

13 Food Allergens

Ricki M. Helm

*Research Support Center, Office of Vice Chancellor for Academic Affairs and Research Administration,
University of Arkansas for Medical Sciences, Little Rock, Arkansas, U.S.A.*

A. Wesley Burks

Pediatric Allergy and Immunology, Duke University Medical Center, Durham, North Carolina, U.S.A.

INTRODUCTION

Food allergy is an immune system-mediated adverse reaction to food proteins that can affect multiple organs. Organ/systemic involvement includes cutaneous, gastrointestinal, respiratory, oral, and generalized reactions, including potentially lethal anaphylaxis (1,2). Reports of anaphylactic episodes are updated in a registry of the American Academy of Allergy Asthma & Immunology and the Food Allergy and Anaphylaxis Network (3). This registry has been maintained continuously to represent a systematic, although incomplete, accounting of fatal food-induced allergic reactions in the United States.

Similar reports from the United Kingdom stress the importance of identifying and reporting fatal food reactions (4). Risk factors include a family history of atopic disorders with environmental factors seemingly modulating the expression of food allergies (5,6). In young children, the most common food allergies are cow's milk, egg, peanut, wheat, soy, tree nuts, fish, and shellfish. Early childhood allergies to milk, egg, soy, and wheat usually resolve by school age (7). Adult food allergies primarily include shellfish, peanut, tree nuts, and fish. However, individuals with known food allergies experience food-induced anaphylaxis primarily because they are unaware that the food they eat contains the allergen to which they are allergic (8). In the British Anaphylaxis Campaign, foods were implicated in 112 of 126 reported cases in children (aged 1–15.9 years) and adults (aged 16–72 years); 14 of these reactions were to unidentified substances. There was a similar distribution among the two groups for common allergenic foods; however, adults reported more reactions to more unusual but internationally well-recognized allergenic foods (citrus foods, peas, bananas, kiwi, sesame, mustard, and food additives) (9). In a multicenter airway research collaboration of 21 North American emergency departments, a chart review of a randomly selected cohort of 678 out of 5396 cases, documentation of a known food allergy was identified in 41% of patients using food allergy or ICD-9 codes (10). Biphasic reactions, a recurrence of symptoms after resolution of the initial presentation, occur in up to 25% of fatal or near-fatal anaphylactic food reactions (11).

Absorption of dietary protein is important in the development and elicitation of food-induced allergic reactions. Investigations by Dirks et al. (12) were performed to determine the absorption and kinetics of allergenic peanut proteins and a possible local uptake from the oral cavity in healthy adults. In brief, donor mononuclear cells containing basophils from nonallergic individuals were stripped of cell-bound IgE, subsequently sensitized by adding serum IgE from double-blind, placebo-controlled food challenged (DBPCFC) positive peanut-allergic subjects and challenged with peanut allergen to establish a histamine-releasing standard curve. Individuals with negative case histories for peanut allergy, negative skin prick test responses, negative specific IgE assay results for peanuts, soy, and pollen, and fasted eight hours were asked to chew peanuts for two minutes and spit out the contents without swallowing. Plasma samples were collected at various time points and used to determine histamine-releasing capacity of the IgE-sensitized basophils. The histamine released was determined by fluorometric analyses and expressed as the percentage of the total cellular histamine content. Histamine release greater than 10% above negative serum controls was considered positive. Assay sensitivity was determined to be 5 pg of peanut protein per milliliter of serum. Results showed that peanut allergens with biologic activity can be measured in the plasma of subjects as early as 10 minutes after chewing peanuts and spitting

them out without swallowing. These findings demonstrate allergen absorption from the buccal mucosa, which is regarded to explain the early onset of many systemic reactions (12).

In another sensitization scheme, Strid et al. (13) demonstrated that the epicutaneous exposure to peanut proteins in a murine model prevents oral tolerance and enhances allergic sensitization. In experiments to determine transepithelial transport of peanut proteins in a murine model, ileal loops containing Peyer's patches were filled with solutions containing digested peanuts. Combined immunohistochemical analysis reveals both peanut allergens and M cells at the highly inductive Peyer's patch sites of the normal, presensitized mucosal immune system (14).

In a discussion of the classification of digestion resistance of food allergens, Jensen-Jarolim and Untersmayr (15) reported that both the quality and extent of clinical reactivity to food allergens correlate with the following classification of food allergens. Class 1 food allergens both sensitize and trigger allergic reactions via the oral route because they are quickly absorbed and distributed to the systemic immune system. However, class 2 food allergens do not sensitize orally because they are easily digested into small peptides and lose their sensitization potential. They elicit allergy indirectly when ingested by the fact that they cross-react with other allergens, typically inhalant allergens. Examples include birch pollen, Bet v 1, and its numerous homologues in apple, Mal d 1; celery, Api g 1; and carrot, Dau c 1.

Not all proteins in foods are allergenic of the more than 70 foods that cause food allergies (16). Historically, major food allergens are water-soluble glycoproteins with molecular weights ranging from 10 to 60 kDa. However, structural characteristics are important for a protein's allergenicity, and many food allergens occur naturally as dimers or trimers with molecular weights of 150 to 200 kDa (17). These oligomeric forms might have a higher allergenic potential than monomers because larger molecules have additional epitopes for IgE-mediated histamine release. For example, inhibition experiments demonstrate extensive IgE cross-reactivity of recombinant mugwort profilin and profilin from various pollens and food extracts (18). The parvalbumin allergen, Gad m 1, of the Atlantic cod (*Gadus morhua*) forms oligomers consisting of multiples of a single 12.5-kDa protein, in native (gel filtration) and under reducing conditions that does not completely dissociate the oligomeric structure consisting of 24, 38, and 51 kDa (19). This is considered a novel finding as no aggregation of fish parvalbumins is previously described, and work to determine whether oligomerization of this protein has an influence on allergenicity is underway.

Using size exclusion chromatography, Ara h 1, a major peanut allergen, appears to exist in an oligomeric structure rather than its stable trimeric state (20). Hydrophobic interaction chromatography causes the oligomers to partly dissociate into trimers, which leads to a change in tertiary structure of the monomeric subunits of the allergen. Monomers of Ara h 1 oligomers have a more compact tertiary structure compared with monomers in Ara h 1 trimers. Thus, as indicated in both Gad m 1 and Ara h 1, structural characteristics of oligomerization may be important for protein allergenicity.

There are no known unique biochemical or immunochemical characteristics for food allergens versus other allergens. Comparisons of primary structure (amino acid sequences) of allergenic proteins do not reveal typical patterns that could be related to allergenicity. Food allergens tend to be resistant to the usual food processing and preparation conditions and are comparatively resistant to heat and acid treatment, proteolysis, and digestion. For example, treatment of food allergens with acid concentrations simulating stomach acid typically has little effect on the specific IgE binding of the class 1 food allergens. There are, however, important exceptions, such as the major IgE-binding allergens in fresh fruits and some vegetables (class 2 allergens), which are affected by these physical conditions, resulting in dissociation into smaller peptides and loss of allergenicity. For example, Mal d 1, Api g 1, and Dau c 1 are rapidly digested (15).

IgE immunogenicity is determined by factors that can be unrelated to the primary structure (amino acid sequence) of the protein and must take into account secondary and tertiary structure (folding, disulfide bonding, and oligomerization) and the food source from which the allergen has been characterized, e.g., Ara h 1 from peanuts. Additionally, the way allergens enter the body, epicutaneously or by ingestion, may have a significant impact on the immune response. Essentially, the protein must induce B cells to produce IgE to be an allergen and on reexposure induce an allergic response in sensitized individuals.

Table 1 Plant and Animal Food Allergens

Plant food allergens	
Prolamin superfamily	2S albumins, nsLTP
2S albumins	Ber e 1 (walnut), Ses i 2 (sesame)
Nonspecific lipid transfer proteins	Pru av 2 (cherry), Mal d 2 (apple)
Cereal α -amylase/trypsin inhibitors	Tri a 19 (wheat), Sec c 20 (rye), STI (soybean)
Cupin superfamily	7S and 11S seed storage proteins
Vicilins	Ara h 1 (peanut), Jug r 2 (walnut)
Legumes	Ara h 3/4 (peanut), Cor a 8 (hazelnut)
Profilin family	Api g 4 (celery), Pru av 1 (cherry)
Bet v 1 family	Gly m 4 (soybean), Ara h 8 (peanut), Pru av 1 (cherry)
Others	Gly m Bd 30K (soybean), Act c 1 (kiwi)
Animal food allergens	
Mammalian milk	α -lactalbumin, β -lactoglobulin, casein
Egg	Gal d 1 (ovomucoid, hen's egg)
Seafood	Pen a 1 (tropomyosins, shrimp), Gad c 1 (calcium-binding parvalbumins, cod)

Source: From Refs. 21,25,26.

TAXONOMY OF FOOD ALLERGENS

Food allergens, found in plants and animals, are classified as to their biologic function or protein family group (21). Plant food allergens are contained in 31 of 8296 protein families (22) with the most important animal food allergens present in milk, egg, and seafoods. Examples of animal groups include birds (chicken and duck), crustaceans (crab and lobster), and red meats (beef and veal). Examples of plant groups include the apple family (apple and pear), grass family (corn and wheat), legume family (lentil and peanut), and walnut family (black walnut and pecan). Allergy, because of cross-reacting allergens, to one member of some food groups may result in a variable degree of clinical reactivity to other members of the same group.

