-reactive allergens to foods. It is also hypothesized that food allergens may be incorporated into house dust and become airborne.²⁷¹

Several allergens cause occupational asthma. Milk proteins have been implicated in the development of occupational asthma in two individuals. In one case, inhalation of powdered milk led to the development of nasal symptoms and wheezing.²⁷² The patient also experienced oral itching and burning upon ingestion of milk products. The reactive protein was identified as sodium caseinate. In a second case, Bernal et al.²⁷³ reported an individual who developed occupational asthma as a result of exposure to α -lactalbumin.

Egg proteins have been associated with the "bird-egg syndrome." Individuals with this syn-

drome generally become sensitized through the respiratory route after being exposed to bird serum antigens. Subsequently, they react to ingestion of chicken egg yolk. Although the syndrome is primarily identified in adults,²⁷⁴ it has also been described in children.²⁷⁵ The allergen responsible for this reaction is α -livetins, a serum albumin of 70 kDa. This protein is also present in the yolk of the chicken egg.²⁷⁶

van Toorenbergen et al.²⁷⁷ found a 60-kDa protein in egg yolk to be allergenic in adults who were exposed to bird serum antigens. In contrast, children without bird exposure, but with egg allergy by ingestion, reacted to a 35-kDa protein. These egg yolk proteins were not further characterized.

Organic dusts from legumes and other plants sensitize through the respiratory route. Inhalation of soybean flour has been implicated as a cause of inhalant asthma in a number of individuals.²⁷⁸ Evaluation of the soybean proteins involved in soy bean flour asthma has identified nine proteins with molecular weights ranging from 14.9 to 54.5 kDa.²⁷⁹ Soybean lecithin has also produced occupational asthma in a sensitive individual.²⁸⁰ Epidemic outbreaks of asthma have been attributed to airborne soybean proteins (Barcelona, Spain)²⁸¹ and airborne castor bean dust.²⁸²⁻²⁸⁴ The green bean has been implicated as a cause of occupational asthma in a homemaker preparing and cooking raw green beans.²⁸⁵ Green coffee bean dust has caused occupational asthma²⁸⁶ in coffee processors.

Fish and crustacea are responsible for occupational reactions, particularly in the seafood industry.²⁸⁷ A number of occupational asthma cases have been attributed to the inhalation of airborne proteins from seafood such as Norway lobster (*Nephrops norvegicus*), king crab (*Paralithodes camshaticus*), snow crab (*Chionoecetes opilio*), and squid,²⁰⁴ among others. Sensitization is believed to follow inhalation of proteins in steam generated in processing.

Baker's asthma is observed in individuals working in the baking industry. Proteins of wheat, rye, and barley flour are the most commonly implicated sources of allergens. A number of proteins from these cereal grain flours have been purified,²⁸⁸ and allergens from wheat barley flour²⁸⁹ have been cloned. Blands et al.²⁹⁰ found 53% of

163 bakers examined had allergy to wheat flour, 25% to rye flour and 23% to both. The highest skin-test reactivity was induced by the water-soluble fractions. Forty antigenic components were identified by CIE, some of which may share partial identity due to proteolytic degradation. Franken et al.²⁹¹ demonstrated IgE binding to a 14-kDa protein using immunoblotting techniques. A similar protein was found in rye flour, although there was less IgE binding to the rye than to the wheat material. Pfeil et al.²⁹² found three major wheat allergens in immunoblotting studies with molecular weights of 47, 17, and 15 kDa. Gomez et al.,²⁹³ using immunoblotting techniques, identified the major allergens associated with baker's asthma as members of the α -amylase inhibitor family from wheat endosperm. Consequently, several trypsin α -amylase inhibitor proteins from wheat species (*Triticum durum* Desf cv Agathe,²⁹⁴ *T. aestivum* L. genomes AABBDD cv Chinese Spring,²⁹⁵ *T. turgidum* L. genomes AABB cv Senatoree-Capelli,²⁹⁵ *T. aestivum* cv Timgalen²⁹⁶) have been molecularly cloned and their DNA sequences reported.

