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4 Toxicants in Food: Food Allergens

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Adverse reactions to foods

Adverse reactions to foods, although caused by different mechanisms, can be confused with one another since they elicit similar symptoms (Sampson and Metcalfe, 1991). In the broadest terms, an adverse reaction to a food is defined as a clinically abnormal response attributed to exposure to foods or food additives. This includes both immunologic and non-immunologic reactions (Table 4.1). 'True' food allergy, or food hypersensitivity, is an immunologically mediated adverse reaction and occurs only in specifically sensitised individuals. Most food allergies are mediated by IgE antibodies and have a rapid onset, typically within minutes of exposure (Sampson and Metcalfe, 1991). Allergens, as any other antigens, are able to induce a specific humoral and/or cellular immune response. However, allergens induce a Th-2 rather than a Th-1 lymphocyte response, thus resulting in the production of allergen-specific IgE. IgE antibodies bind to specific receptors on mast cells or basophils (the effector cell) and when allergen reaches the sensitised mast cell, it cross-links surface-bound IgE ('bridging') triggering the release of preformed and newly synthesised mediators. These mediators, in turn, elicit the clinical signs and symptoms of allergic diseases including asthma, eczema, hay fever and anaphylaxis (Geha, 1984; Roitt *et al.*, 1985). There is evidence for genetic control of IgE antibody production to specific allergens (Marsh *et al.*, 1974; Blumenthal *et al.*, 1981) even though the immunological mechanisms involved or the structural features of allergens that distinguish them from other tolerated food antigens are not fully understood.

Other adverse reactions to foods are food intolerance, an abnormal physiological response to ingested food or food additive; food poisoning, which is basically a toxic reaction; and pharmacological food reactions, due to

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Table 4.1 Classification of adverse reactions to foods

Type of adverse reaction		Mechanism
Immunologically mediated (food allergy)	IgE-mediated	Cross-linking (bridging) of cell-bound allergen-specific IgE by allergen
	Not IgE-mediated	Diseases such as gastroenteropathy and coeliac disease; role of food in immunological mechanism is unknown
Not immunologically mediated (food intolerance)	Toxic	Caused by naturally occurring toxins, food-processing-induced compounds, or contaminants
	Enzymic	Lactose intolerance
	Pharmacological	Individuals with high reactivity to substances such as vasoactive amines (histamine)
	Undefined	Mechanism unknown, e.g. some reactions to food additives

Adapted from Anderson (1996).

substances such as biogenic amines (e.g. histamine) that produce a drug-like effect and mimic symptoms of food allergy.

Diagnosis of food allergy

The diagnosis of food allergy is still considered difficult. A major problem is that many extracts used for *in vivo* and *in vitro* diagnosis are neither well-characterised and most are not well-standardised; the allergenic potency of some of these extracts is at best questionable. Even the value of the 'gold standard' of food allergy diagnosis, the double-blind, placebo-controlled food challenge (DBPCFC) may be impaired by low-quality extracts since DBPCFC is only as accurate as the activity of the food material used in an oral challenge test (Atkins *et al.*, 1985; Sampson and Metcalfe, 1991).

EXTRACTS FOR DIAGNOSTIC USE

In contrast to extracts of many inhalant and stinging insect allergens such as grass pollen, house dust mite and bee venom extract, extracts of allergenic foods are only used in food allergy diagnosis but not for therapy (immunotherapy of food allergy is not considered to be safe and advantageous). The most important requirement of a well-prepared extract is that it contains relevant allergens in an immunologically active form in sufficient amounts. An extract

containing immunologically inactive allergens or only a small amount of active allergen may yield false negative results. For example, apple extracts and extracts of other fruits are frequently inactive, and fresh fruits rather than extracts are often recommended for use in skin prick testing. Other extracts such as peanut or shrimp, that contain more stable food allergens are more reliable, but can also vary in their allergen content (Jeoung *et al.*, 1997). In general, the quality of food extracts has to be improved to obtain reliable, standardised reagents since the reliability of *in vitro* and *in vivo* diagnostic tests and procedures depends on high-quality extracts.

IN VIVO DIAGNOSIS

A skin test is the most common *in vivo* method to demonstrate sensitisation to an allergen. Allergen is pricked (skin prick test) or injected intracutaneously (intracutaneous test) into the skin. If the subject is sensitised, a localised allergic reaction (wheal-and-flare reaction) occurs. The skin prick test is the preferred method over intracutaneous testing since it does not result in as many false positive reactions and the risk of systemic reactions is diminished.

As mentioned earlier, the DBPCFC is considered the gold standard for the diagnosis of adverse reactions to foods (Bock *et al.*, 1988). The subject ingests masked allergen or placebo, and any reactions and symptoms are noted. Neither subject nor physician have prior knowledge whether the oral provocation is performed with allergen or with placebo. It is important that the allergen is carefully masked, so that the subject cannot identify the allergen-containing sample by smell, taste or texture.

IN VITRO DIAGNOSIS

In vitro tests measure allergen-specific IgE. The detection of specific IgE in an of itself is not sufficient to diagnose an allergy since the presence of specific IgE does not always correlate with clinical symptoms. RAST (radio allergosorbent test) is the most commonly used assay to measure allergen-specific IgE. CAP and ELISA are performed according the same principle, using solid-phase bound allergen which is probed with patient's serum and a labelled, IgE specific antibody or antiserum. Immunoblotting is useful as a research and diagnostic tool to identify IgE antibody reactivity to individual allergenic molecules in crude, multi-allergen extracts.

Radio allergosorbent test (RAST)

In the RAST, the allergen extract is covalently bound to a suitable solid phase, frequently a paper disc. The disc is incubated with serum from an allergic subject, allowing the allergen-specific IgE antibodies to bind to the allergen. The bound IgE antibodies are then detected with ^{125}I-labelled anti-human IgE

Histamine release test

This test measures the amount of histamine released from blood basophils. Allergen is added to peripheral blood cells from allergic subjects, basophils carrying allergen-specific IgE antibodies degranulate, and released histamine is measured by fluorescence or radioimmuno assay (RIA). This test is not routinely used in allergy *in vitro* diagnosis as the test requires viable blood cells and must be performed within a short time after the blood is drawn.