Food allergens are named using the first three letters of the genus, followed by a single letter for the species and a number indicating the chronologic order of allergen purification (23). Standardization of food allergen nomenclature was proposed by the World Allergy Organization and refers to food hypersensitivity reactions as "food allergy" only when immunologic mechanisms are demonstrated. "The term *hypersensitivity* should be used to describe *objectively reproducible symptoms or signs initiated by exposure to a defined stimulus at a dose tolerated by normal persons*. An allergen is an antigen causing allergic disease. If IgE is involved in a food-related reaction, the term IgE-mediated food allergy is appropriate." As a consequence, entities within the field of environmental medicine, such as multiple chemical sensitivity, multisymptomatic reactions (amalgam in tooth fillings), and electrical waves do not fulfill these criteria. All other reactions are described as "nonallergic food hypersensitivity" (24). How nomenclature and structural biology play a crucial role in defining allergens for research studies and for the development of new clinical products is reviewed in Ref. 25. An abbreviated listing of the families and examples of plant and animal proteins is presented in Table 1. A format with listed allergens is also available in the Allergome e-mail Newsletter (Table 2).

MAJOR AND MINOR FOOD ALLERGENS

The most common foods that cause IgE-mediated reactions in childhood are cow's milk, eggs, peanut, soybean, wheat, fish, and tree nuts (Table 3). Approximately 80% of these reactions are secondary to milk, eggs, and peanut alone. In adulthood, the most common food allergens are peanut, tree nuts, fish, and shellfish. Worldwide, there are some differences in which foods cause problems in both children and adults primarily based on different diets (29).

Cow's Milk

A number of milk proteins are allergenic and patients react to various cow's milk proteins by either skin prick tests or challenge. Caseins and β -lactoglobulin are the major allergens in cow's

Table 2 AllFam Allergen Family Chart

Protein family name	Number of allergens
Prolamin superfamily	56
Profilins	44
EF hand domain	32
Tropomyosins	27
Cupin superfamily	25
Expansins, C-terminal domain	23
Bet v 1-related proteins	22
PR-1 proteins	21
Lipocalins	20
Subtilisin-like serine proteases	17

Source: From Refs. 27,28.

Table 3 Major Food Allergens in Children and Adults

Children	Adults
Milk	Peanut
Egg	Tree nuts
Peanut	Fish
Soybean	Shellfish
Wheat	
Fish	
Tree nuts	

milk. Casein, a phosphoprotein, is the major protein of bovine milk that exists in equilibrium of soluble and complex colloidal aggregates (micelles). Its heterogeneity has long been recognized as consisting of α -, β -, γ -caseins (75%, 22%, and 3%, respectively). The major α - and β -caseins have molecular weights of approximately 23 kDa, and there are several genetic variants of each. β -Lactoglobulin (17 kDa), the most abundant whey protein, also has several genetic variants. α -Lactalbumin (14 kDa) and bovine serum albumin (67 kDa), both whey proteins, are minor cow's milk allergens. The IgE-binding epitopes on the milk caseins (30), lactalbumin and lactoglobulin, are identified (31). So too are specific IgE-binding epitopes that may differentiate between patients with persistent and transient cow's milk allergy (32,33).

Confusion can arise in the determination of cow's milk allergy because of the different forms of cow's milk used in challenges, e.g., liquid cow's milk, nonfat dry milk, and infant formula. Similarly, reported incidences of inadvertent or unexpected exposure to different milk proteins, e.g., casein in sausage equivalent to 60 mg of casein, have led to fatal anaphylaxis (34). Reliable analytical results for milk allergens (casein, lactalbumin, and lactoglobulin) in nondairy allergic reactions are needed for them to be temporally associated with milk allergy. Data from Morisset et al. (35) suggest that in patients allergic to egg, peanut, and milk, detection tests should show a sensitivity of 10 parts per million (ppm) for egg, 24 ppm for peanut, and 30 ppm for cow's milk, respectively, when 110 g of a food source is consumed. Wal's (36) update on the biochemistry and immunochemistry of milk proteins indicates that no single allergen or structure accounts for milk allergenicity or provides for a predicted allergic response. The great variability in the polysensitization and IgE responses to cow's milk and potential immunologic cross-reactions to milk of other species, such as buffalo's, goat's, ewe's, and camel's milk, will vary according to the characteristics of the population studied. Any milk product that contains native or denatured milk proteins or fragments derived thereof may trigger an allergic reaction, even those present in nondairy foods.

Egg

Several studies have identified the major egg allergens (37,38). Ovomucoid (Gal d 1), a glycoprotein with a molecular weight of 28 kDa and an acidic isoelectric point, is the major egg allergen (39). In a study of 18 children with egg allergy, ovomucoid was found to be a more potent allergen than purified ovalbumin as determined by skin prick test and radioallergosorbent test (RAST). While previous studies indicate that ovalbumin was the major egg

allergen
this di
weight
varian
ovomu
molecu
iron-bi
that in
minor
Additi
particu
epitope
IgG-bin

F
ovomu
were id
protein
yolk pr
ovomu
was sta
differen
differen

A
presche
nut all
the all
allerge
inhibit
protein

Peanut

Peanut
history
the recu
higher
(44). Pe
(saline
conarac
as a mo
monom
into tw

He
peanut
the adv
systema
number
glycopr
linked i
relative
acid sec
membe
acidic is
sequenc
glycinin
patients

A
5X-38 ce
soluble

allergen, this study demonstrates ovomucoid contamination of the ovalbumin, accounting for this discrepancy. Ovalbumin (Gal d 2) is a monomeric phosphoglycoprotein with a molecular weight of 43 to 45 kDa and an acidic isoelectric point. Purified ovalbumin has three primary variants, A₁, A₂, and A₃. It is difficult to determine the exact role of this allergen because of ovomucoid contamination of ovalbumin (39). Ovotransferrin (Gal d 3) or conalbumin has a molecular weight of 77 kDa and an acidic isoelectric point. It has antimicrobial activity and iron-binding properties. Lysozyme (Gal d 4) is a lower molecular weight allergen (14.3 kDa) that in some studies appears to be a major allergen, but in other studies is thought to be a minor allergen. Other minor allergens in eggs include apovitellin, ovomucin, and phosvitin. Additional studies show that the carbohydrate portion of the glycoproteins in eggs, particularly in ovomucoid, does not play a primary role in specific IgE binding. B- and T-cell epitopes for ovalbumin and ovomucoid are mapped in a limited way, as are the major IgE- and IgG-binding epitopes of ovomucoid (40).

Four sets of distinct egg allergic groups (A, lysozyme and ovalbumin; B, ovomucoid; C, ovomucin; and D, ovotransferrin and the yolk proteins apovitelenins I and VI and phosvitin) were identified in 40 subjects that react to four discrete hen's egg protein sets (1, egg white proteins lysozyme and ovalbumin; 2, egg white ovomucoid; 3, egg white ovomucin; and 4, egg yolk proteins apovitelenins I and IV and egg white ovotransferrin) (41). Both lysozyme and ovomucin bind significant amounts of IgE in the sera of patient groups A and C. Lysozyme was statistically correlated with ovalbumin and was a significant allergen for group A. Other differences in IgE binding were found that may explain why various investigators report different allergens to be important in egg hypersensitivity.

Allen et al. (42) reviewed and identified key points of egg allergy. It primarily affects preschool children. Life-threatening reactions are less common in egg than in peanut/tree nut allergy, and heat and digestion alters the allergenicity of egg proteins. Heating reduces the allergenicity of ovomucoid and ovalbumin, but does not affect lysozyme. Ovomucoid allergenicity may also be reduced by gastric pH. It is possible that the age and/or the use of inhibitors of gastric acid secretion in young children may be a factor that promotes egg protein food sensitization.

Peanut

Peanut allergy in 14 of 22 preschool children in Southampton and South Manchester with a history of mild-to-moderate peanut allergy spontaneously resolved with time (43). However, the recurrence rate in patients who outgrow peanut allergy is 7.9%, and the risk is significantly higher in patients who avoid known peanut foods following resolution of their peanut allergy (44). Peanut proteins are customarily classified as albumins (water soluble) and globulins (saline soluble). The globulin proteins are made up of two major fractions, arachin and conarachin, also known as legumin and vicilin, respectively. Arachin, in its native state 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. Conarachin can be divided by ultracentrifugation into two fractions, 2S and 8.4S.

Historically, peanut-1 and concanavalin A-reactive glycoprotein were some of the first peanut allergens partially characterized using peanut-specific IgE from allergic subjects. With the advent of more refined methods for allergen purification, allergens were named using a systematic nomenclature—abbreviated Linnean genus and species names and an Arabic number to indicate the chronology of allergen purification. Thus, Ara h 1, is a 63.5-kDa glycoprotein identified as the first major peanut allergen using immunoblotting and enzyme-linked immunosorbent assay (ELISA) (45). This allergen has an acidic isoelectric point and is relatively resistant to enzymatic degradation. There are multiple IgE-binding sites in the amino acid sequence of Ara h 1, which has at least 23 specific IgE-binding epitopes. Ara h 1 is a member of the vicilin family of seed storage proteins. Ara h 2 is a 17-kDa allergen with an acidic isoelectric point that has at least 10 specific IgE-binding epitopes along its amino acid sequence and is a member of the conglycinin family of seed storage proteins. Ara h 3 is a glycinin seed storage protein with a molecular weight of 60 kDa. Approximately 45% of patients with peanut allergy have specific IgE to this allergen (46,47).