Armentia et al.²⁸⁸ purified 11 proteins from wheat and barley flour that comprised a family of α -amylase/trypsin inhibitors. Most of the flour allergens were found in the albumin and globulin fractions. Subsequently, the prominent allergens have been identified, including a monomeric form from wheat and a dimeric form from barley. Mena et al.²⁸⁹ cloned the major barley allergen, a 14.5-kDa barley endosperm protein. This is a glycosylated monomeric member of a multigene family of inhibitors of α -amylase/tryptase from cereal grains. Its deduced amino acid sequence contains 132 residues. Five continuous IgE-binding epitopes have been identified on the wheat amylase inhibitor.⁵⁰

VII. CROSS-REACTIVITY

A. Cow's Milk

Individuals allergic to cow's milk will often have serum IgE antibodies to goat or sheep's milk.^{13,14,21} For the most part this has not been confirmed by DBPCFC, but nine of ten cow's milk-allergic children ingesting goat's milk had

reactions similar to those provoked by cows' milk (H. A. Sampson, personal communication).

B. Fish

The degree of allergic cross-reactivity between different fish species varies widely between individuals.²⁹⁷ Several studies have attempted to assess the reactivity of fish-allergic subjects to different species of fish. de Martino and associates²⁹⁸ performed skin tests in 20 cod-allergic children with 17 different species of fish. Eel extract was the most reactive (85%), although parents in every case indicated that their children had never eaten eel. Pascual et al.²⁹⁹ studied sole, whiff, witch, hake, cod, and albacore skin-test reactivity in 79 children with fish hypersensitivity. All of the subjects reacted to the six types of fish. It is apparent that skin testing alone cannot adequately predict clinical cross-reactivity for fish species.

Research using DBPCFC and other tests^{56,298} in fish-allergic children has shown that subjects are not uniformly sensitive to all species. Bernhisel-Broadbent et al.⁸³ found variable reactions to DBPCFC in that positive oral challenges resulted with only one fish species in seven subjects, two fish species in one subject, and three fish species in two subjects. Skin tests in these subjects were positive to all ten fish species evaluated in 8 of the 11 subjects. The three remaining subjects had positive skin tests to at least two species. However, among nine fish-allergic subjects in one study,⁶⁰ only one did not react to oral challenge to the type of fish they reacted to in ST.

The *in vitro* cross-reactivity of fish extracts has also been investigated using SDS-PAGE and immunoblotting. One study⁸³ examined extracts from nine species of raw and cooked fish. With the exception of raw and cooked tuna, all extracts had a prominent band in SDS-PAGE at 13 kDa that appeared to be analogous to the major codfish allergen, *Gad c 1*. Immunoblotting results using serum from fish-allergic individuals showed the most pronounced IgE binding was to this 13-kDa band. Tuna did not appear to contain this 13-kDa protein, possibly explaining why tuna does not cross-react extensively with other species.

Immunoblotting further indicated IgE binding to extracts of fish to which the subjects had no

clinical sensitivity as determined by oral challenge. In ELISA inhibition assays, the concentration of fish extract required to achieve 50% inhibition of specific IgE binding was similar for fish to which patients were clinically allergic and those to which they were not clinically allergic.⁸³

RAST inhibition studies have shown a variable amount of cross-reactivity among species of fish. Tuna or albacore has been the least effective inhibitor in RAST studies,^{298,300} showing the fewest reactions in skin tests.²⁹⁹ Helbling et al.³⁰⁰ found that tuna extract inhibited a trout RAST by 45% and a mackerel RAST by only 26%. When RAST inhibition was performed with *Gad c 1* against hake, whiff, sole, witch, and albacore, it was observed that although parvalbumin is important as an allergen in cod, it is not as important in another gadiform, hake, and even less so for other types of fish.²⁹⁹

Helbling et al.³⁰⁰ found that many individuals with a history of adverse reactions to fish also report adverse reactions to crustacea, mainly shrimp. However, RAST inhibition studies showed that fish extracts (salmon, anchovy, tuna, trout, pollock, and mackerel) could not inhibit a shrimp RAST, indicating that the individuals probably had multiple food allergies.