Allergen characterisation

Identifying and characterising individual allergens in allergenic foods is a step to improve extract quality and to study and understand the interactions between the immune system and allergen on a molecular level. Allergen characterisation encompasses the detection of individual allergens in a given allergenic source, allergen identification, and analysis of immunological properties of its structural features. This encompasses the characterisation of their primary structures and the identification of portions of the allergen molecule (epitopes) which interact with antibodies or cells of the immune system. Epitopes that bind to peptide-specific receptors on T cells are called T-cell epitopes, whereas epitopes that bind to B cell-produced antibodies are called B-cell epitopes. In general, it is thought that T-cell epitopes do not depend on protein conformation (linear epitopes) whereas B-cell epitopes do (conformational epitopes). However, there may be exceptions to this rule (King, 1994).

ALLERGEN CLASSIFICATION AND NOMENCLATURE

The most general classification of allergenic proteins used is the division into major and minor allergens. According to this definition, major allergens are defined as allergens to which >50% of specifically sensitised allergic subjects react; minor allergens are detected by < 50% of allergic subjects. For example, Pen a 1 is a major shrimp allergen because >80% of all shrimp-allergic subjects have Pen a 1-specific IgE antibodies. This classification does not include the percentage of specific IgE bound by a particular allergen molecule and may not necessarily reflect the clinical importance of a particular allergen.

According to nomenclature rules (King *et al.*, 1995), allergens are designated according to the accepted taxonomic name of their source. The first three letters of the genus, followed by a space, the first letter of the species' name, another space, and an arabic number. Numbers are assigned in chronological order of characterisation and homologous allergens from related species are generally assigned the same number. For example, the first allergen described in brown shrimp, *Penaeus aztecus*, was designated Pen a 1 and the homologous allergens in Indian shrimp, *P. indicus*, and greasyback shrimp (*Metapenaeus ensis*) are named Pen i 1 and Met e 1, respectively. However, these nomenclature rules are not strictly applied to all major allergens, some well-known proteins that were studied for other purposes than their later discovered allergenicity are not named according to these roles. For example, the well-characterised milk allergen β-lactalbumin is not named according to the nomenclature rules.

METHODS TO DETECT INDIVIDUAL ALLERGENS

The first step of allergen characterisation is to analyse the IgE antibody response to individual proteins. This is usually achieved by separating proteins according to their biophysical properties and probing the separated proteins with sera of allergic subjects who are allergic to a particular allergen.

Crossed radio-immuno electrophoresis (CRIE) was one of the first methods to identify individual allergens (Weeke, 1973; Løwenstein *et al.*, 1976; Aukrust and Aas, 1997). CRIE provides qualitative and semiquantitative information concerning the IgE reactivity and the amount of allergen present in the extract; however, it is not widely used anymore, since this method is not suitable for general extract standardisation and allergen identification because polyclonal antisera from different immunisations or laboratories used to precipitate the allergens may vary, and allergens may be missed if the antiserum does not precipitate a particular allergen or binds to IgE-binding sites.

Immunoblotting

Immunoblotting is now the most frequently used methodology to identify and characterisation allergens. It consists basically of four steps: electrophoretic separation of proteins; transfer of separated proteins onto a carrier membrane; incubation with the subject's serum; and detection of IgE antibodies which bind to individual bands (allergens).

Allergens can be separated according to molecular size (sodium dodecylsulphate polyacrylamide gel electrophoresis, SDS–PAGE), isoelectric point (pI.) (isoelectric focusing, IEF), or two-dimensionally (2D) using IEF for the first dimension and SDS–PAGE for the second dimension. SDS–PAGE is the most frequently used separation technique even though treatment with SDS and reducing agents such as β-mercaptoethanol, dithiothreitol (DTT) or dithioerythriol (DTE) may destroy allergenic epitopes with a loss of IgE binding. Separated proteins are transferred from the gel onto carrier membranes electrophoretically (semi-dry blotting) (Kyhse-Andersen, 1984), tank blotting or if an agarose gel is used, by capillary force (Peltre *et al.*, 1982). Nitrocellulose, CNBr-activated nitrocellulose (Demeulemester *et al.*, 1987) and polyvinylidine difluoride (PVDF) membranes are the most frequently used carrier membranes. 2D immunoblotting can be a valuable tool to purify small amounts of allergen sufficient for N-terminal sequencing (Petersen *et al.*, 1993, 1994).

RAST inhibition

RAST inhibition is a modified version of the standard RAST. Serum is incubated in the presence of different concentrations of allergen extract in the fluid phase. If IgE antibodies bind to either solid phase and fluid phase allergens, IgE binding to solid phase allergens decreases with increasing inhibitor concentration. This assay format is used to trace allergens contaminating other foods (Yunginger *et al.*, 1983; Keating *et al.*, 1990; Gern *et al.*, 1991; Jones *et al.*, 1992; Nordlee *et al.*, 1993) to assess the effect of processing on the allergenicity of various products made from peanuts and soybeans (Nordlee *et al.*, 1981; Herian *et al.*, 1993), and to study cross-reactivities among allergens. RAST inhibition test was also used to identify Brazil nut allergen in transgenic soybeans (Nordlee *et al.*, 1994, 1996).

ALLERGEN IDENTIFICATION AND MOLECULAR CHARACTERISATION

The characterisation of allergen structure is a prerequisite to study the interaction of immune system and allergen on a molecular level. Without the knowledge of the amino acid sequence of an allergen, it is impossible to identify IgE binding sites (B-cell epitopes) and T-cell-binding peptides (T-cell epitopes). Different strategies have been employed; peptides, obtained from enzymatically digested, purified allergens were sequenced, and expression cDNA expression libraries were screened with IgE antibodies. Food allergens that have been characterised are summarised in Table 4.2. Since many food proteins have been studied for reasons other than their allergenicity (i.e. because they are important storage, structural and functional proteins), it is often possible to identify a food allergen by searching protein databases using only a small part of its entire amino acid sequence. In this context, electrophoretically purified and blotted allergens may provide a simple approach to obtain sufficient amounts of purified proteins for N-terminal sequencing. For example, the major shrimp allergen Pen a 1 was isolated by preparative SDS–PAGE, eluted from the gel, digested with proteinase Lys-C, and a 21 amino acid residue-long peptide was sequenced. This sequence identified Pen a 1 as the muscle protein tropomyosin (Daul *et al.*, 1994).