Ara h 2 is the most potent allergen in a functional IgE cross-linking assay using RBL SX-38 cells with sera from 12 highly sensitive peanut-allergic individuals (48). Mechanistically, soluble peanut antigen (PNAg) and Ara h 1 were prepared from defatted peanut flour and

both induce the Erk1/2 phosphorylation of monocyte-derived dendritic cells consistent with T helper 2 (Th2) adjuvant activity (49). Altogether, eight allergens are identified—Ara h 1 to Ara h 8 (50), with Ara h 1, 2, and 3 considered to be the main allergens (51,52). Advances in peanut allergy are reviewed in Ref. 53.

Soybean

Globulins are the major proteins of soybean. These globulins can be separated into ultracentrifugation fractions 2S, 7S, 11S, and 15S. α -Conglycinin is a primary protein of the 2S fraction while β -conglycinin is the primary fraction of the 7S component. The glycinin fraction is the primary component of the 11S ultracentrifugation fraction.

Soybeans, like peanut, are legumes that contain multiple allergens (54,55). When examining specific IgE to ultracentrifugation components, the 2S or 7S fraction contain the primary allergens. Gly m 1 is a 30-kDa allergen that is a component of the 7S fraction. The majority of patients have soybean-specific IgE to Gly m 1 (56). Gly m 1 has an acidic isoelectric point and sequence homology to a soybean seed 34-kDa oil-body-associated protein, soybean vacuolar protein P34. There are at least 16 distinct soybean-specific IgE-binding epitopes along the amino acid sequence of this allergen. The Kunitz soybean trypsin inhibitor binds soybean-specific IgE in soybean-allergic patients, although only in a minority of patients, making it a minor allergen. A comprehensive review is available for the identification and characterization of soybean allergens with current techniques to reduce allergenicity including thermal, enzymatic, chemical, traditional breeding, and genetic modification of the allergens (57).

Wheat

Wheat and other cereal grains are often implicated as food allergens, particularly in children (58). The proteins of wheat include the water-soluble albumins, the saline-soluble globulins, the aqueous ethanol-soluble prolamins, and the glutelins. Patients with wheat allergy have specific IgE to wheat fractions 47 kDa and 20 kDa, proteins not recognized by the sera of patients with grass allergy. Wheat α -amylase inhibitor (15 kDa) is also a major wheat allergen. This protein does not bind IgE from wheat-tolerant control patients, including those with grass allergy (59,60). Battais et al. (61) identified major wheat allergens in the water/salt soluble, gluten fractions, and isolated wheat fractions by IgE-binding studies. IgE from wheat-dependent exercise-induced anaphylaxis and urticaria reacts with sequential epitopes (QQX1PX2QQ) in the repetitive domain of gliadins whereas IgE from atopic eczema/dermatitis patients recognizes conformational epitopes (62,63).

Fish

Fish and shellfish allergy are extensively reviewed in Ref. 64 with parvalbumins and tropomyosins identified as the major allergens, respectively. The clinical manifestations, diagnosis, immunologic mechanisms, and molecular biology of seafood allergens are discussed in Ref. 65.

The consumption or inhalation of fish allergen is a common cause of IgE-mediated food reactions. The incidence of fish allergy is believed to be much higher in countries where fish consumption is greatest. For example, codfish allergy is common in the Scandinavian countries (66). Red and golden snapper, local species of snapper consumed in Malaysia, commonly cause food allergy with parvalbumin being considered as a minor allergen (67).

One of the most comprehensive descriptions of a food allergen is by Esayed and Apol of codfish allergen, Gad c 1 (originally called Allergen M) (68). Gad c 1 belongs to a group of muscle proteins known as parvalbumins. The parvalbumins control the flow of calcium in and out of cells and are only found in the muscles of amphibians and fish. This allergen has an acidic isoelectric point and a molecular weight of 12 kDa. The tertiary structure of Gad c 1 has three domains. There are at least five IgE-binding sites on the allergen and the carbohydrate moiety does not appear to be important in its allergenicity. Fish consumed in India include pomfret and hilsa, which contain heat-labile allergens, while bhetki and mackerel have more heat-stable allergen characteristics (69).

Shrimp

Shrimp is the most studied of the Crustacea allergens (70,71). The IgE-binding epitopes of the shrimp allergen Pen a 1 are now identified (72,73). Deduced amino acid sequence of 284 amino acids

from
verte
respe
both
bindi

Tree

Tree
fish
and
reacti
Nang
of tree
utero
aged
Poten
and tr
Two r
protein
a high
3-kDa
vicilin

OTHE

Protein
reacti
allergy
import
antige
and tu
specifi
Table

HIDDE

Hidden
labels
emerg
reports
food al
to be
known
predict

Table 4

Pollen
Pollen
Flour
Latex
Crustacean
Respiratory
Flour
Seafood

from recombinant allergens and amino acid sequences from allergenic and nonallergenic vertebrate tropomyosins reveal 80% to 99% and 51% to 58% amino acid sequence homology, respectively. Analysis of secondary structure for natural and recombinant Pen a 1 shows that both have α -helical conformation that is typical for tropomyosins with essentially the same IgE-binding capacity as determined by RAST (74).

Tree Nuts

Tree nuts cause food-allergic reactions in both children and adults. Just as allergic reactions to fish and peanuts can persist for life, so too can reactions to tree nuts. Hazelnut, walnut, cashew, and almond are the most common tree nuts responsible for allergic reactions with less frequent reactions to pecan, chestnut, Brazil nut, pine nut, macadamia nut, pistachio, coconut, and Nangai (75). Clark and Ewan (76) reviewed the development, sensitization, and clinical impact of tree nut allergens suggesting that multiple nut sensitizations and allergies can take place in utero or soon after birth. Findings from the study reveal that a large proportion of children aged 0 to 1 year were already sensitized (nut-specific IgE) to almond, Brazil, hazel, and walnut. Potential sensitization routes included breast milk, peanut- or soya-containing infant formula, and trace contamination of infant diet and use of peanut- or soya-containing eczema creams. Two major allergens in almonds are identified: 70-kDa heat-labile and 45- to 50-kDa heat-stable proteins. Although several different Brazil nut proteins are allergens, the major one, Ber e 1, is a high-methionine-containing protein (77). This 12-kDa protein has two subunits, a 9-kDa and a 3-kDa protein. A major walnut allergen is a 65-kDa glycoprotein, Jug r 2, similar to other plant vicilins, as well as another walnut allergen, Jug r 1, a 2S albumin seed storage protein (78).

OTHER ALLERGENS

Protein and oil components of sesame seed cause immediate and delayed hypersensitivity reactions (79). Lupine allergens extensively cross-react with other legume species (80,81). Kiwi allergy is increasing (82,83) with kiwellin, a cysteine-rich 28-kDa protein, isolated as the important allergen (84). Apple peel extract from 10 different apple varieties show both antigenic and allergenic activity associated with the oral allergy syndrome (85). Oilseed rape and turnip napin 2S allergens have been identified (86). An N-linked glycan from oranges has specific IgE-binding properties (87). Examples of pollen-food related syndromes are listed in Table 4.

HIDDEN ALLERGENS

Hidden allergens are protein sources that often are not recognized or included on product labels. In a United States study, there was a known allergy to the triggering food in 41% of emergency department visits for allergic reactions to foods (10). Of the 32 food-related fatalities reported in Ref. 95, at least 87% of subjects had a prior history of a reaction to the responsible food allergen. In a retrospective study of the 530 food-allergic reactions, 22.4% were considered to be due to hidden allergens (96) (Table 5). Although food-induced anaphylaxis is well known, continued ongoing research seeks to improve both diagnostic tools and to improve predictors of anaphylaxis risk.

Table 4 Pollen- and Respiratory Food-Related Syndromes

	Reference
Pollen	
Pollen/food allergy syndrome	88
Ficus fruit syndrome	89
Latex fruit syndrome	90,91
Oral allergy syndrome	92
Respiratory	
Flour	93
Seafood	94

Table 5 Source of Hidden Food Allergens

Food group	Food source
Shellfish	Oil, contaminated equipment
Fish	Cooking oil, salmon cream canape
Nuts	Chocolates, cookies, pastries, cakes
Fruits	Ice creams
Legumes	Ham, sausage, cheese puffs
Egg	Ice cream, pastries

Other examples of hidden allergens include uncooked anchovy, fish, and shellfish, contaminated with *Anisakis simplex* larvae, a fish worm truly responsible for the allergic reactions, accounting for the majority of anaphylactic reactions in a Spanish geographical area (96). In another case, a lupine allergen with a molecular weight close to 14 kDa was detected in extracts from cookies, a chicken bouillon cube, and a chicken dehydrated soup (97). Similarly, a human sera-based immunoassay identified the presence of unknown peanut allergens in products belonging to various food categories, such as cereals, cookies, cakes, and snacks (98). In most cases, food producers are able to detect and eliminate this sort of contamination and implement measures to prevent the presence of hidden allergens in their products, thereby increasing food safety for sensitized persons.