C. Crustacea and Mollusks

1. Crustacea

Histories of IgE hypersensitivity to multiple types of shellfish are frequently reported. Shrimp-allergic individuals react to other species of crustacea. They exhibit positive skin tests and RAST to other crustaceans.^{98,301} Studies using RAST inhibition assays and additional immunochemical techniques indicate common antigenic/allergenic epitopes in shrimp and other crustacea.^{101,302,303} Halmeupuro et al.¹⁰¹ found that five of six IgE-binding CIE precipitins from crawfish share partial immunologic identity to precipitins in spiny lobster, white shrimp, and blue crab extracts, and that three of four spiny lobster IgE-binding CIE precipitins had partial immunological identity with crawfish, white shrimp, and blue crab extracts. In addition, extracts from shrimp, blue crab, and crawfish all inhibit *Pen a 1* RAST to a similar

extent.⁵ *Pen a 1*-reactive IgE as well as *Pen a 1*-specific monoclonal antibodies can detect a 36-kDa protein present in crawfish, blue crab, and spiny lobster, perhaps indicating the presence of common IgE-binding epitopes.⁹³ The presence of IgE to unique and shared class allergens may explain an individual's clinical sensitivity to one or more members of the crustacea.

2. Mollusks

Although mollusks are much less allergenic than crustacea, a study of cross-reactivity of oyster and crustacea extracts indicated some common antigenic/allergenic epitopes based on RAST inhibition studies. Shrimp, blue crab, spiny lobster, and crawfish were all highly cross-reactive with oyster.²⁰⁷

Squid-allergic patients in one study also experienced symptoms after ingesting shrimp, and demonstrated strong positive skin-test reactions to boiled squid extracts and various commercial crustacea extracts. Specific IgE-binding inhibition studies showed cross-reactivity between shrimp, lobster, crab, oyster extracts, and boiled squid extract, although cross-reactivity was not demonstrated between squid and octopus (another cephalopod), nor squid and round clam, mussel, or other mollusks.²⁰⁵

Limpet-sensitive subjects in one study could consume squid, clam, cockle, octopus, snail, oyster, shrimp, lobster, and crawfish without adverse reaction.²⁰⁴ Cross-allergenicity between grand keyhole limpet, abalone, and keyhole limpet hemocyanin was demonstrated using sera from limpet-sensitive subjects in a raw limpet RAST.²⁰⁸

3. Seafood/Insect Cross-Reactions

Crustacea and mollusks may share allergenic determinants with some species of arthropods, as phylogenetic conservation may occur. Shared allergenic determinants have been demonstrated between the major shrimp allergen and fruit fly (*Drosophila*) antigens. *Pen a 1* and *Pen i 1* share 86 to 87% sequence similarity with fruit fly tropomyosin.^{5,92} Patients allergic to Chironomids (nonbiting midges) often demonstrate positive skin

tests to crustacea. Chironomid extracts inhibit shrimp RAST, and vice versa,³⁰⁴ although other researchers report low cross-reactivity using this assay method.³⁰⁵

Witteman et al.³⁰⁶ found that a monoclonal antibody raised against *Dermatophagoides pteronyssinus* (dust mite) extract cross-reacted with a shrimp allergen, presumably *Pen a 1*, and also reacted with extracts of chironomids, mosquitos, and cockroaches. The authors concluded that tropomyosin is involved in cross-reactions between mite, shrimp, and insects in shrimp-allergic individuals. Aki et al.³⁰⁷ recently cloned a recombinant mite (*Dermatophagoides farinae*) protein that is a major allergen for the 31 mite-allergic subjects in the study. The deduced amino acid sequence from a cDNA fragment had 76% homology to fruit fly tropomyosin and was coincident with partial sequence fragments of purified native mite tropomyosin. The deduced amino acid sequence had 11 of 17 and 6 of 9 identical amino acids, respectively, to two IgE-binding segments of shrimp tropomyosin (shrimp residues 50 to 66 and 163 to 161). It was concluded that this new allergen was mite tropomyosin. Some snail-sensitive serum samples show positive IgE binding to mite, and inhibition of snail RAST was elicited by *Dermatophagoides* extract (14 to 66% inhibition).²⁰³ Specific IgE binding for another gastropod, limpet, was significantly inhibited by *D. pteronyssinus*.²⁰⁵ However, this same group²⁰⁴ reported no significant inhibition of squid-specific IgE by *D. pteronyssinus* or cockroach extracts.