Common properties of food allergens

Most foods contain a large number of different proteins, though only a few of these elicit allergic reactions. This prompts the question whether allergens share common properties that distinguish them from other, non-allergenic food proteins. In our view, there is no convincing answer to this question today. However, some very broad characteristics common to several food allergens have been identified. These include abundance of a given protein in a particular food, and physicochemical properties, such as a molecular

Table 4.2 Identified and characterised major food allergens

Allergen source	Allergens (systematic and original names)	Molecular weight (kDa)	Sequence data	References[a]
Gadus calarias (cod)	Gad c 1; allergen M	12	C	Elsayed and Bennich, 1975
Gallus domesticus (chicken)	Gal d 1; ovomucoid	28	C	Hoffman, 1983
	Gal d 2; ovalbumin	44	C	Langeland, 1983b
	Gal d 3; conalbumin (Ag22)	78	C	Williams *et al.*, 1982
	Gal d 4; lysozyme	14	C	Blake *et al.*, 1965
Penaeus aztecus (brown shrimp)	Pen a 1; tropomyosin	36	P	Daul *et al.*, 1993, 1994
Penaeus indicus (Indian shrimp)	Pen i 1; tropomyosin	34	P	Shanti *et al.*, 1993
Metapenaeus enis (greasyback shrimp)	Met e 1; tropomyosin	34	C	Leung *et al.*, 1994
Brassica juncea (oriental mustard)	Bra j 1; 25 albumin	14	C	Monsalve *et al.*, 1993
Hordeum vulgare (barley)	Hor v 1; BMAI-1	15	C	Mena *et al.*, 1992
Sinapis alba (yellow mustard)	Sin a 1; 25 albumin	14	C	Menendez-Arias *et al.*, 1988
Arachis hypogea (peanut)	Ara h 1	63.5	C	Burks *et al.*, 1995a,b,c
	Ara h 2	17.5	C	Stanley *et al.*, 1997
Malus domestica (apple)	Mal d 1	17.7	C	Vanek-Krebitz *et al.*, 1995
Apium graveolens (celery)	Api g 1	16.2	C	Breiteneder *et al.*, 1995

Modified from Lehrer *et al.* (1997).
[a]References for partial (P) or complete (C) sequence data.

weight (10–70 kDa), acidic isoelectric point, glycosylation and resistance to heat and digestion (Taylor *et al.*, 1987). These characteristics have been associated with protein allergenicity; many of these properties, however, characterise a vast number of non-allergenic proteins as well and thus are not unique to food allergens.

Abundance

Food allergens frequently account for a major fraction of the total protein content within a given food. For example, in peanuts and soybeans (Herian *et al.*, 1990; Burks *et al.*, 1991, 1992) important allergens were identified as major storage proteins. Similarly, the muscle protein tropomyosin, the major allergen in shrimp, accounts for about 25–30% of the total shrimp tail muscle protein (Daul *et al.*, 1991, 1992). An exception to this rule is the major allergen of codfish parvalbumin (Gad c 1) that is not a dominant protein in codfish muscle (Elsayed and Bennich, 1975).

Molecular size

Three reasons make molecular size relevant for protein allergenicity. First, the protein must be large enough to elicit an immune response. Second, it must be of sufficient size for at least two IgE binding sites to bridge mast cell-bound IgE; and third, the protein must be small enough to cross the gut mucosal membrane barrier. Most known food allergens have molecular weights between 10 and 70 kDa, thus fulfilling these requirements. The molecular weight of 10 kDa probably represents a lower limit of immunogenicity; the upper limit of 70 kDa probably reflects restricted mucosal absorption of large molecules (Taylor *et al.*, 1987). However, there are exceptions to these size restrictions. For example, native peanut allergens Ara h 1 and Ara h 2 are large polymers with molecular weights between 200–300 kDa (Burks *et al.*, 1991, 1992). It is not known whether the polymer itself or the subunits cleaved during digestion act as allergens. In contrast, mellitin, a 21-amino acid residue peptide from bee venom, induces histamine release from basophils and mast cells (King *et al.*, 1993). Even though this 3-kDa peptide is not a food allergen, it demonstrates that a protein smaller than 10 kDa can act as an allergen; it induces an immune response, binds specific IgE and causes mast cell and basophil degranulation.

Acidic isoelectric point and glycosylation

Most allergens are glycoproteins with an acidic isoelectric point (pI). However, these characteristics are not unique to allergens and many other non-allergenic proteins also exhibit them.

Heat resistance

Heat-resistance is probably the most common feature of potent food allergens The fact that heat denaturation may cause loss of the native protein's conformation, yet patients' IgE antibodies still react with these denatured food proteins, indicates that the native conformation may not always be crucial for IgE binding. Thus, food allergens may contain a large number of non-conformational, sequential epitopes. Cow's milk caseins and whey proteins, for example, retain their allergenicity after heating (Kilshaw *et al.*, 1982, Ford *et al.*, 1983; Host and Samuelsson, 1988; Lee, 1992).

Resistance to digestion

The ability of food allergens to cross the mucosal membrane of the intestinal tract is most likely an important feature. As mentioned earlier, size is one parameter in this context. Another property may be resistance to digestion. Studies that used a gastric model for mammalian digestion to study the digestibility of food allergens point in this direction (Fuchs and Astwood, 1996, Metcalfe *et al.*, 1996). In this study, the digestibility of allergens from egg, milk, peanut, soybean and mustard were evaluated. Food allergens tested resisted digestion for up to an hour whereas the few non-allergens that were tested were digested within a minute. However, not all allergens are resistant to digestion and many non-allergenic proteins may be resistant so that there is still insufficient information to conclude that the resistance to digestion is the most important property that characterises a food allergen.

Important food allergens

A number of plant- and animal-derived foods have been identified as major sources of food allergens. Important plant-derived food allergens have been identified in legumes, particularly peanuts and soybeans; seeds and nuts; fruit; vegetables; grains; and spices. A number of foods from animal sources have been implicated to cause allergic reactions; however, only a few have been well studied. Major sources of allergens in this group are milk, eggs, fish and shellfish, particularly crustaceans.

PEANUT ALLERGENS

Peanut (*Arachis hypogaea*) ranks first as the cause of severe and lethal, anaphylactic reactions to foods; very small amounts may trigger an allergic reaction. Several children die every year from allergic reactions to peanut. Peanut allergens are subject to intense analysis due to their high allergenic potential and the economic importance of peanuts as a protein source for the food processing industry. Peanuts contain a large number of allergens; up to 37

allergenic peanut components have been identified (Barnett *et al.*, 1983; Bush *et al.*, 1983). Four peanut allergens have been described as major allergens. Peanut 1, Concanavalin A-reactive protein, Ara h 1 and Ara h 2.