DIAGNOSIS

Food allergy often manifests as gastrointestinal symptoms, and patients therefore often consult a family physician or a gastroenterologist who may not be sufficiently knowledgeable to diagnose food allergy (99–101). Allergic reactions to foods are the most common cause of life-endangering anaphylaxis (102). Asero et al. (103) in a review of food allergy identified a major problem in the diagnosis of food allergy, i.e., the relatively poor “clinical specificity” of both skin and in vitro tests. Another major problem in appropriately diagnosing food allergy is the fact that there are no standardized food allergen extracts (104).

A detailed medical history, physical examination, and appropriate laboratory tests are necessary to diagnose food allergy (Table 6). Skin prick test and RAST are sensitive indicators of food-specific IgE; however, they are not very predictive of clinical sensitivity (105). A positive skin test to a food indicates the possibility that the patient has symptomatic reactivity to that specific food, although overall the positive predictive accuracy is less than 50%. A negative skin test confirms the absence of an IgE-mediated reaction with an overall negative predictive accuracy of greater than 95%. The definitive diagnosis of food allergy is based on standardized oral challenges.

On the basis of previously established 95% predictive decision points for egg, milk, peanut, and fish allergy, greater than 95% of food allergies diagnosed in prospective study of 100 children were correctly identified by quantifying serum food-specific IgE concentrations (106). Using allergen-specific IgE values for egg, 6 kUA/L; milk, 32 kUA/L; peanut, 15 kUA/L; and fish, 20 kUA/L as diagnostic decision points, the positive predictive values in this prospective study ranged from 96% to 100%. By using decision points of 100 kUA/L for wheat and 65 kUA/L for soy, the predictive accuracy was 100% and 86%, respectively. Using these predictive values, the authors have been able to reduce DBPCFC by 40% to 50%.

Table 6 Important Information from Medical History

The food suspected to have provoked the reaction
The quantity of the food ingested
The length of time between ingestion and development of symptoms
A description of the symptoms provoked
If similar symptoms developed on other occasions when the food was eaten
If other factors (e.g., exercise) are necessary
The length of time since the last reaction

large
clini
(107).
negat
positi
DBPC

allergi
1.2 kU
Celik-
milk t
studie
sensiti

confor
intens
determ

state,
genera
60 min
portion
to 10 g
negativ
negativ
serum-
S
reacts t
and rA
rAra h

Table 7

Time (m)
0:00
0:15
0:30
0:45
60

^aFor a re
the reac
jacionlin

Table 8

Therapy
TNX-901
Chinese
Birch pol
Peptide(s)
Chimeric
Probiotic
Dietary m

Source: F

Over a nine-year period in a tertiary clinic in Australia, infants and young children with a large skin prick test wheal (mean size 8–10 mm) was associated with a >95% likelihood of clinical reactivity to cow's milk, egg, and peanut, as confirmed by an open food challenge (107). For each food it was possible to identify a skin wheal diameter at and above which negative reactions did not occur: cow's milk, 8 mm; egg, 7 mm; and peanut, 8 mm. In contrast, positive reactions could occur even with a skin wheal diameter of 0 mm. Therefore, the DBPCFC remains the gold standard to determine food allergy.

A retrospective chart review of 584 food challenges where 253 (43%) resulted in an allergic reaction, the median food-specific IgE for a failed challenge was 2.0 kUA/L for milk, 1.2 kUA/L for egg, 1.9 kUA/L for peanut, 9.3 kUA/L for soy, and 19.6 kUA/L for wheat (108). Celik-Bilgili S et al. (109) calculated a 90% positive predictive serum-specific IgE level for cow's milk to be 88.8 kU/L and for hen's egg to be 6.3 kU/L that correlated with positive challenge studies. In each case, caveats include the age of the population under investigation and sensitivity and specificity of the allergen-specific IgE.

A newer method to confirm food allergy is by food allergen epitope recognition patterns, conformational versus sequential and number of epitopes recognized, combined with the intensity of IgE binding by microarray analysis. They are considered to be important determinants of severity and duration of food allergy (51).

Food challenges, when necessary, should be administered with the patient in a fasting state, starting with a challenge dose of the food in question unlikely to provoke symptoms, generally 125 mg to 500 mg of lyophilized food. This dose is then increased every 15 to 60 minutes depending on the historical reaction. A similar scheme is followed with the placebo portion of the study. Clinical reactivity can be ruled out when the blinded patient tolerates up to 10 g of lyophilized food in capsules or liquid. If the blinded portion of the challenge is negative, it must be confirmed by an open feeding under observation to rule out rare false-negative challenges (Table 7). Retrospective studies have been investigated to determine serum-specific IgE concentrations in subjects that underwent oral food challenges (Table 8).

Shreffler et al. (51) demonstrated that 97% of subjects with peanut allergy have IgE that reacts to at least one peanut allergen, whereas 77%, 75%, and 77% recognized rAra h 1, rAra h 2, and rAra h 3, respectively. Subsequently, Astier et al. (118) showed that cosensitization with rAra h 1, 2, and 3 is more predictive of clinical reactions and even more severe reactions.

Table 7 Sample Schedule for Double-Blind, Placebo-Controlled Food Challenge^a

Time (min)	Food	Time (hour of day) (PM)	Placebo
0:00	125–500 mg	3:00	500 mg
0:15	1 g	3:15	1 g
0:30	2 g	3:30	2 g
0:45	3 g	3:45	3 g
60	3.5 g	4:00	3.5 g

^aFor a review of DBPCFC recipe validation of food challenge materials for children, the reader is referred to the Journal of Allergy Online Repository ([http://www.jacionline.org/article/S0091-6749\(03\)02481-3/fulltext](http://www.jacionline.org/article/S0091-6749(03)02481-3/fulltext)).

Table 8 Novel Food Therapies

Therapy	Mode of action	Reference
TNX-901	Anti-IgE antibody	111
Chinese herbal medicine	Unknown	112
Birch pollen immunotherapy	Tolerance	113
Peptide(s)	Tolerance	110
Chimeric antibodies	Tolerance	114
Probiotics and Th adjuvants	Tolerance/Th switch	115
Dietary manipulation	Avoidance	116,117

Source: From Ref. 110.

Table 9 Published Values for Diagnosis (IgE), Exposure, and Threshold Response Concentrations

Food	Diagnosis (kUA/L specific antibody)	Exposure (grams food/protein)	Threshold response (milligrams protein)
Egg	≥7 (120); 6 (121)	43 g (4.3 g protein) (122)	1/20 (2000 mg protein) (123)
Milk	32 (121)	226 g (7.2 g protein) (122)	30 mL (965 mg protein) (123)
Peanut	15 (121)	14 g (3 g protein) (122)	1½ (500 mg protein) (124)
Fish	20 (121)	NA ^a	NA

^aNot available.

THERAPIES

Nowak-Wegrzyn and Sampson (119) reviewed novel therapies for food allergy (Table 9). The immunotherapeutic strategies to treat peanut allergy suggest that a combination therapy of anti-IgE and other therapies could work (125). In a randomized, uninterrupted avoidance versus oral desensitization protocol of children with IgE-dependent milk or egg allergy, oral desensitization helped children overcome their food allergy while those on avoidance diets often develop a lower threshold of reactivity to the incriminated food as evidenced by single-blind, placebo-controlled food challenge (SBPCFC) (126). A SBPCFC to milk was positive in 11.1% of those following oral desensitization versus 40% after avoidance, whereas for egg allergy, a SBPCFC was positive in 30.6% after oral desensitization versus 48.6% for avoidance.

THRESHOLD DOSES

A better knowledge of clinical reactive thresholds is still needed (123). Small quantities of hidden allergens in foods and their concentrations are calculated to be in the range of 0.003% to 2.3% (wt/wt) (127). Taylor et al. (128) presents a comprehensive review of problems encountered by industry and the rational link with the determination of threshold doses as well as a thorough report of the experience of several clinical groups with a daily practice of standardized food diagnostic challenges. Morisset et al. (129) reports minimal reactive quantities that guarantee a 95% safety for patients who are allergic to egg, peanut, or milk. On the basis of consumption of 100 g of food, the detection tests should ensure a sensitivity of 10 ppm for egg, 24 ppm for peanut, and 30 ppm for milk. Lowest reactive threshold doses from 125 positive oral challenges to egg, 103 to peanut, 59 to milk, and 12 to sesame were 2 mg of egg, 5 mg of peanut, 0.1 mL of milk, and 30 mg of sesame seed, respectively.

Factors that are likely to modify threshold or response levels include the allergen(s) in the food, exercise, simultaneous intake of aspirin, regular treatment with β -blockers or angiotensin-converting enzyme inhibitors, association with other food/pollen allergens, alcohol, and content of fat in the food or meal. Additional problems include processing, storage, and genetically modified food technologies that may alter a protein or food itself exposing neoallergens (130). Guidelines to assist clinicians and allergic individuals continue to be challenging in light of these many variables.