It is believed that the invertebrate hemoglobin (erythrocrucorin) molecule might be involved in the reported cross-reactivity of caddis fly and mollusk.³⁰⁸ It is a potent allergen for chironomid-sensitive individuals, and serum from caddis-sensitive patients in one study reacted with a component of similar molecular weight in mollusk and bee venom extracts.³⁰⁸

D. Legumes

Extensive *in vitro* allergenic cross-reactivity in the legume family has been documented. For example, Barnett et al.³⁰⁹ found that 25% of sera from legume-sensitive patients reacted strongly

with peanut, garden pea, soybean, and chick pea extracts in RAST studies. Bernhisel-Broadbent et al.,³¹⁰ using immunoblotting and dot-blotting methods, found extensive *in vitro* cross-allergenicity with peanuts, soybeans, peas, and lima beans in 57 of 62 patients with legume sensitivity. However, an earlier study by the same researchers found that clinical and *in vitro* results did not correlate in evaluating allergenic cross-reactivity in the legume family; 59% of skin-test-positive patients reacted to oral challenge, and only 2.8% reacted in oral challenge to more than one legume. Peanut hypersensitivity accounted for 31% of the positive reactions, soybean 23%, and pea 5%. Green and lima beans gave no positive oral challenge reactions.⁶

The interrelationships of proteins in each legume as subunits or degradation products of one another, or as a result of influence of posttranslational glycosylation on molecular weight, are not understood. The cross-reacting sequences or epitopes in these legumes which cause clinical and *in vitro* reactions are also not known.

The studies reviewed above reveal that although IgE antibodies can cross-react with proteins in related foods and cause positive skin-test and RAST results, the clinical manifestations of such cross-reactivity are apparently rare. However, peanut-allergic individuals can develop severe IgE-mediated reactions to taupeh (sprouted small green beans), a typical component of egg rolls.³¹¹ A case of lupin-fortified pasta causing a reaction in a peanut-sensitive child has recently been reported.²¹⁶ The subjects experiencing a positive skin-test reaction to the lupin extract also reported a history of adverse reactions to green peas. Therefore, while it appears from the literature that clinical sensitivity to one legume does not always warrant elimination of all legumes from the diet in most cases, this should be evaluated on an individual basis.

E. Cereal Grains

Molecular techniques have led to increasing insights into the cross-reactivity of cereal grain proteins. The major allergens in baker's asthma have been identified as a group of α -amylase inhibitor proteins. α -Amylase inhibitors from

barley and wheat share 37% amino acid sequence homology.²⁹⁴ The deduced amino acid sequence homology of rice α -amylase/trypsin inhibitor and wheat is 40%, and between rice and barley it is 20%.²³² A monoclonal antibody directed against a 14-kDa wheat α -amylase inhibitor also recognizes a similar component in rye flour,²⁹¹ suggesting shared epitopes. Franken et al.³¹² confirmed the importance of α -amylase inhibitor as a major allergen of wheat. The extent of IgE cross-reactivity and clinical sensitivity to cereal grains has been explored recently.³¹³ Approximately 25% of wheat-allergic children reacted to another cereal grain (barley, oat, or rye).³¹³

F. Tree Nuts, Vegetables, Fruits, and Pollen Allergy

It is well documented that individuals with tree pollen allergies (birch, alder, hazel, hornbeam, and oak) also frequently suffer intolerance to nuts, fruits, and vegetables.³¹⁴⁻³¹⁸ In Northern Europe, up to 70% of patients with birch pollen allergy demonstrate intolerance to these foods, in contrast to 19% of patients not allergic to birch pollen.³¹⁴ Apples^{314,319} and hazelnuts^{315,316} are the most common offenders, although reactions to botanically unrelated fruits, such as kiwi, have been reported.^{263,320} Individuals who suffer from grass or weed pollen allergies often show sensitivity to carrot, celery, potato, and some spices,^{266,321,322} and many individuals with ragweed allergy report intolerance to fruits in the gourd family, and banana, a nongourd.^{244,323} Most of these cross-reactions manifest as OAS, but a certain percentage of individuals experience systemic symptoms.³²⁴ Although many of these cross-reacting allergens are inactivated by cooking, the cross-reacting allergens of tree nuts are heat stable.