Burks and coworkers (Burks *et al.*, 1991, 1995a,b) recently identified Ara h 1 and Ara h 2; their ongoing studies are some of the most advanced with regard to understanding the interaction of molecular structure of food allergens and the immune system. Ara h 1, a 63.5-kDa glycoprotein (Burks *et al.*, 1991, 1995a) is vicilin, a peanut storage protein, a major protein from the globulin fraction (Burks *et al.*, 1995b). Ara h 2, a second major peanut allergen (Burks *et al.*, 1992), is smaller than Ara h 1 with a molecular weight of 17.5 kDa and an isoelectric point of 5.2 (Burks *et al.*, 1992); it showed homology to the conglutinin family of seed storage proteins. Experiments with Ara h 1- and Ara h 2-specific monoclonal antibodies and human IgE identified three (Burks *et al.*, 1994; Stanley *et al.*, 1995) and two antigenic sites on Ara h 1 and Ara h 2 (Burks *et al.*, 1995c), respectively.

In recent studies, the major linear IgE-binding epitopes of Ara h 1 and Ara h 2 were systematically mapped using overlapping peptides synthesised on an activated cellulose membrane and pooled serum IgE from peanut-sensitive patients (Burks *et al.*, 1997; Stanley *et al.*, 1997). Twenty-three Ara h 1 and ten different linear Ara h 2 IgE-binding epitopes, located throughout the lengths of the molecules, were identified; no obvious sequence motif was shared by all peptides. Four Ara h 1 and three Ara h 2 peptides were immunodominant epitopes as they were recognised by more than 80% of the patients tested. Mutational analysis of the immunodominant epitopes revealed that single amino acid changes within these peptides could have dramatic effects on IgE-binding characteristics: single amino acid substitutions resulted in loss or increase of IgE binding.

SOYBEAN ALLERGENS

Similar to peanuts, soybeans (*Glycine max*) contain multiple allergens. A small study with four subjects suggested the 2S-globulin fraction as a major source of allergens (Shibasaki *et al.*, 1980); further, significant IgE binding to proteins with molecular weights between 14 and 70 kDa was demonstrated in the 7S fraction with major binding to a 30-kDa band (Ogawa *et al.*, 1991). This band was designated as Gly m 1. The 15-amino acid N-terminal sequence of Gly m 1 has been determined and is identical with the 34-kDa oil body-associated protein from soybean. This soybean protein also reacts with human IgE and Gly m 1-specific monoclonal antibodies. Some 65% of soybean-reactive subjects had specific Gly m 1-specific IgE antibodies; however, none of these individuals experienced severe or anaphylactic reactions to soybeans.

Other proteins were also identified as soybean allergens. The Kunitz soybean trypsin inhibitor was identified as a soybean allergen by skin test, RAST and RAST inhibition in a soybean-allergic subject (Moroz and Yang, 1980); the IgE

reactivity to soybean was inhibited completely by the Kunitz soybean trypsin inhibitor. However, the RAST reactivity of two other sera from soy-allergic individuals indicate that the Kunitz soybean trypsin inhibitor is a relatively minor allergen.

A 68-kDa allergen was identified as a minor soy allergen (Ogawa *et al.* 1995). It reacted with sera from approximately 25% of soy-allergic subject and was identified as the α-subunit of β-conglycinin. IgE only bound to the α subunit but not to the α_1 and β-subunits, even though these structures are highly homologous.

Allergenic activity of other legumes was reported. For example, albumins i pea (*Pisum sativum*) were described as allergens (Grant *et al.*, 1976). Th allergens appeared to be stable as they retained their allergenic activity whe heated or boiled. A 1.8-kDa allergen from green peas with a carbohydrat content of 30% was purified from pea dialysate (Malley *et al.*, 1975, 1976 but was not further characterised.

RICE

The major allergens from rice (*Oryza sativa*) are encoded by a multigene famil (Adachi *et al.*, 1993). Rice allergens range from 14 to 60 kDa (Matsuda *et a* 1993) and are resistant to heat and proteolysis. The cDNA sequence coding f the major rice allergen Ory s 1 has been determined (Izumi *et al.*, 1992; Adac *et al.*, 1993). The mature protein has a molecular weight of approximate 14 kDa and has an amino acid sequence homology of 20% and 40% wi the barley trypsin inhibitor and the wheat α-amylase inhibitor, respectivel Attempts have been made to select hypoallergenic strains (Izumi *et al.*, 199 Adachi *et al.*, 1993; Matsuda *et al.*, 1993; Watanabe, 1993). Protease treatme was used to reduce the allergenicity of the rice (Watanabe, 1993) though tl process requires large amounts of enzyme. To reduce the allergenicity of ric synthesis of rice allergen was suppressed (Matsuda and Nakamura, 199 Matsuda and colleagues have cloned and sequenced a 16-kDa rice seed pr tein that was identified as the major rice allergen. Based on its nucleoti sequence, an antisense RNA strategy was applied to repress expression this allergen in maturing rice seeds. Seeds from transgenic rice plants w the antisense gene have substantially reduced amounts of the allerg (Adachi *et al.*, 1993; Matsuda and Nakamura, 1993; Matsuda *et al.*, 19 Watanabe, 1993).

CORN

Corn (*Zea mays*) is generally not considered as an important food allergen. I recent study, however, the IgE-binding proteins in corn were identified (Lel *et al.*, 1997b). As with most cereals, corn contains mostly alcohol-solu proteins. These proteins have not been considered in terms of their allerg

city. In order to assess IgE-reactive corn proteins, aqueous and alcohol extracts were prepared of corn seeds according to established biochemical procedures and analysed by immunoblotting. Forty-seven sera of corn-reactive individuals were tested. Individuals were considered corn-reactive if they met two out of three criteria: a history of food allergy consistent with corn allergy, positive skin test to corn, or positive IgE response to corn. Two-thirds of these subjects had significant IgE antibodies to proteins present in aqueous extracts and, more interestingly, approximately 60% of the corn-reactive subjects also showed significant reactivity to proteins in the alcohol extracts. The allergenic reactivity to alcohol-soluble proteins did not necessarily correlate with that to proteins present in the aqueous extract. Twenty water-soluble and eight alcohol-soluble IgE-reactive bands were identified in corn. Two proteins of the aqueous corn fraction with molecular weights of 28 kDa and 10 kDa reacted with more than 50% of the subjects' sera, whereas one 28-kDa band of the alcohol fraction can be considered a major allergen. These results demonstrate the need for proper extraction methods for preparing food allergen extracts that are based on properties of the proteins present, rather than merely employing historically used standard extraction procedures. However, to establish the clinical relevance of corn IgE reactivities, double-blind, placebo-controlled food challenges must be performed.