MECHANISMS

Allergen-reactive Th2 cells play a central role in the pathophysiology of allergic diseases. A summary of food allergens mapped for T-cell epitopes indicates that T-cell activating peptides also bind IgE antibodies (131). T-cell subset patterns reveal that the childhood IL-4 cytokine response is predominantly from CD4⁺ CD45RO⁺ cells, whereas IL-4 and IFN- γ secretion of nonallergic controls involves CD4⁺ and CD8⁺ CD45RO⁺ cell populations (132). Food-allergic IL-4 cytokine response to relevant allergens is predominantly from a memory population of CD4⁺ CD45RO⁺ cells, whereas IFN- γ and IL-4 of nonallergic controls is predominantly from mixed CD4⁺ and CD8⁺ CD45RO⁺ cells.

Using carboxyfluorescein succinimidyl ester labeling and cloned T cells to investigate food antigen-specific T-cell responses in peanut-allergic and nonallergic children and children

who outgrew their food allergy, nonallergenic foods (β -lactoglobulin, ovalbumin) induce a Th1-skewed cytokine profile regardless of the allergic status of the donors (133). This Th1-skewing induced by nonallergenic food antigens is characterized by a high production of IFN- γ and TNF- α , almost undetectable production of IL-4 and IL-5, and relatively low production of IL-13. Peanut-allergic donors show a Th2 polarization of cytokine production by peanut-specific cells (IFN- γ^{low} , TNF- α^{low} , IL-4 $^{\text{high}}$, IL-5 $^{\text{high}}$, IL-13 $^{\text{high}}$), confirming that these cells are indeed peanut specific by cloning.

Children, age two to five years, with active egg allergy atopic dermatitis and those who had outgrown their egg allergy, were used to detail their peripheral blood lymphocyte immunologic response (134). A marked increase in IL4 and a decrease in IFN- γ synthesis by peripheral blood lymphocytes following ovalbumin specific in vitro stimulation were observed in active atopic dermatitis. In contrast, ovalbumin-induced IL4 synthesis in patients in remission was comparable to that in normal individuals. Children with clinically resolving milk allergy had a persisting Th2 cytokine response. Th2-cell-dependent, peanut-specific IL-5, IL-13, and CCL22 were common in peanut-tolerant individuals regardless of whether they had a positive or negative skin test (135). This led the authors to suggest that the continuum and spectrum of Th2 responses among individuals with negative and positive skin tests might be more important than the Th1/Th2 balance.

Children who outgrew their milk allergy (tolerant children) had higher frequencies of circulating CD4(+)CD25(+) T cells and decreased in vitro proliferative responses to bovine β -lactoglobulin in peripheral blood mononuclear cells (PBMCs) compared with children who maintained clinically active allergy. Clearly, there is additional research needed to address the significance of Th1/Th2 cytokine balance, T regulatory cells defined as regulatory T (Treg) cells, and allergen-specific IgE and skin test responses that could be related to the allergen and mucosal response.

Comparison of phenotypic and functional characterization of PBMCs before and after in vivo milk challenges from children with clinical active or resolved cow's milk allergy suggests a possible mechanism for mucosal induction of tolerance against β -lactoglobulin (136). Gastrointestinal lymphoid tissue activation and expression of specific effector T cells and CD4+CD25+ Treg are thought to be responsible for the reduced in vitro proliferative response to β -lactoglobulin in children who outgrow their cow's milk allergy.

Factors necessary for a food protein antigen to function as an allergen remain unidentified, even though structural and biochemical characteristics that provoke allergic responses continue to be discovered. Allergen-specific antibody-binding sites are routinely limited to short amino acid sequences of proteins identified as sequential or conformational. Specific cross-reactivity with proteins sharing sufficient sequential and/or conformational homology can exist with the original immunogen/allergen as well as other related proteins (137). Continuous epitopes of allergens consist of amino acids in the primary sequences, while conformational epitopes exist as a consequence of three-dimensional folding of the proteins. Therefore, divergent patterns of cross-reactivity and clinically relevant allergic reactions to foods can occur in individual patients (138) (Table 10).

To trigger an allergic reaction, at least two different epitopes on the surface of the allergen must cross-link IgE to result in a biologic response. Sequential epitopes, consisting of linear amino acid sequences, have been characterized by preparing overlapping peptides derived from the primary allergen sequence (139). Conformational epitopes have nonlinear

Table 10 Food Allergen Cross-Reactivity

	Specific IgE to multiple members of the family	Clinical reactivity
Milk	Common	Common
Legumes	Common	Uncommon
Wheat	Common	Uncommon
Fish	Common	Uncommon
Crustacea—mollusks	Common	Unknown or questionable
Tree nuts	Common	Uncommon
Egg—chicken	Occasional	Rare
Milk—beef	Occasional	Uncommon

amino acid sequences due to secondary and tertiary structure. Evidence for conformational epitopes have been determined using resolution of antigen-antibody complex structures (140), site-directed mutagenesis using mismatched oligonucleotides prepared by polymerase chain reaction to alter the amino acid sequences (141,142), and peptide mimics (individual peptides isolated from peptide libraries that frequently yield peptides mimicking the binding site of the cognate antigen for the specific antibody but does not correspond to the linear sequence) (143). Using a Bet v 1-related food allergy as a model, five patients were investigated by cross-competitive ELISA for specific and cross-reacting IgE to Bet v 1 and its homologues, Gly m 4 (soybean), Ara h 8 (peanut), and Pru av 1 (cherry) (144). By competitive immunoscreening and epitope mapping of these birch pollen-related food, the model revealed patient-specific IgE epitopes; however, one surface binding area was recognized by all patients and two areas were recognized by three patients. Cross-reactivity can further be assessed by structural similarity using an in silico homology search in combination with in vitro IgE antibody assays (145). Using peptides or mimotopes for allergen molecules, epitopes in a three-dimensional format can be studied by crystallographic comparisons. IgE epitopes identified in this manner were all conformational and responsible for high-affinity interactions with specific IgE preferentially forming di-, tri-, or multimers that display repetitive IgE epitopes (146). As B lymphocytes are pattern recognizers, this feature is essential for a memory response, but may also be critical for the very first allergen contact and initiation of the IgE response.

CONCLUSION

The incidence of food-allergic-induced anaphylaxis is increasing. The natural history of food allergy varies; some go away with time while others are lifelong. New food allergens continue to be recognized, identified, and characterized at the molecular level, and the immunologic mechanisms responsible for both food tolerance and hypersensitivity are under investigation. Although several experimental treatments are promising, none are currently clinically available for the practicing physician.

Detailed studies on how foods sensitize will become more feasible as the structure and molecular properties of food allergens are finalized and more standardized food allergens become available for diagnosis. Continuing studies on the patterns of sensitization will give more insight into the development and possible treatment of protein-related food allergy.

SALIENT POINTS

- Food allergens, found in plants and animals, are classified on the basis of their biologic function or protein membership families.
- A complete medical history and physical examination combined with laboratory testing is essential to diagnose food allergy.
- Many food allergens occur naturally as dimers or trimers often making their molecular weight 150 to 200 kDa.
- IgE immunogenicity is accurately determined by factors unrelated to the primary structure of the protein and must take into account the food source and how the allergen enters the body—essentially, the protein must induce B cells to produce IgE.
- Food allergies are different throughout the world because of different foods and diets.
- A retrospective study of food-allergic reactions reveals that 22.4% are due to hidden allergens.

REFERENCES

1. Sampson HA. Update on food allergy. *J Allergy Clin Immunol* 2004; 113:805-819.
2. Sicherer SH, Sampson HA. Food allergy. *J Allergy Clin Immunol* 2006; 117:S470-S475.
3. Bock SA, Munoz-Furlong A, Sampson HA. Further fatalities caused by anaphylactic reactions to food, 2001-2006. *J Allergy Clin Immunol* 2007; 119:1016-1018.
4. Pumphrey RSH, Gowland MH. Further fatalities caused by anaphylactic reactions to food, 2001-2006. *J Allergy Clin Immunol* 119:118-119.

