

Food allergens

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A number of advances in the scientific knowledge concerning adverse food reactions have been made in the past few years. Understanding about the nature of the food allergen itself, the molecular characterization of the epitopes on these allergens, the pathophysiology of the clinical reaction, and the diagnostic methods have all been significantly enhanced. *Curr Opin Allergy Clin Immunol* 1:243–248. © 2001 Lippincott Williams & Wilkins.

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Abbreviation

RAST radioallergosorbent test

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Introduction

Part of the difficulty in understanding adverse food reactions has been the nomenclature used in this literature. Standardized definitions are beginning to ensure that the scientific literature is more uniform (Table 1). An adverse food reaction is a generic term referring to any untoward reaction after the ingestion of a food. Adverse food reactions may be secondary to food allergy (hypersensitivity) or food intolerance. A food-allergic reaction is presumed to be the result of an abnormal immunological response after the ingestion of a food, whereas food intolerance is the result of non-immunological mechanisms [1,2].

The true prevalence of adverse food reactions is unknown. In American households approximately one-third of families believe some family member to be affected. The best studies to date indicate that approximately 6–8% of young children and 1–2% of adults have some type of food allergy [3].

Food allergens

Foods are composed of proteins, carbohydrates, and lipids. The major food allergens have been identified as water-soluble glycoproteins having molecular masses ranging from 10 000 to 60 000 M_r. There are no known, unique biochemical or immunochemical characteristics of food allergens. Comparisons of primary amino acid sequences of allergenic proteins have not revealed conserved patterns. However, food allergens tend to be resistant to usual food processing and preparation conditions. These proteins are thus comparatively resistant to heat and acid treatment, proteolysis, and digestion. The treatment of food allergens with acid concentrations simulating stomach conditions typically has little effect on the specific IgE binding of the allergen. There are, however, important exceptions, such as the major allergens in fresh fruits and some vegetables.

Food allergens, in general, are soluble in water or saline solutions, thus belonging to the classes known as albumins (water soluble) or globulins (saline soluble). Although the level of exposure to a specific protein necessary to sensitize an individual is unknown, extremely low levels of the offending food can initiate a reaction in individuals with pre-existing IgE-mediated food allergies. Microgram to milligram quantities of peanut has elicited an adverse reaction in food challenges in selected individuals. The immunochemical or physicochemical properties that account for such unique allergenicity of food allergens are poorly understood.

Recent work has identified plant food allergens that are homologous to pathogenesis-related proteins that are induced by pathogens [4,5]. Examples include: the chitinases (PR-3 family) from avocado, banana, and chestnut; antifungal proteins such as the thaumatin-like proteins (PR-5) from cherry and apple; and the lipid transfer proteins (PR-14) from fruits and vegetables [6,7].

Major food allergens

The most common foods to cause documented IgE-mediated reactions in childhood are cow's milk, eggs, peanuts, soybeans, wheat, fish, and tree nuts (Table 2). Approximately 80% of these reactions are secondary to either milk, eggs, or peanuts. In adulthood, the most common food allergens are peanuts, tree nuts, fish, and shellfish. Worldwide, there are some differences in the foods that cause problems in both children and adults in different countries primarily because of the diet of the population.

Cow's milk

The prevalence of cow's milk allergy in infants and children, worldwide, is estimated at between 2.0 and 2.5%. Allergic symptoms related to cow's milk often begin in early childhood, but children typically lose their sensitivity in the first 3–5 years of life. Cow's milk consists of a number of different proteins, traditionally divided into caseins, which constitute 80% of the total protein, and whey proteins, which constitute 20% of the total protein. Most patients allergic to cow's milk have specific-IgE antibodies to more than one of the milk proteins. Caseins were originally defined as phosphoproteins that precipitate from raw skim milk upon acidification to pH 4.6 at 20°C; whey proteins are those proteins remaining in the milk after the precipitation of caseins. 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 milk 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 (Table 3).

A number of milk proteins have been identified as allergens in humans. Using either skin-prick testing or oral challenge many patients have been found to have reactivity to multiple cow's milk proteins. Caseins and beta-lactoglobulin appear to be the major allergens in cow's milk. The caseins are a family of proteins (alpha, beta, and kappa) that are chemically related. The major alpha- and beta-caseins have a molecular mass of approximately 23 000 M_r. There are several genetic variants of each of these caseins. Beta-lactoglobulin (17 000 M_r), the most abundant whey protein, also has several genetic variants. Alpha-lactalbumin (14 000 M_r) and bovine serum albumin (67 000 M_r), both whey proteins, appear to be minor cow's milk allergens. Bovine serum albumin has also been identified as a distinct milk allergen. This protein is heterogeneous in nature and has a molecular mass of 67 000 M_r making up approximately 1% of the total milk protein.