NUTS

Brazil nuts (*Bertholletia excelsa*) can cause systemic anaphylaxis in some individuals. Several allergenic fractions were identified by immunoblotting using sera from Brazil nut-allergic subjects (Arshad *et al.*, 1991). The major allergen from Brazil nut Ber e 1 is a high-methionine, 2S protein (Nordlee *et al.*, 1994, 1996) with a molecular weight of 12 kDa. Ber e 1 is a dimer that consists of 9-kDa and 3-kDa subunits. The cDNA sequence of Ber e 1 was determined (Altenbach *et al.*, 1987) and its amino acid sequence deduced. Ber e 1 is homologous to high-methionine proteins from castor bean and rapeseed.

Other nuts are also a source of allergens. Bargman *et al.* (1992) used immunoblotting techniques to detect IgE binding proteins in almond extracts. A number of IgE-binding proteins ranging from 38 to 70 kDa were detected. A 70-kDa heat-labile and a 40–50-kDa heat-stable allergen were identified. Furthermore, chestnuts and pistachio were implicated as potential allergens. Reactivity to chestnuts was demonstrated in latex-allergic subjects (Anibarro *et al.*, 1993; Fernandez de Corres *et al.*, 1993).

EGG ALLERGENS

Food allergy to proteins from egg of the domestic chicken (*Gallus domesticus*) is one of the most frequently implicated causes of immediate food allergic reactions in children in the United States and Europe (Crespo *et al.*, 1994).

Egg sensitivity frequently disappears by the fourth or fifth year of life; howe one-third of individuals have clinical sensitivity that lasts over 6 years (Cre *et al.*, 1994). Egg white (albumin) appears to be more allergenic than yolk, egg white proteins have been extensively studied and sequenced (Yungir 1990).

Four proteins, ovomucoid, ovalbumin, ovotransferrin (conalbumin) and l zyme, have been identified as major egg white allergens. Ovomucoid (Gal is a glycoprotein with a molecular weight of 28 kDa, and an isoelectric poi 4.1. Its primary structure (Kato *et al.*, 1987) is a polypeptide chain of 186 ar acids. The tertiary structure consists of three tandem domains each homolo to the pancreatic secretory trypsin inhibitor. Ovalbumin (Gal d 2) (Langel 1983a,b) is a monomeric phosphoglycoprotein with a molecular w between 43 and 45 kDa and an isoelectric point of 4.5. Gal d 2 con 385 amino acid residues (McReynolds *et al.*, 1978; Nisbet *et al.*, 1 Ovotransferrin (Conalbumin, Gal d 3) has a molecular weight of 77 kD isoelectric point of 6.0 and is 686 amino acid residues long (Jeltsch Chambon, 1982; Williams *et al.*, 1982). Lysozyme (Gal d 4) has a mole weight of 14.3 kDa and an isoelectric point of 10.7. Gal d 4 contains amino acid residues in a single polypeptide chain cross-linked by four phide bounds (Canfield, 1963). The importance of Gal d 4 as an allergen i not well established. In one study (Miller and Campbell, 1950), lysozyme found to be a major allergen by skin testing whereas in another (Langeland, 1983a) no sera from egg-allergic patients had positive IgE reac to Gal d 4. In contrast, a third study (Anet *et al.*, 1985) showed that about h the egg-sensitive patients reacted to lysozyme by RAST.

Only minimal information is available concerning epitopes of egg aller ovalbumin has been the best egg allergen studied so far. IgE was found to the two CNBr-fragments of ovalbumin (residues 41–172 and 301–385) (K *et al.*, 1992), and in another study (Honma *et al.*, 1996) a ten-amino residue peptide (OVA357-366) inhibited histamine release from baso Two T-cell reactive ovalbumin peptides (OVA 105-122 and OVA 323 were reported (Shimojo *et al.*, 1994; Holen and Elsayed, 1996) whic not bind IgE.

In addition to ovomucoid, ovalbumin, ovotransferrin and lysozyme, a v of other egg proteins have been described as minor allergens. These in ovomucin, ovoinhibitor, ovaflavoprotein, apovitellenin and phosvitin (A *al.*, 1985; Scott *et al.*, 1987; Taylor *et al.*, 1987; Walsh *et al.*, 1987, 198 these proteins were not studied in more detail.

COW'S MILK ALLERGENS

Cow's milk is one of the most common food allergens. It is estimate between 0.3 and 7.5% of infants and young children suffer from cow' allergy (Wershil and Walker, 1988; Amonette *et al.*, 1993). Cow's milk is

complex mixture of proteins; two major groups of cow's milk proteins, caseins and β-lactoglobulin, have been identified as major allergens (Savilathi, 1981; Alexander *et al.*, 1989; Amonette *et al.*, 1992; Savilathi and Kuitonen, 1992). Caseins are phosphoproteins that precipitate from raw skim milk upon acidification to pH 4.6 at 20°C. They comprise 80% of the total milk protein. Whey proteins are those proteins remaining in the fluid ('serum') after casein precipitation (Whitney, 1988).

The biochemistry of milk proteins has been extensively studied; many cow's milk proteins have been sequenced (Swaisgood, 1985; Whitney, 1988), and computer-based molecular modelling of the three-dimensional structure of several milk proteins has also been reported (Kumosinski *et al.*, 1991a,b, 1993). Surprisingly, even though cow's milk is a major food allergen and the structures of cow's milk allergens are well studied, the major IgE binding sites and T-cell epitopes of milk allergens have not been determined in humans.

Caseins and β-lactoglobulin appear to be the major allergens in cow's milk (Savilathi, 1981; Amonette *et al.*, 1992; Savilathi and Kuitonen, 1992). The caseins are a family of chemically related proteins. The frequency of reactivity to different casein variants has not been systematically studied. α-S1 casein has at least five genetic variants, with varying degrees of post-translational phosphorylation, and four α-S2 casein variants have been identified. β-Caseins are a group of proteins which have one major component with seven genetic variants and eight minor components which are proteolytic fragments of the major component. The molecular weight of the major component is 24 kDa. β-Lactoglobulin is a whey protein that comprises approximately 20% of total milk proteins. It has a molecular weight of 18 kDa and at least six genetic variants have been identified. The primary structure has been determined (Swaisgood, 1985; Whitney, 1988) and the protein has a 91% sequence homology with egg β-lactoglobulin (Alexander *et al.*, 1989).

The whey proteins α-lactalbumin and bovine serum albumin (BSA) have been identified as minor cow's milk allergens (Goldman *et al.*, 1963). α-Lactalbumin has a molecular weight of 14 kDa and its amino acid sequence has been determined (Swaisgood, 1985; Hurley and Schuler, 1987; Whitney, 1988). BSA has a molecular weight of 67 kDa and comprises 1% of total milk protein.