T-cell epitope mapping has been investigated for birch, hazel nut, and alder pollens. The specific amino acid sequences resulting in sensitivity, however, have not been identified. Exposure to tree pollens can lead to the development of IgE antibodies that recognize epitopes on a variety of food proteins containing similar amino acid sequences. The primary sensitization is to the pollen, and not to the food.

1. *Bet v 1*

Bet v 1 is the major birch pollen allergen and is the most important allergen for birch pollen-food cross-reactions. It is a 17-kDa cytosolic protein whose cDNA sequence³²⁵ is highly conserved in dicotyledonous plants,³²⁶ and has homology to a family of mRNAs that are induced in somatic tissues of some higher plants by pathogen infection.³²⁷

Using immunoblotting techniques, Ebner et al.²³⁵ found that serum from 81 of 83 birch pollen-allergic subjects showed IgE-binding to *Bet v 1* and also apple (double bands) in the molecular weight range of 17 to 18 kDa. Complete inhibition of IgE binding to these apple allergens was observed after preincubation of the sera with *Bet v 1*.^{235,324} In addition, birch pollen and apple allergen-encoding nucleic acids cross-hybridized in Northern blots.²³⁵ Other researchers have found that the 18- and 31-kDa apple allergens share about 50% identity with *Bet v 1*, in addition to disease resistance genes in other plants.²³⁸ Immunoblotting studies have also revealed that the 18-kDa hazelnut allergen shares IgE-binding similarities with *Bet v 1*, as binding of IgE from patients with hazelnut allergy to the 18 kDa allergen could be blocked by preincubation of sera with recombinant *Bet v 1*.¹⁹⁵

In a study of 43 patients with birch pollen allergy and a history of intolerance to fruits, two groups were discovered; those whose serum reacted with a 20-kDa protein in birch pollen and fruits, and those who reacted to an 18-kDa protein in birch pollen, fruits, grass pollen, and potato.³¹⁵ RAST inhibition and immunoblot analysis indicated that there was antigenic similarity between the allergens of birch pollen and fruits, in particular apple, cherry, peach, and pear, which are all members of the Rosaceae family.

No biological properties of *Bet v 1* are yet known. It 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 birch-allergic patient serum.³²⁸

2. *Profilin* (*Bet v 2*)

Profilin is involved in birch pollen-fruit sensitivity, but also plays a wider role in cross-

reactivity with other foods.³²⁹ In contrast to other major allergens in botanically related species, profilins represent important cross-sensitizing allergens responsible for allergic symptoms,³²⁶ as approximately 20% of pollen-allergic patients show IgE binding to allergen.³³⁰ *Bet v 2*, a birch pollen protein identified as a profilin with a molecular weight of 14 kDa, has also been identified as a cross-reactive allergen in a variety of fruits and vegetables.³²⁹ Profilins, which are highly conserved ubiquitous proteins found in almost all eukaryotic organisms, control actin polymerization. They have been isolated from a variety of pollens.

Pollen profilins appear to share cross-reactivity with a number of foods. Ebner et al.³²⁴ found in immunoblotting studies that preincubation of birch pollen-allergic serum with recombinant *Bet v 2* reduced cross-reactive IgE binding to pear, celery, carrot, and potato proteins. Vallier et al.²⁴¹ found that of 63 mugwort-allergic subjects, 18 had specific IgE to a 15-kDa protein in mugwort and to two birch pollen proteins at 14- and 16-kDa. Of the 36 that were positive for celery RAST, 18 had specific IgE binding to two celery proteins at approximately 15 kDa. These 18 sera also showed IgE-binding to the 15-kDa protein in mugwort and the 14- and 16-kDa birch pollen proteins. In a later study, the same researchers purified the 15-kDa celery allergen and showed that preincubation with it prevented IgE binding to the 15-kDa allergen present in mugwort and birch pollen. In addition, IgE from three *Bet v 2*-allergic subjects bound to the purified 15 kDa celery allergen, and this binding could be prevented by preincubation with recombinant *Bet v 2*.²⁴⁰