Eggs

Egg allergy is one of the most commonly implicated causes of food allergic reactions both in the United

Table 1. Definitions of adverse food reactions

Adverse food reaction	Generic term referring to any untoward reaction after the ingestion of a food
Food allergy (hypersensitivity)	The result of an abnormal immunological response after the ingestion of a food
Food intolerance	The result of non-immunological mechanisms after the ingestion of a food

Table 2. Major food allergens in children and adults

Children	Adults
Milk	Peanuts
Egg	Tree nuts
Peanuts	Fish
Soybeans	Shellfish
Wheat	
Fish	
Tree nuts	

Table 3. Purified antigens in foods

Protein fraction	Molecular mass
Cow's milk	
Caseins	19 000–24 000
α-casein	27 000
αs-casein	23 000
β-casein	24 000
κ-casein	19 000
γ-casein	21 000
Whey	
β-lactoglobulin	36 000
α-lactoglobulin	14 400
Bovine serum albumin	69 000
Chicken egg white	
Ovalbumin	45 000
Ovomucoid	28 000
Ovotransferrin	77 700
Lysozyme	14 300
Peanut	
Ara h 1	63 500
Ara h 2	17 500
Ara h 3	56 000
Soybean	
Gly m 1	34 000
Soybean trypsin inhibitor	20 500
Fish	
Allergen M (Gad c 1)	12 328
Shrimp	
Antigen I	42 000
Antigen II	38 000
Pen a 1	36 000

States and Europe. Eggs from chickens (*Gallus domesticus*) are widely used for human consumption. Although there is extensive cross-reactivity among the various birds, hen eggs tend to be slightly more allergenic than duck eggs. Eggs are composed of egg white and egg yolk. The egg white (albumin) appears to be more allergenic than the yolk. The major protein in the egg white is ovalbumin, with other proteins including ovotransferrin, ovomucoid, ovomucin, and lysozyme. Egg yolk can be separated into two fractions using ultracentrifugation. This results in a granular fraction that contains primarily protein and a supernatant fraction that contains primarily lipid. The granular fraction contains lipovitellin, phosvitin and LDL.

Ovomucoid (Gal d 1), a glycoprotein with a molecular mass of 28 000 M_r and an acidic isoelectric point has been implicated as the major allergen in egg. In a recent study [8], purified ovomucoid was found to be a more potent allergen than purified ovalbumin by skin-prick testing and radioallergosorbent test (RAST) in a group of children with egg allergy. Whereas previous studies had shown that ovalbumin was the major egg allergen, the recent work demonstrated ovomucoid contamination in the previously isolated ovalbumin.

Ovalbumin (Gal d 2) is a monomeric phosphoglycoprotein with a molecular mass of 43 000–45 000 M_r and an acidic isoelectric point. Purified ovalbumin has three primary variants, A₁, A₂, and A₃. Because of the previous ovomucoid contamination of ovalbumin, it is difficult to determine the exact role of this allergen. Ovotransferrin (Gal d 3; conalbumin) has a molecular mass of 77 000 M_r and an acidic isoelectric point. It has antimicrobial activity and iron-binding properties. Lysozyme (Gal d 4) is a lower molecular mass allergen (14 300 M_r), which in some studies has appeared to be a major allergen, but in other studies has been thought to be a minor allergen. Other minor allergens in eggs include apovitellin, ovomucin and phosvitin. Additional studies have shown that the carbohydrate portion of the glycoproteins in eggs, particularly in ovomucoid, do not play a primary role in specific IgE binding. B and T cell epitopes have been mapped in a limited way for both ovalbumin and ovomucoid.

Peanuts

The peanut is an annual plant in the family Leguminosae. In the United States several varieties including the Virginia, Spanish and runner are grown. Most of the peanut crop in the United States is used for the production of peanut butter. Runner types are used most frequently for oil production and peanut butter. Children are increasingly being exposed to peanut products at an early age. Allergic reactions to peanuts are often very acute and severe, accounting for many of

the cases of food-induced anaphylaxis documented each year [9].

Peanut proteins are customarily classified as albumins (water soluble) and globulins (saline soluble). The globulin proteins consist of two major fractions, arachin and conarachin (also known as legumine and vicilin, respectively). Arachin in its native state exists as a molecule of at least 600 000 M_r and readily dissociates into a 340 000–360 000 M_r dimer and a monomer of approximately 170 000–180 000 M_r . Conarachin can be divided by ultracentrifugation into two fractions, one 2S and one 8.4 S.