FISH ALLERGENS

The consumption of fish (Elsayed *et al.*, 1972) is a frequent cause of IgE-mediated reactions. Fish is one among the most commonly implicated allergenic foods, and have been incriminated in fatal anaphylactic reactions (Yunginger *et al.*, 1988). Species-specific analysis of IgE reactivities have not been performed since most studies were either performed with cod, or information about the species tested are not provided.

One of the first and most comprehensive analyses of a food allergen was th purification and characterisation of the major codfish allergen, Gad c 1. Th was originally designated allergen M from Baltic cod, *Gadus callarius*. belongs to a group of muscle proteins called parvalbumins (Elsayed ar Bennich, 1975) and constitutes approximately 0.05–0.1% of the white co muscle tissue. Gad c 1 has a molecular weight of 12.3 kDa and an isoelectr point of 4.75. Its amino acid sequence has been established; it contains 1 amino acid residues (Elsayed and Aas, 1970; Elsayed and Bennich, 197 Elsayed *et al.*, 1976). Gad c 1 contains at least five IgE binding sides (Elsay and Apold, 1983). Studies using synthetic peptides established that region 49 64 encircled two repetitive sequences. These two tetrapeptides appear to mutually important for IgE binding as region 49–64 showed relatively hi RAST inhibition compared with Gad c 1 and could produce a Praunit Küstner reaction.

Minor cod fish allergens distinct from Gad c 1 (Aukrust *et al.*, 1978) w identified but were not further characterised. Some 25% of fish-allergic subje bound to an allergen designated as Ag-17-cod (Aukrust *et al.*, 1978) a approximately 10% of cod-allergic individuals reacted to a cod blood ser protein (Aas and Elsayed, 1975). Protamine sulphate, a low-molecular-wei sperm protein of fish species belonging to the families Salmonidae Clueidea, has been implicated as a fish allergen. However, based on the res of several studies, (Caplan and Beckman, 1976; Knape *et al.*, 19 Greenberger *et al.*, 1989; Levy *et al.*, 1989), it can be concluded that protan sulphate is rarely allergenic for fish-allergic subjects.

CRUSTACEA ALLERGENS

Crustacea such as prawns, crabs, lobster and crawfish are a common c of food hypersensitivity. Like fish, a higher incidence of crustacea allergy be expected in geographical areas where more shellfish are consumed regular basis, such as the Gulf coast region of the United States.

Several studies (Hoffman *et al.*, 1981; Nagpal *et al.*, 1989; Daul *et al.*, 1 Leung *et al.*, 1994) described the identification of shrimp allergens. As wit other allergenic foods, several allergens were found in shrimp; it is now kn that the muscle protein tropomyosin is the most important major allergen. results of our studies indicate that the structural features responsible fo allergenicity of Pen a 1 are located in the phylogenetically diverse pa tropomyosin rather than the conserved parts. This was confirmed for diff shrimp species; Pen a 1 from brown shrimp (*Penaeus aztecus*) (Daul 1994), Pen i 1 from Indian shrimp (*Penaeus indicus*) (Shanti *et al.*, 1993 Met e 1 from greasyback shrimp (*Metapenaeus ensis*) (Leung *et al.*, 1 There is very good evidence that clinically relevant cross-reactivity is d virtually identical primary structures: the cDNA of Pen a 1 showed 26 bas substitutions when compared with the sequence of Met e 1; these bas

can have clinical symptoms after ingesting lobster and crab (Lehrer, 1986; Daul *et al.*, 1987; Halmepuro *et al.*, 1987) whereas cross-reactivities among legumes seem to be of less clinical importance; peanut-allergic subjects who show *in vitro* IgE antibody reactivity to beans or peas usually do not show clinical symptoms after ingestion of beans or peas (Barnett *et al.*, 1987; Taylor *et al.*, 1987; Bernhisel-Broadbent and Sampson, 1989).

EXAMPLES OF CROSS-REACTING, PHYLOGENETICALLY RELATED FOODS

Legumes

Positive skin reactions and RASTs are frequently observed in peanut-allergic subjects to other leguminosae such as soybean, peas and beans (Barnett *et al.*, 1987; Bernhisel-Broadbent and Sampson, 1989; Bernhisel-Broadbent *et al.*, 1989; Bock and Atkins, 1990; Bernhisel-Broadbent, 1995). However, multiple positive skin tests to different leguminosae did not necessarily correlate with positive oral provocation (Bernhisel-Broadbent and Sampson, 1989) and the IgE reactivities to different leguminosae, analysed by Western blotting, did not correlate with clinically relevant symptoms either (Bernhisel-Broadbent *et al.*, 1989). An example of a seemingly clinically relevant cross-reactivity among legumes was reported by Hefle and Bush (1994) when a peanut-allergic child reacted to a lupin-fortified pasta product. The IgE-binding proteins of the lupin have an approximate molecular weight of 21 kDa (range from 35 to 55 kDa), and are heat-stable.

Fishes

The majority of consumed fishes belong to one of five taxonomic orders (Moody *et al.*, 1993): Perciformes (e.g. mackerel, tuna fish), Gadiformes (e.g. codfish), Pleuronectiformes (e.g. flounder), Cypriniformes (e.g. carp, catfish) and Clupeiformes (e.g. trout, salmon, herring). The reactivity to different fishes by RAST and skin test suggest cross-reactivity; however, the majority of fish-allergic subjects could eat other fish species or did not react during food challenge (Aas, 1996; Bernhisel-Broadbent *et al.*, 1992) indicating that the *in vitro* cross-reactivity may be of limited clinical relevance.

Crustacea

The substantial cross-reactivity among crustacea appears to be clinically important (Waring *et al.*, 1985; Daul *et al.*, 1987); for example shrimp-allergic subjects may react to other crustaceans without prior exposure. The cause for this cross-reactivity is probably the major allergen tropomyosin, a highly conserved muscle protein. Tropomyosin has been identified in three shrimp species: brown shrimp (*P. aztecus*) (Daul *et al.*, 1993, 1994), Indian shrimp (*P. indicus*) (Shanti *et al.*, 1993) and greasyback shrimp (*M. ensis*) (Leung *et al.*, 1994). Pen a 1-like proteins were detected in crab, crawfish and lobster using sera of shrimp-allergic subjects and Pen a 1-specific monoclonal antibodies (Daul *et al.*, 1993, 1994). The amino acid sequence similarity among these different shrimp tropomyosin is very high; for example, Met e 1 and Pen a 1 only differ in one position (Leung *et al.*, 1994; Reese *et al.*, 1997).