5. Sicherer SH, Munoz-Furlong A, Sampson HA. Prevalence of peanut and tree nut allergy in the United States determined by means of a random digit dial telephone survey. *J Allergy Clin Immunol* 2003; 112:1203–1207.
6. Sicherer SH, Leung DYM. Advances in allergic skin disease, anaphylaxis, and hypersensitivity reactions to foods, drugs, and insects. *J Allergy Clin Immunol* 2007; 119:1463–1469.
7. Wood RA. The natural history of food allergy. *Pediatrics* 2003; 111:1631–1637.
8. Wang J, Sampson HA. Food anaphylaxis. *Clin Exp Allergy* 2007; 37:651–660.
9. Uguz A, Lack G, Pumphrey R, et al. Allergic reactions in the community: a questionnaire survey of members of the anaphylaxis campaign. *Clin Exp Allergy* 2005; 35:746–750.
10. Clark S, Bock SA, Gaeta TJ, et al. Multicenter study of emergency department visits for food allergies. *J Allergy Clin Immunol* 2004; 113:346–352.
11. Golden DB. Patterns of anaphylaxis: acute and late phase features of allergic reactions. *Novartis Found Symp* 2004; 257:101–115.
12. Dirks CG, Pedersen MH, Platzer MH, et al. Does absorption across the buccal mucosa explain early onset of food-induced allergic systemic reactions. *J Allergy Clin Immunol* 2005; 115:1321–1323.
13. Strid J, Hourihane J, Kimber I, et al. Epicutaneous exposure to peanut protein prevents oral tolerance and enhances allergic sensitization. *Clin Exp Allergy* 2005; 35(6):757–766.
14. Chambers SJ, Wickman MS, Regoi M, et al. Rapid in vivo transport of proteins from digested allergens across pre-sensitized gut. *Biochem Biophys Res Commun* 2004; 325:1258–1263.
15. Jensen-Jarolim E, Untersmayr E. Food safety: in vitro digestion tests are non-predictive for allergenic potential of food in stomach insufficiency. *Immunol Lett* 2006; 102:118–119.
16. The InformAll Database. Available at: <http://food.allergens.ifr.ac.uk>.
17. Lemanske RF Jr., Taylor SL. Standardized extracts, foods. *Clin Rev Allergy* 1987; 5(1):23–36.
18. Wopfner N, Willeroidee M, Hebenstreit D, et al. Molecular and immunological characterization of profilin from mugwort pollen. *Biol Chem* 2002; 383(11):1779–1789.
19. Das Dore S, Chopin C, Villaume C, et al. A new oligomeric parvalbumin allergen of Atlantic cod (*Gad m I*) encoded by a gene distinct from that of *Gad c I*. *Allergy* 2002; 57(suppl 72):79–83.
20. van Boxtel EL, van Beers MM, Koppelman SJ, et al. Allergen Ara h 1 occurs in peanuts as a large oligomer rather than as a trimer. *J Agric Food Chem* 2006; 54(19):7180–7186.
21. Breiteneder H, Radauer C. A classification of plant food allergens. *J Allergy Clin Immunol* 2004; 113:821–830.
22. Jenkins JA, Griffiths-Jones S, Shewry PR, et al. Structural relatedness of plant food allergens with specific reference to cross-reactive allergens: an in silico analysis. *J Allergy Clin Immunol* 2005; 115:163–170.
23. King TP, Hoffman D, Lowenstein H, et al. Allergen nomenclature. *Bull World Health Organ* 1994; 72:797–780.
24. Johansson SG, Bieber T, Dahl R, et al. Revised nomenclature for allergy for global use: report of the Nomenclature Review Committee of the World Allergy Organization, October, 2003. *J Allergy Clin Immunol* 2004; 113:832–836.
25. Chapman MD, Pomes A, Breiteneder H, et al. Nomenclature and structural biology of allergens. *J Allergy Clin Immunol* 2007; 119:414–420.
26. Zuidmeer L, van Rhee R. Lipid transfer protein allergy: primary food allergy or pollen/food syndrome in some cases. *Curr Opin Allergy Clin Immunol* 2007; 7:269–273.
27. AllFam allergen family chart. Downloaded from AllFam version of May 5, 2007. Available at: <http://www.meduniwien.ac.at/allergens/allfam/>.
28. Radauer C, Marl A, Breiteneder H. The AllFam database—a resource for classifying allergenic proteins. *Allergy* 2007; 62(suppl 83):154.
29. Strobel S, Mowat AM. Immune responses to dietary antigens: oral tolerance. *Immunol Today* 1998; 19(4):173–181.
30. Busse PJ, Jarvinen KM, Vila L, et al. Identification of sequential IgE-binding epitopes on bovine alpha(s2)-casein in cow's milk allergic patients. *Int Arch Allergy Immunol* 2002; 129:93–96.
31. Jarvinen KM, Chatchatee P, Bardina L, et al. IgE and IgG binding epitopes on alpha-lactalbumin and beta-lactoglobulin in cow's milk allergy. *Int Arch Allergy Immunol* 2001; 126(2):111–118.
32. Jarvinen KM, Beyer K, Vila L, et al. B-cell epitopes as a screening instrument for persistent cow's milk allergy. *J Allergy Clin Immunol* 2002; 110(2):293–297.
33. Chatchatee P, Jarvinen KM, Bardina L, et al. Identification of IgE- and IgG-binding epitopes on alpha(s1)-casein: differences in patients with persistent and transient cow's milk allergy. *J Allergy Clin Immunol* 2001; 107(2):379–383.
34. Taylor SL, Hefle SL, Bindslev-Jensen C, et al. Factors affecting the determination of threshold doses for allergenic foods: how much is too much? *J Allergy Clin Immunol* 2002; 109:24–30.
35. Morisset M, Moneret-Vautrin DA, Kanny G, et al. Thresholds of clinical reactivity to milk, egg, peanut, and sesame in immunoglobulin E-dependent allergies: evaluation by double-blind or single-blind placebo-controlled oral challenges. *Clin Exp Allergy* 2003; 33:1046–1051.

36. Wal JM. Bovine milk allergenicity. *Ann Allergy Asthma Immunol* 2004; 93:2-11.
37. Anet J, Back JF, Baker RS, et al. Allergens in the white and yolk of hen's egg. A study of IgE binding by egg proteins. *Int Arch Allergy Appl Immunol* 1985; 77(3):364-371.
38. Hoffman DR. Immunochemical identification of the allergens in egg white. *J Allergy Clin Immunol* 1983; 71(5):481-486.
39. Bernhisel-Broadbent J, Dintzis HM, Dintzis RZ, et al. Allergenicity and antigenicity of chicken egg ovomucoid (Gal d III) compared with ovalbumin (Gal d I) in children with egg allergy and in mice. *J Allergy Clin Immunol* 1994; 93(6):1047-1059.
40. Mine Y, Wei ZJ. Identification and fine mapping of IgG and IgE epitopes in ovomucoid. *Biochem Biophys Res Commun* 2002; 292(4):1070-1074.
41. Walsh BJ, Hill DJ, Macoun P, et al. Detection of four distinct groups of hen egg allergens binding IgE in sera of children with egg allergy. *Allergol Immunopathol (Madrid)* 2005; 33:183-191.
42. Allen CW, Campbell DE, Kemp AS. Egg allergy: are all childhood food allergies the same? *J Paediatr Child Health* 2007; 43:214-218.
43. Hourihane JO, Roberts SA, Warner JO. Resolution of peanut allergy: case-control study. *Br Med J* 1998; 316:1271-1275.
44. Fleischer DM, Conover-Walker MK, Christie L, et al. Peanut allergy: recurrence and its management. *J Allergy Clin Immunol* 2004; 114:1195-1201.
45. Burks AW, Cockrell G, Stanley JS, et al. Recombinant peanut allergen Ara h I expression and IgE binding in patients with peanut hypersensitivity. *J Clin Invest* 1995; 96(4):1715-1721.
46. Rabjohn P, West CM, Connaughton C, et al. Modification of peanut allergen Ara h 3: effects on IgE binding and T cell stimulation. *Int Arch Allergy Immunol* 2002; 128(1):15-23.
47. Rabjohn P, Helm EM, Stanley JS, et al. Molecular cloning and epitope analysis of the peanut allergen Ara h 3. *J Clin Invest* 1999; 103(4):535-542.
48. Palmer GW, Dibbern DA, Burks AW, et al. Comparative potency of Ara h 1 and Ara h 2 in immunochemical and functional assays of allergenicity. *Clin Immunol* 2005; 115:302-312.
49. Shreffler WG, Castro RR, Kucuk ZY, et al. The major glycoprotein form *Arachis hyogaea*, Ara h 1, is a ligand of dendritic cell-specific ICAM-grabbing nonintegrin and acts as a Th2 adjuvant in vitro. *J Immunol* 2006; 177:3677-3685.
50. Allergen Nomenclature—The Official "List of Allergy." WHO/IUIS Allergen Nomenclature Subcommittee, 2006. Available at: <http://www.allergen.org>.
51. Shreffler WG, Beyer K, Chu TH, et al. Microarray immunoassay: association of clinical history, in vitro IgE function, and heterogeneity of allergenic peanut epitopes. *J Allergy Clin Immunol* 2004; 113:776-782.
52. Shreffler WG, Lencer DA, Bardina L, et al. IgE and IgG4 epitope mapping by microarray immunoassay reveals the diversity of immune response to the peanut allergen Ara h 2. *J Allergy Clin Immunol* 2005; 116:893-899.
53. Palmer K, Burks W. Current development in peanut allergy. *Curr Opin Allergy Clin Immunol* 2006; 6:202-206.
54. Burks AW Jr., Brooks JR, Sampson HA. Allergenicity of major component proteins of soybean determined by enzyme-linked immunosorbent assay (ELISA) and immunoblotting in children with atopic dermatitis and positive soy challenges. *J Allergy Clin Immunol* 1988; 81(6):1135-1142.
55. Ogawa A, Samoto M, Takahashi K. Soybean allergens and hypoallergenic soybean products. *J Nutr Sci Vitaminol (Tokyo)* 2000; 46(6):271-279.
56. Ogawa T, Tsuji H, Bando N, et al. Identification of the soybean allergenic protein, Gly m Bd 30K, with the soybean seed 34-kDa oil-body-associated protein. *Biosci Biotechnol Biochem* 1993; 57(6):1030-1033.
57. L'Hocine L, Boye JI. Allergenicity of soybean: new developments in identification of allergenic proteins, cross-reactivities and hypoallergenization technologies. *Crit Rev Food Sci Nutr* 2007; 47:127-143.
58. Sutton R, Hill DJ, Baldo BA, et al. Immunoglobulin E antibodies to ingested cereal flour components: studies with sera from subjects with asthma and eczema. *Clin Allergy* 1982; 12(1):63-74.
59. Jones SM, Magnolfi CF, Cooke SK, et al. Immunologic cross-reactivity among cereal grains and grasses in children with food hypersensitivity. *J Allergy Clin Immunol* 1995; 96(3):341-351.
60. James JM, Helm RM, Burks AW, et al. Comparison of pediatric and adult IgE antibody binding to fish proteins. *Ann Allergy Asthma Immunol* 1997; 79(2):131-137.
61. Battais F, Pineau F, Popineau Y, et al. Food allergy to wheat: identification of immunoglobulin E- and immunoglobulin G-binding proteins with sequential extracts and purified proteins from wheat flour. *Clin Exp Allergy* 2003; 33:962-970.
62. Battais F, Mothes T, Moneret-Vautrin DA, et al. Identification of IgE-binding epitopes on gliadins for patients with food allergy to wheat. *Allergy* 2005; 60:815-821.
63. Battais F, Richard C, Szustakowski G, et al. Wheat flour allergy: an entire diagnostic tool for complex allergy. *Eur Annals Allergy Clin Immunol* 2006; 38:59-61.