A number of peanut allergens have been identified and characterized. Peanut-1 and concanavalin A-reactive glycoprotein were two of the first peanut allergens to be partly characterized. Ara h 1 is a 63 500 M_r glycoprotein identified as a major peanut allergen using immunoblotting and enzyme-linked immunosorbent assay methods [10]. This allergen has an acidic isoelectric point and is relatively resistant to enzyme degradation. Molecular studies have identified multiple IgE binding sites in the amino acid sequence of Ara h 1. This peanut allergen has at least 23 specific IgE binding epitopes along its amino acid sequence. Ara h 1 has been identified as a member of the vicilin family of seed storage proteins. Ara h 2 is a 17 000 M_r allergen with an acidic isoelectric point. This allergen has at least 10 specific IgE binding epitopes along its amino acid sequence. Ara h 2 appears to be a member of the conglutin family of seed storage proteins. Ara h 3 is another peanut allergen of 56 000 M_r , and is a member of the glycinin family of seed storage proteins.

Soybeans

Soybeans, although not implicated as often as milk, eggs, and peanuts, are one of five major allergens in the United States causing food allergic reactions in children. Soybean globulins are the major proteins of the soybean. Soybean globulins can be separated into ultracentrifugation components identified as 2S, 7S, 11S, and 15S fractions. Alpha-conglycinin is a primary protein of the 2S fraction whereas beta-conglycinin is the primary fraction of the 7S component. The glycinin fraction is the primary component of the 11S ultracentrifugation fraction.

Soybeans, like peanuts, are legumes that have multiple allergens. When examining specific IgE to the ultracentrifugation components, authors have primarily identified either the 2S or 7S fraction as containing the primary allergens. Gly m 1 is a 30 000 M_r allergen, which is a component of the 7S fraction. Gly m 1 has an acidic isoelectric point. It has sequence homology to a soybean seed 34 000 M_r oil-body-associated protein (called soy-

bean vacuolar protein P34). There appear to be at least 16 distinct soybean-specific IgE binding epitopes along the amino acid sequence of this allergen. The Kunitz soybean trypsin inhibitor binds to soybean-specific IgE in soybean allergic patients, although only in a minority of patients (making it likely a minor allergen).

Wheat

Although not the most common source of food allergy, wheat and other cereal grains are often implicated as food allergens, particularly in children. The proteins of wheat include the water-soluble albumins, the saline-soluble globulins, the ethanol-soluble prolamins, and the glutelins. It is not uncommon for children to have multiple positive skin-prick tests to various cereal grains, while having clinical reactivity to only one of the foods. This extensive cross-reactivity is probably caused by non-specific IgE binding to the lectin fractions in cereal grains. Patients with wheat allergy apparently have specific IgE binding to wheat fractions of 47 000 and 20 000 M_r (proteins not recognized by the serum from patients with grass allergy) [11]. Wheat alpha amylase inhibitor (15 000 M_r) also appears to be a major wheat allergen. This protein does not bind IgE from wheat-tolerant control patients, including those with grass allergy.

Fish

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 in which fish consumption is greatest. For example, codfish allergy is extremely common in the Scandinavian countries. One of the most comprehensive descriptions of a food allergen has been the work by Elsayed and Apol [12] on the codfish allergen, Gad c 1 (originally designated allergen M). Gad c 1 belongs to a group of muscle proteins known as parvalbumins [13•]. 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 mass of 12 000 M_r. The tertiary structure of Gad c 1 exhibits 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. Recent work has characterized fish parvalbumin as a major IgE-binding fish allergen. This protein has been identified as a calcium-binding protein that has remarkable stability and refolding capacity. More than 95% of fish-allergic patients had specific IgE to this protein. Interestingly, many of the IgE-binding epitopes on this allergen were present on other species of fish.

Tree nuts

Tree nuts are common causes of food allergic reactions in both children and adults. Like allergic reactions to fish

and peanuts, reactions to tree nuts tend to persist throughout the lifetime of an individual. Two major allergens have been identified in almonds. The allergens are a 70 000 M_r heat-labile protein and a 45 000–50 000 M_r heat-stable protein. In Brazil nuts, the major allergen is Ber e 1, a high-methionine, 12 000 M_r protein. Work with the walnut allergens has identified a major allergen as a 65 000 M_r glycoprotein, similar to other plant vicilins.

A recent study [14] has identified Jug r 2 as a major allergen in walnuts. This protein has significant homology with the vicilin family of seed storage proteins like major allergens in peanuts and soybeans.