Raw maple syrup induced angioedema of the tongue in an individual who had tree pollen sensitivity.³³¹ It was theorized that a heat-labile allergen, possibly profilin, accounted for this reaction.

3. Cross-Reacting Carbohydrate Determinants

Besides *Bet v 1* and profilin, carbohydrate structures on glycoproteins are also involved in pollen-vegetable cross-reactivity, but their clinical relevance is doubtful.^{321,332}

G. Cross-Reactions between Latex and Foods

With the increase in latex IgE-mediated allergy noted in the last 10 years, cross-reactions of latex with various foods have been reported. Blanco et al.³³³ reported that among 25 patients with latex sensitivity, 7 experienced systemic reactions to avocado, 4 to chestnut, 5 to bananas, and 2 each to kiwi, papaya, and figs. RAST inhibition analyses showed cross-reactivity with latex and avocado, chestnut, and banana; therefore, latex shows shared antigenic determinants with other fruits that are not botanically related. Papaya, fig, and kiwi showed a lower association in the RAST inhibition tests.

Anibarro et al.³³⁴ reported a latex-allergic subject who reacted to an oral challenge of chestnut, manifested by urticaria and angioedema. Immunoblotting using this patient's serum showed strong IgE binding to a band at 14 kDa and several in the range of 25-30 kDa less strongly in addition to binding to latex proteins in these same molecular weight ranges.³³⁵ In another study, 7 out of 10 latex-allergic subjects experienced anaphylaxis to avocado. These individuals also reported allergies to banana, chestnut, kiwi, and papaya. RAST inhibition suggested common epitopes among avocado, latex, chestnut, and banana.³³⁵ Latex-allergic subjects have also reported anaphylaxis to banana or chestnuts.³³⁶ In addition to the cross-reactivities noted above, latex cross-reaction has been associated with celery, passion fruit, and peach.³³⁷

VIII. RECOMBINANT ALLERGENS AND IMPLICATIONS FOR FOOD TECHNOLOGY

A. Introduction: Advantages and Disadvantages of Recombinant Allergens

The dramatic increase in knowledge of allergen structure since 1983 is due primarily to the successful application of recombinant DNA technologies. The molecular techniques have helped define primary antigenic structure, as well as B- and T-cell epitopes from a variety of allergenic proteins.³³⁸

These technologies have revolutionized efforts to develop reliable sources of allergenic material for laboratory study and clinical use.

It is now possible to create a reliable, reproducible allergen source that has batch-to-batch consistency and is available in stable, large quantities, a remarkable achievement.³³⁹ This should result in improved allergenic extracts for diagnosis and immunotherapy. 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. An additional advantage of recombinant allergens is that they may be added to natural allergenic extracts to improve the quality of current extracts.³⁴⁰ However, another method would be to use a few (two to four) recombinant allergens cloned from a given source for diagnostic purposes.

Perhaps the greatest value of biotechnology, however, will be to provide recombinant allergens to be used instead of allergens that are present in natural products in limited quantities, such as vespid venoms. Recombinant allergens will provide better tools for allergen standardization, structural investigation, and T- and B-cell epitope identification. Such studies could yield new concepts in immunotherapeutic approaches in the treatment of allergic diseases. Recombinant DNA technology will also be useful in analyzing cross-reactivity between allergens. Such techniques have demonstrated that a number of pollen allergens have inter- and intraspecies and intergenic variability and cross-reactivity.³⁴¹

A major potential disadvantage of recombinant allergens is that their IgE binding affinity may be less than their native counterparts. Also, because many allergenic extracts contain multiple allergens, substantial resources could be required to prepare all recombinant allergens in a specific food, if that was necessary. Finally, the safety and efficacy of recombinant allergens are major concerns and are issues that must be considered prior to use in humans.