64. Wild LG, Lehrer SB. Fish and shell fish allergy. *Curr Allergy Asthma Rep* 2005; 5:74-79.
65. Chua KH, Tang CY, Wu A, et al. Seafood allergy: lessons from clinical symptoms, immunological mechanisms and molecular biology. *Adv Biochem Eng Biotechnol* 2005; 97:205-235.
66. Aas K. Studies of hypersensitivity to fish. A clinical study. *Int Arch Allergy Appl Immunol* 1966; 29(4): 346-363.
67. Rosmilah M, Shahnaz M, Masita A, et al. Identification of major allergens of two species of local snappers: *Lutjanus argentimaculatus* (mera/red snapper) and *Lutjanus johnii* (janahak/golden snapper). *Trop Biomed* 2005; 22:171-177.
68. Elsayed S, Apol J. Immunochemical analysis of cod fish allergen M: locations of the immunoglobulin binding sites as demonstrated by the native and synthetic peptides. *Allergy* 1983; 38:449-459.
69. Chatterjee U, Mondal G, Chakraborti P, et al. Changes in the allergenicity during different preparations of pomfret, hilsa, bhetki and mackerel fish as illustrated by enzyme-linked immunosorbent assay and immunoblotting. *Int Arch Allergy Immunol* 2006; 141:1-10.
70. Lehrer SB, Ibanez MD, McCants ML, et al. Characterization of water-soluble shrimp allergens released during boiling. *J Allergy Clin Immunol* 1990; 85(6):1005-1013.
71. Daul CB, Morgan JE, Waring NP, et al. Immunologic evaluation of shrimp-allergic individuals. *J Allergy Clin Immunol* 1987; 80(5):716-722.
72. Ayuso R, Lehrer SB, Reese G. Identification of continuous, allergenic regions of the major shrimp allergen Pen a 1 (tropomyosin). *Int Arch Allergy Immunol* 2002; 127(1):27-37.
73. Reese G, Ayuso R, Leong-Kee SM, et al. Characterization and identification of allergen epitopes: recombinant peptide libraries and synthetic, overlapping peptides. *J Chromatogr B Biomed Sci Appl* 2001; 756(1-2):157-163.
74. Reese G, Schickanz S, Lauraer I, et al. Structural, immunological and functional properties of natural recombinant Pan a 1, the major allergen of Brown shrimp. *Panaeus aztecus*. *Clin Exp Allergy* 2006; 36:517-524.
75. Roux KH, Teuber SS, Sathe SK. Tree nut allergens. *Int Archives Allergy Immunol* 2003; 131:L234-L244.
76. Clark AT, Ewan PW. The development and progression of allergy to multiple nuts at different ages. *Pediatr Allergy Immunol* 2005; 16:507-511.
77. Nordlee JA, Taylor SL, Townsend JA, et al. Identification of a Brazil-nut allergen in transgenic soybeans. *N Engl J Med* 1996; 334(11):688-692.
78. Robotham JM, Teuber SS, Sathe SK, et al. Linear IgE epitope mapping of the English walnut (*Juglans regia*) major food allergen, Jug r 1. *J Allergy Clin Immunol* 2002; 109(1):143-149.
79. Gangur V, Kelly C, Navulure L. Sesame allergy: a growing allergy of global proportions? *Ann Allergy Asthma Immunol* 2005; 95:11-13.
80. Guarneri F, Guarneri C, Benvenga S. Identification of potentially cross-reactive peanut-lupine proteins by computer-assisted search of amino acid sequence homology. *Int Arch Allergy Immunol* 2005; 138:273-277.
81. Magni C, Herndl A, Sironi E, et al. One- and two-dimensional electrophoretic identification of IgE-binding polypeptides of *Lupinus albus* and other legume seeds. *J Agric Food Chem* 2005; 53: 4567-4571.
82. Lucas JS, Grimshaw KE, Collins K, et al. Kiwi fruit is a significant allergen and is associated with differing patterns of reactivity in children and adults. *Clin Exp Allergy* 2004; 34:1115-1121.
83. Lucans JS, Lewis SA, Trewin JB, et al. Comparison of the allergenicity of *Actinidia deliciosa* (kiwi fruit) and *Actinidia chinensis* (gold kiwi). *Pediatr Allergy Immunol* 2005; 16:647-654.
84. Tamburrini M, Cerasuolo I, Carratore V, et al. Kiwellin, a novel protein from kiwi fruit. Purification, biochemical characterization and identification as an allergen. *Protein J* 2005; 24:423-429.
85. Carnes J, Ferrer A, Fernandez-Caldas E. Allergenicity of 10 different apple varieties. *Ann Allergy Asthma Immunol* 2006; 96:564-570.
86. Puumalainen TJ, Poikonen S, Kotovuori A, et al. Napins, 2S albumins, is major allergens in oilseed rape and turnip rape. *J Allergy Clin Immunol* 2006; 117:426-432.
87. Ahrazem O, Ibanez MD, Lopez-Torrejon G, et al. Orange germin-like glycoprotein Cit s 1: an equivocal allergen. *Int Arch Allergy Immunol* 2006; 139:96-103.
88. Egger M, Mutschlechner S, Wopfner N, et al. Pollen-food syndromes associated with weed pollinosis: an update from the molecular point of view. *Allergy* 2006; 61:461-476.
89. Hemmer W, Focke M, Gotz M, et al. Sensitization to *Ficus benjamina*: relationship to natural rubber latex allergy and identification of foods implicated in the Ficus-fruit syndrome. *Clin Exp Allergy* 2004; 34:1251-1258.
90. Blanco C. Latex-fruit syndrome. *Curr Allergy Asthma Rep* 2003; 3:467-453.
91. Yagami T, Osuna H, Kuono M, et al. Significance of carbohydrate epitopes in a latex allergen with beta-1,3-glucanase activity. *Int Arch Allergy Immunol* 2002; 129:27-37.
92. Purohit-Sheth TS, Carr WW. Oral allergy syndrome (pollen-food allergy syndrome). *Allergy Asthma Proc* 2005; 26:229-230.