Shrimp

Shrimp is the most studied of the crustacea allergens. The original two fractions characterized in shrimp were antigen I (45 000 M_r) and antigen II (38 000 M_r). SA-II was the next major allergen characterized in shrimp, SA-II appears similar to antigen I that had previously been described. Pen a 1 was identified as a major allergen from boiled brown shrimp (isolated in boiled water) and thus thought to be similar to SA-II. This allergen has a molecular mass of 36 000 M_r and constitutes 20% of the soluble protein in crude cooked shrimp. Pen a 1 binds shrimp-specific IgE in over 85% of patients with shrimp allergy studied to date. Another shrimp allergen, Met e 1, has been isolated from another species of shrimp and has a molecular mass of 34 000 M_r. Pen a 1 and Met a 1 allergens are highly homologous with tropomyosin from a variety of species. Immunological relationships between crustaceans, cockroaches and housedust mites have been established and may suggest tropomyosin as an important cross-sensitizing pan allergen [15].

Food allergen cross-reactivity

Within certain food groups, we have a much better understanding of the cross-reactivity of specific foods.

Cow's milk

Immunoblotting and crossed-radioimmuno-electrophoresis studies have shown extensive milk-specific IgE cross-reactivity among milk proteins in cows, goats, and sheep (Table 4). We know that at least 50% of cow's milk allergic individuals were also allergic to goat's milk. Clinical practice indicates that patients allergic to one type of milk protein will not tolerate milk proteins of other species.

Legumes

Extensive in-vitro allergenic cross-reactivity in the legume family has been documented. A clinical study of 57 patients with legume sensitivity to peanuts [16], soybeans, peas, and lima beans revealed extensive in-vitro IgE cross-reactivity but minimal in-vivo clinical

Table 4. Food allergen cross-reactivity

	Specific IgE to multiple members of the family	Clinical cross-reactivity
Milk	Common	Common
Legumes	Common	Uncommon
Wheat	Common	Uncommon
Fish	Common	Uncommon
Crustacea: molluscs	Common	??
Tree nuts	Common	Uncommon
Egg: chicken	Occasional	Rare
Milk: beef	Occasional	Uncommon

cross-reactivity. Specifically 59% of skin-test-positive patients reacted to oral challenge to one of the legumes, but only 5% of the patients reacted to oral challenge to more than one legume. Whereas patients with peanut-specific IgE may have clinical reactions to other legumes, these reactions will be quite uncommon and should be evaluated on an individual basis.

Fish

In one study of 11 children allergic to fish on oral blinded challenge, one reacted to two fish, two reacted to three fish, and one patient did not react to any fish [17]. In general, fish-allergic adults have more in-vivo cross-reactivity than do children. Not only do adults have fish-specific IgE to multiple species of fish, they are also more likely to have adverse reactions to more than one species on oral challenges.

Crustacea and molluscs

Patients who have positive skin-prick tests or RAST to crustacea tend to react positively to multiple members of this family. In particular, individuals with shrimp allergy exhibit positive skin-prick tests and RAST to other crustaceans. Extracts from shrimp, blue crab, and crawfish all inhibit Pen a 1 RAST to a similar extent. There is little oral challenge data to know the extent of clinical reactivity among the different crustacea.

Although molluscs are much less commonly allergenic than crustacea, there is some in-vitro cross-reactivity among oyster (molluscs) and the crustacea. Shrimp, blue crab, spiny lobster, and crawfish were all highly cross-reactive with oyster. Again, the extent of clinical cross-reactivity has not been studied sufficiently. Therefore, clinical advice to patients must be individualized.

Tree nuts

A variety of nuts have caused anaphylactic reactions in children and adults. In one study [18], 14 children underwent 19 blinded challenges to nuts; one patient reacted to five nuts, one to two nuts, and the remaining 12 children to one nut each. Overall, there were seven reactions to walnuts, six to cashews, three to pecans, two to pistachios, and one to filbert. Adults allergic to nuts

generally do not need to avoid peanuts (a legume), and vice versa, although children with peanut allergy appear to be more likely to develop allergy to tree nuts than the general population.

Tree nuts: coconuts

Interestingly, a recent report [19] showed that two patients with walnut allergy had systemic reactions after the ingestion of coconut. The investigators were able to identify a 35 000 M_r allergen that was immunologically similar to soy glycinin (in the legumin family of seed storage proteins).

Conclusion

The application of molecular biology to the study of food allergens has transformed this area of research. Each year a number of new allergens continues to be identified.

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