EXAMPLES OF CROSS-REACTING, NON-RELATED FOODS

Cross-reactivity between foods and pollens

Birch, mugwort and ragweed pollen have been associated with various food allergies. For example, ragweed pollen cross-reacts with melons and bananas (Anderson *et al.*, 1970; Enberg *et al.*, 1987); grass and mugwort pollens cross-react with celery and a variety of vegetables (Wütrich and Dietschi, 1985; Calkhoven *et al.*, 1987, 1991); and birch pollen cross-reacts with a number of fruits (Halmepuro *et al.*, 1984; Calkhoven *et al.*, 1991). As the interest in cross-reactivity between foods and non-food allergens has increased, more and more information about the structural basis of cross-reactivity has become available, and these cross-reactivities are now the best studied examples of the cross-reactions between food and non-food allergens.

The association of allergic reactions to pollen allergens and apple, hazelnut, potatoes, celery and carrots has been attributed to cross-reactivities among pathogenesis-related plant proteins and profilin, an ubiquitous cyctoskeleton protein. The structural relationship of birch pathogenesis-related plant protein (Bet v 1), profilin (Bet v 2) and the reactivity to fruits has been studied in more detail (Vieths *et al.*, 1994, 1995; Ebner *et al.*, 1995; Fahlbusch *et al.*, 1995; Vanek-Krebitz *et al.*, 1995). The structural and immunological homology of the major apple allergen Mal d 1 and Bet v 1 has been demonstrated (Vieths *et al.*, 1994, 1995; Fahlbusch *et al.*, 1995; Vanek-Krebitz *et al.*, 1995). The immunological properties of recombinant Mal d 1 were tested and cross-reactivity with Bet v 1 was shown (Vanek-Krebitz *et al.*, 1995). The association between celery, apple, peanut and kiwi fruit, and mugwort pollen was ascribed to the homologous mugwort allergen Art v 1 (Valenta and Kraft, 1995) and pollen profilins (Vallier *et al.*, 1992; Valenta *et al.*, 1992; Breiteneder *et al.*, 1995).

Cross-reactivity between foods and latex

Over the past decade, latex allergy has increased and studies have shown that some allergic reactions to fruits such as avocado, chestnut and banana are due to cross-reacting allergens (Anibarro *et al.*, 1993; Fernandez de Corres *et al.*, 1993; Fisher, 1993; Blanco *et al.*, 1994a,b; Makinen-Kiljunen, 1994; Lavaud *et al.*, 1995). Papaya, fig, celery, passion fruit and peach have been suggested as sources for cross-reacting allergens.

substitutions, however, resulted in only one amino acid substitution (Figure 4.1). Furthermore, Pen a 1 and tail muscle tropomyosin of American lobster (*Homarus americanus*) differ only in three amino acid positions. Slow tropomyosin from the claws of American lobster differ from Pen a 1 in 18 positions mainly clustered around position 57 (Mykles *et al.*, 1998); the differences in the allergenicity have not been studied but based on epitope studies on Pen i 1 and Pen a 1, its allergenicity is probably not critically affected.

In Pen i 1 (Shanti *et al.*, 1993) two peptides, of 17 and 9 amino acid residues each, were identified as important IgE-binding epitopes, since they inhibited more than 50% of specific IgE reactivity to Pen i 1. Four IgE-binding Pen a 1 epitopes were identified recently (Reese *et al.*, 1997). One of two IgE-reactive peptides, previously identified in the Indian shrimp, *P. indicus* as peptide 153–161 (Daul *et al.*, 1994), partially overlaps with IgE-reactive peptide from *P. aztecus* 157–166 (Reese *et al.*, 1997), indicating that this part of shrimp tropomyosin is a major IgE binding site (Figure 4.1).

In our view, the shrimp allergen tropomyosin provides a unique opportunity to study the contribution of protein structure to a protein's allergenicity since sequences of non-allergenic tropomyosins from pork, beef and poultry are known and shrimp-allergic subjects usually do not suffer from allergies to vertebrate meats (Ayuso *et al.*, 1998). Comparison of allergenic Pen a 1 epitopes with homologous sequences of vertebrate tropomyosins and sequential amino acid substitution experiments may help to understand and predict the allergenicity of proteins.

Food cross-reactivities

Cross-reactivities are found among foods of related phylogenetic origin and foods of seemingly unrelated non-food allergens. Allergens from legumes, fishes, crustaceans, fruits, tree nuts are examples for phylogenetically related, cross-reacting allergens. In addition to cross-reactivity of foods within the same group, cross-reactivity has been described between foods that are unrelated or only distantly related. For example, it has been reported that ragweed pollen cross-reacts with melons and bananas (Anderson *et al.*, 1970; Enberg *et al.*, 1987); grass and mugwort pollens cross-react with celery and a variety of vegetables (Wütrich and Dietschi, 1985; Calkhoven *et al.*, 1987, 1991); and birch pollen cross-reacts with a number of fruits (Halmepuro *et al.*, 1984; Calkhoven *et al.*, 1991). Studies from our laboratory have shown that marine animals belonging to different phyla such as oysters and crustacea (Lehrer and McCants, 1988), or clams and shrimp (Desjardins *et al.*, 1995), cross-react.

The origin of food allergen cross-reactivity is still unknown; it is thought to be due to similar protein structure or multiple sensitisation to similar proteins in cross-reacting foods. The clinical relevance of food allergen cross-reactivity depends on the food in question. For example, the *in vitro* cross-reactivities among crustacea are thought to be clinically relevant – shrimp-allergic subjects