B. Requirements of Recombinant Allergens

A major objective for any recombinant allergen produced is that it be comparable to its natu-

ral counterpart. Recombinant allergens used *in vivo* should be adequately analyzed for the following properties: the purity of the protein must be defined as described in the World Health Organization (WHO) document on recombinant proteins.³⁴² Recombinant allergen samples must be demonstrated to lack toxicity using animal model systems (see "Principles and Characteristics of Food Allergens" by Taylor and Lehrer in this issue). These molecules should be stable and the half-life should be assessed with *in vitro* assays normally used in allergen standardization.

Because a number of allergens possess inherent biological activity, such as enzymes or lectins, their recombinant counterpart may possess these activities as well. If there exists undesired traits, such as toxicity, these traits may have to be removed. Site-directed mutagenesis could be used to accomplish this goal. Finally, it is critical that recombinant allergens have similar IgE-binding activity to their natural counterparts.³⁴⁰

C. Molecularly Cloned Allergens

A host of allergens have been cloned in the past few years. Emphasis has been directed at inhalant allergens (for review, see Stewart³⁴³ and Scheiner³⁴⁴). Among the first allergens to be cloned was the major dust mite allergen, *Der p 1*.³⁴⁵ Clones were detected by rabbit anti-sera and oligonucleotide probes. In the expression system used, however, IgE binding to the expressed protein could not be readily demonstrated. This suggested that posttranslational events of conformational structure are necessary for IgE binding. Other systems have shown that recombinant allergens can be detected using IgE binding for identification of relevant clones.

A number of nonfood inhalant allergens have been identified including numerous grass pollen antigens.³⁴⁶ The sequence homology of birch pollen allergens with food such as hazelnut and the profilins was discussed in Section VII.F. Several ragweed pollen allergens have also been cloned. The use of PCR-based technology has led to the identification of a full-length gene that encodes for the major cat allergen, *Fel d 1*. The information derived from these studies has led to the identification of T-cell epitopes in the *Fel d 1*

molecule. Currently, synthetic peptides based on these amino acid sequences are under evaluation for immunotherapy to reduce allergic reactions in cat-allergic individuals. Fungal allergens have also been cloned from *Alternaria* and *Aspergillus*. The *Aspergillus* protein is one of the few in which the recombinant allergen has been shown to produce a positive skin test in allergic subjects.³⁴⁷ Molecular cloning of stinging insect venom proteins has also been reported. The sequence homologies of some of these proteins with plant-derived proteins, and cockroach allergens with food proteins, have been discussed.

Recombinant food allergens have also been produced. One peanut allergen, *Ara h 2*, has been produced using recombinant technology.³⁴⁸ Genes coding for major allergenic proteins from rice seeds have been reported.^{231,232} Several cDNA and genomic clones were prepared. The sequence analysis of these indicated a deduced amino acid sequence that has homology to barley trypsin inhibitor and wheat α -amylase inhibitor. The clones were detected by oligonucleotide probes based on the amino acid sequence of the purified 16-kDa major rice allergen. However, neither of the studies examined the ability of the molecularly cloned protein to bind IgE from human serum. Gonzalez de la Pena et al.²⁶⁹ described the cloning and expression of a major allergen from yellow mustard seed (*Sin a I*). Cloning was carried out by PCR using nondegenerate oligo primers and coding for the N- and C-terminal regions of the mature protein. Nucleotide sequence analysis indicated that there was polymorphism, suggesting the existence of multiple isoforms of the allergen. However, IgE-binding experiments were not conducted.

A list of allergens cloned and sequenced to date is shown in "Assessment of the Allergenic Potential of Foods Derived from Genetically Engineered Crop Plants" by Dean D. Metcalfe et al. in this issue (see Tables 1 and 2). This list will undoubtedly increase with time and will provide invaluable information on allergens and facilitate new therapies for the treatment of allergic diseases.

ACKNOWLEDGMENTS

The authors thank Samuel B. Lehrer, Ph.D., for his contributions to this article.

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