93. Hoffman HJ, Skjold T, Raithel M, et al. Response of respiratory flour allergics in an ingested flour challenge may involve plasmacytoid dendritic cells, CD25+ and CD152+ T cells. *Int Arch Allergy Immunol* 2006; 140:252-260.
94. Goetz DW, Whisman BA. Occupational asthma in a seafood restaurant worker: cross-reactivity of shrimp and scallops. *Ann Allergy Asthma Immunol* 2000; 85:461-466.
95. Bock SA, Munoz-Furlong A, Sampson. Fatalities due to anaphylactic reactions to foods. *J Allergy Clin Immunol* 2001; 107:191-193.
96. Anibarro B, Seone FJ, Mugica MV. Involvement of hidden allergens in food allergic reactions. *J Investig Allergol Clin Immunol* 2007; 17:168-172.
97. Rojas-Hijazo B, Garcés MM, Caballero ML, et al. Unsuspected lupine allergens hidden in food. *Int Arch Allergy Immunol* 2006; 141(1):47-50.
98. Schäppi GF, Konrad V, Imhof D, et al. Hidden peanut allergens detected in various foods: findings and legal measures. *Allergy* 2001; 56:1216-1220.
99. Bischoff SC. Food allergies. *Curr Treat Options Gastroenterol* 2007; 10:34-43.
100. Bischoff S, Crowe SE. Gastrointestinal food allergy: new insights into pathophysiology and clinical perspectives. *Gastroenterology* 2005; 128:1089-1013.
101. Sicherer SH. Clinical aspects of gastrointestinal food allergy in childhood. *Pediatrics* 2003; 111:1609-1616.
102. Bock SA, Munoz-Furlong A, Sampson HA. Fatalities due to anaphylactic reactions to foods. *J Allergy Clin Immunol* 2001; 107:191-193.
103. Asero R, Ballmer-Weber BK, Beyer K, et al. IgE-mediated food allergy diagnosis: current status and new perspectives. *Mol Nutr Food Res* 2007; 51:135-147.
104. Kiel T. Epidemiology of food allergy: what's new? A critical appraisal of recent population-based studies. *Curr Op Allergy Clin Immunol* 2007; 7:259-263.
105. Nowak-Węgrzyn A. Food allergy to proteins. In: Cooke RJ, Vanderplas Y, Wahn U, eds. *Nutrition Support for Infants at Risk*. Nestle Nutr Workshop Ser Pediatr Program 2007; 59:17-35.
106. Sampson HA. Utility of food-specific concentrations in predicting symptomatic food allergy. *J Allergy Clin Immunol* 2001; 107:891-896.
107. Sporik R, Hill DJ, Hosking CS. Specificity of allergen skin testing in predicting positive food challenges to milk, egg, and peanut in children. *Clin Exp Allergy* 2000; 30:1540-1546.
108. Perry TT, Matsui EC, Conover-Walker M, et al. The relationship of allergen-specific IgE levels and oral food challenge outcome. *J Allergy Clin Immunol* 2004; 114:144-149.
109. Celik-Bilgili S, Mehl A, Verstege A, et al. The predictive value of specific immunoglobulin E levels in serum for the outcome of oral food challenges. *Clin Exp Allergy* 2005; 35:268-273.
110. Nowak-Węgrzyn A. Food allergy to proteins. *Nestle Nutr Workshop Ser Pediatr Program* 2004; 59:17-36.
111. Leung DY, Sampson HA, Yunginger JY, et al. Effect of anti-IgE therapy in patients with peanut allergy. *New Eng J Med* 2003; 348:986-993.
112. Li XM, Zhang TF, Huang CK, et al. Food allergy herbal formula-1 (FAHF-1) blocks peanut-induced anaphylaxis in a murine model. *J Allergy Clin Immunol* 2001; 108:639-646.
113. Bolhaar T, Tiemessen MM, Zuidmeer L, et al. Efficacy of birch-pollen immunotherapy on cross-reactive food allergy confirmed by skin tests and double-blind food challenges. *Clin Exp Allergy* 2004; 34:761-769.
114. Zhu D, Kepley CL, Zhang K, et al. A chimeric human-cat fusion protein blocks cat-induced allergy. *Nat Med* 2005; 60:11:446-449.
115. Frick OL, Teuber SS, Buchanan BB, et al. Allergen immunotherapy with heat-killed *Listeria monocytogenes* alleviates peanut and food-induced anaphylaxis in dogs. *Allergy* 2005; 60:243-250.
116. Muraro A, Dreborg S, Halken S, et al. Dietary prevention of allergic disease in infant and small children. Part III: Critical review of published peer-reviewed observations and interventional studies and final recommendations. *Pediatr Allergy Immunol* 2003; 15:291-307.
117. American Academy of Pediatrics Committee on Nutrition. Hypoallergenic infant formulas. *Pediatrics* 2000; 106:346-349.
118. Astier C, Morisset M, Roitel O, et al. Predictive value of skin prick tests using recombinant allergens for diagnosis of peanut allergy. *J Allergy Clin Immunol* 2006; 118:250-256.
119. Nowak-Węgrzyn A, Sampson HA. Food allergy therapy. *Immunol Allergy Clin North Am* 2004; 113:705-725.
120. Sampson HA. Utility of food-specific IgE concentrations in predicting food allergy. *J Allergy Clin Immunol* 2001; 107:891-896.
121. Sampson HA, Ho DG. Relationship between food-specific IgE concentrations and the risk of positive food challenges in children and adolescents. *J Allergy Clin Immunol* 1997; 100:444-451.
122. McCone KL, Sickles-Wright H, Birch LL, et al. Food portions are positively related to energy intake and body weight in children. *J Pediatr* 2002; 140:340-347.

123. Moneret-Vautrin DA, Kanny G. Update on threshold doses of food allergens: implications for patients and the food industry. *Curr Opin Allergy Clin Immunol* 2004; 4:215-219.
124. Leung DYM, Sampson HA, Yunginger JY, et al. Effect of anti-IgE therapy in patients with food allergy. *NEJM* 2003; 348:986-993.
125. Pons L, Palmer K, Burks W. Towards immunotherapy for peanut allergy. *Curr Opin Allergy Clin Immunol* 2005; 5:558-562.
126. Morriset M, Moneret-Vautrin DA, Guenard L, et al. Oral desensitization in children with milk and egg allergies obtains recovery in significant proportion of cases. A randomized study of 60 children with cow's milk allergy and 90 children with egg allergy. *Allerg Immunol (Paris)* 2007;39:12-19.
127. Malmheden Yman I, Eriksson A, Everitt G, et al. Analysis of food proteins for verification of contamination or mislabeling. *Food Agric Immunol* 1994; 6:167-172.
128. Taylor SL, Hefle S, Bindslev-Jensen C, et al. Factors affecting the determination of threshold doses for allergenic foods: how much is too much? *J Allergy Clin Immunol* 2002; 109:24-30.
129. Morisset M, Moneret-Vautrin DA, Kanny G, et al. Thresholds of clinical reactivity to milk, egg, peanut and sesame in immunoglobulin E-dependent allergies: evaluation by double-blind or single-blind placebo-controlled oral challenges. *Clin Exp Allergy* 2003; 33:1046-1051.
130. Thomas K, Herouet-Guicheney C, Ladics G, et al. Evaluation the effect of food processing on the potential human allergenicity on novel proteins: international workshop report. *Food Chem Toxicol* 2007; 45:1116-1122.
131. Bohle B. T-cell epitopes of food allergens. *Clin Rev Allergy Immunol* 2006; 30:97-108.
132. Scott-Taylor TH, Hourihane JB, Harper J, et al. Patterns of food allergen-specific cytokine production by T lymphocytes of children with multiple allergies. *Clin Exp Allergy* 2005; 35:1473-1480.
133. Turcanu V, Maleki SJ, Lack G. Characterization of lymphocyte responses to peanuts in normal children, peanut-allergic children and allergic children who acquired tolerance to peanuts. *J Clin Invest* 2003; 111:1065-1072.
134. Noma T, Yoshizawa I, Aoki K, et al. Cytokine production in children outgrowing hen egg allergy. *Clin Exp Allergy* 1996;26:1298-1307.
135. Thottungal TB, Setfura BP, Simons FER, et al. Human subjects without peanut allergy demonstrate T cell-dependent, Th2-based, peanut-specific cytokine and chemokine responses independent of th1 expression. *J Allergy Clin Immunol* 2006; 118:905-914.
136. Karlsson MR, Rugtveit J, Brandtzaeg P. Allergen-responsive CD4+CD25+ regulatory T cells in children who have outgrown cow's milk allergy. *J Exp Med* 2004; 199:1679-1688.
137. Vieths S, Scheurer S, Ballmer-Weber B. Current understanding of cross-reactivity of food allergens and pollen. *Ann N Y Acad Sci* 2002; 964:47-68.
138. Holm J, Gajhede M, Ferreras M, et al. Allergy vaccine engineering: epitope modulation of recombinant Bet v 1 reduces IgE-binding but retains protein folding pattern for induction of protective blocking-antibody responses. *J Immunol* 2004; 173:5258-5267.
139. Reese G, Ayuso R, Leon-Kee SM, et al. Characterization and identification of allergen epitopes: recombinant peptide libraries and synthetic, overlapping peptides. *J Chromatogr B Biomed Sci Appl* 2001; 756:157-163.
140. Mirza O, Henriksen A, Ipsen H, et al. Dominant epitopes and allergic cross-reactivity: complex formation between a fab fragment of a monoclonal murine IgG antibody and the major allergen from birch pollen. *J Immunol* 2000; 165:331-338.
141. Wiche R, Gubesch M, Konig H, et al. Molecular basis of pollen-related food allergy: identification of a second cross-reactive IgE epitope on Pru a 1, the major cherry allergen (*Prunus avium*). *Biochem J* 2005; 385:319-327.
142. Neudecker P, Lehmann K, Nerkamp J, et al. Mutational epitope analysis of Pru av 1 and Api g 1, the major allergens of cherry (*Prunus avium*) and celery (*Apium graveolens*): correlating IgE reactivity with three-dimensional structure. *Biochem J* 2003; 15; 376:97-107.
143. Suphioglu C, Schäppi G, Kenrick J, et al. A novel grass pollen allergen mimotope identified by phage display peptide library inhibits allergen-human IgE antibody interaction. *FEBS Lett* 2001; 27:502:46-52.
144. Mittag D, Batori V, Neudecker P, et al. A novel approach for investigation of specific and cross-reactive IgE epitopes on Bet v 1 and homologous food allergens in individual patients. *Mol Immunol* 2006; 43:268-278.
145. Aalberse RC, Stadler BM. In silica predictability of allergenicity: from amino acid sequence via 3-D structure to allergenicity. *Mol Nutr Food Res* 2006; 50:625-627.
146. Untersmayr E, Jensen Jarolim E. Mechanisms of type I food allergy. *Pharmacol Ther* 2006; 112:787-798.