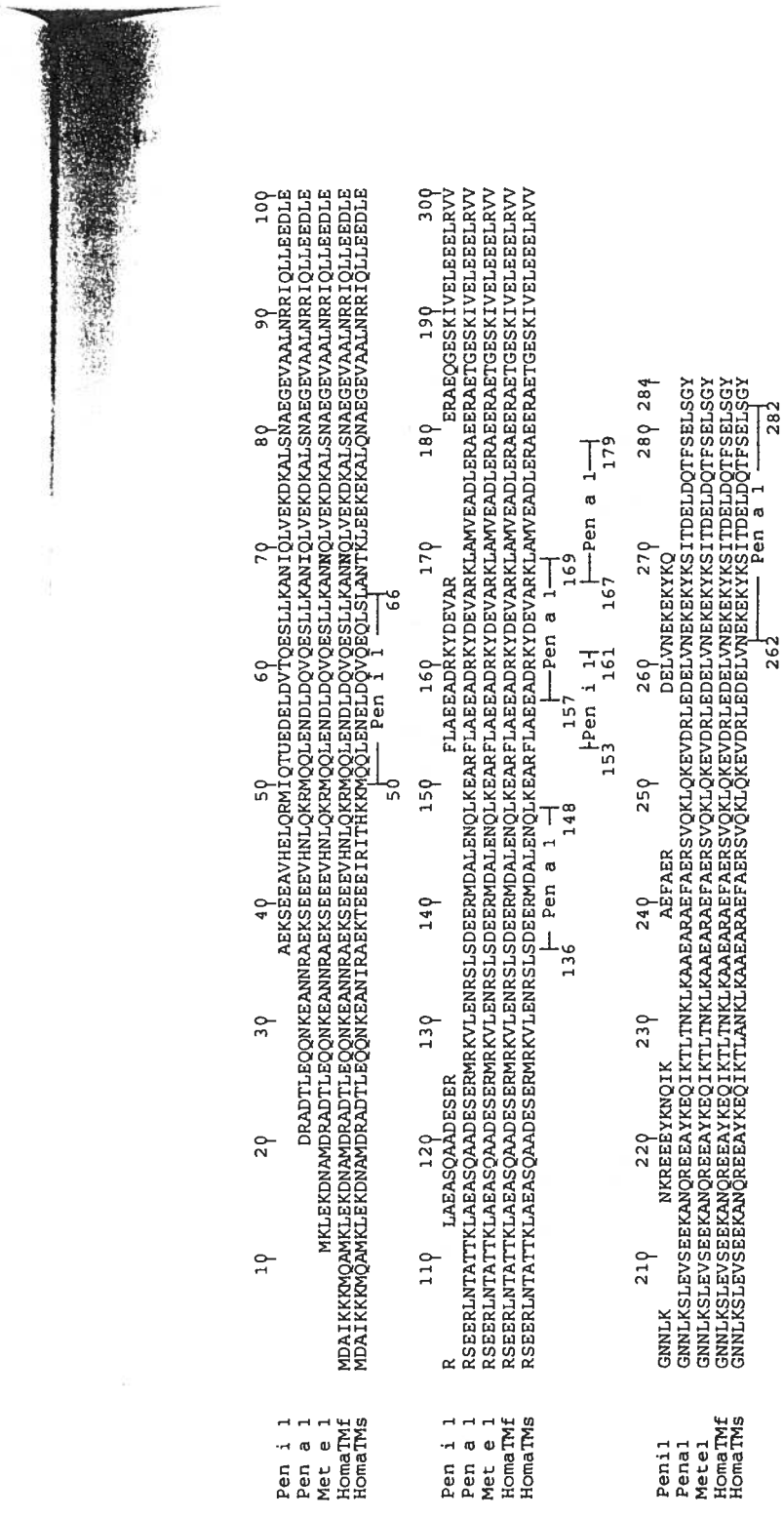

Figure 4.1 Sequence comparison of shrimp allergens Pen i 1, Pen a 1, Met e 1 and slow (HomaTMs; GenBank# AF034953) and fast muscle (HomaTMf; GenBank# AF034954) of American lobster (*Homarus americanus*). Pen i 1 and Pen a 1 epitopes are marked. (From Shanti *et al.*, 1993 and Reese *et al.*, 1997.)

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Transgenic foods

Recombinant DNA technology has been used to introduce traits such as resistance to disease, herbicides, insects or environmental stress; delayed ripening; male sterility; synthesis of modified starch and oils; or increased synthesis of major components into a variety of plants. To date, more than 60 different plant species have been successfully genetically engineered. This field is rapidly expanding and more than 20 new plant products are expected to be introduced into the marketplace within the next 5 years (Table 4.3). Since the introduced traits result from the expression of proteins that are not necessarily part of the species' original genome, there is concern about the safety of transgenic foods as they enter the marketplace. A major aspect in this regard is the potential allergenicity of new products (Harlander, 1991; Kessler *et al.*, 1992; Olempska-Beer *et al.*, 1993; Fuchs and Astwood, 1996; Lehrer *et al.*, 1996; Metcalfe *et al.*, 1996; O'Neil *et al.*, 1998).

It is relatively straightforward to evaluate the allergenicity of genetically engineered foods derived from plants known to be allergenic as information about the allergen and allergen-specific human sera or monoclonal antibodies is available. Using the parental variety as a control, *in vitro* immunological assays can be employed to assess the IgE reactivity of transgenic foods. RAST and RAST inhibition and/or ELISA are the tests of choice to quantify possible differences in total allergen content and allergen composition. Immunoblotting, although less quantitative than other solid phase assays, can also be used to identify allergens and compare IgE reactivity to individual allergens. If results from the *in vitro* testing are negative or equivocal, more definitive *in vivo* testing such as skin prick tests and DBPCFC can be performed. For example, a storage protein from Brazil nut was expressed in soybean to improve the nutritional quality of soy as animal feed. *In vitro* tests, using sera from Brazil nut-sensitive subjects, confirmed that an immunologically functional Brazil nut allergen was produced by the transgenic soybeans (Nordlee *et al.*, 1994, 1996). Another example was the evaluation of two sulphur-rich corn proteins (Lehrer and Reese, 1997). Sera from 42 individuals, demonstrated by skin test, RAST or clinical history and immunoblot to be corn-reactive, were tested for IgE antibody reactivity to the two zein corn proteins using SDS–PAGE/immunoblotting. None of the sera from corn-reactive subjects demonstrated IgE reactivity against either zein proteins, suggesting that products encoding these genes do not pose an increased risk of allergy to consumers.

Another study investigated transgenic soybeans with elevated oleic acid levels (McCants *et al.*, 1997) for differences in their allergenic potency and levels of endogenous allergens. Transgenic and wild-type soybeans were extracted and sera from 31 subjects with histories of soy allergy, positive soy skin test and/or IgE antibody response were tested for reactivity to soy allergens by RAST and immunoblotting. Both wild-type and transgenic soy

Table 4.3 Foods obtained from plant varieties derived through recombinant DNA technology

Plant	Product	Company	Year of FDA evaluation
Corn	Glufosinate-tolerant corn	Pioneer Hi-Bred	Ongoing
	Glyphosate-tolerant corn	Monsanto Co.	Ongoing
	Glufosinate-tolerant corn	AgrEvo Inc.	Ongoing
	Glufosinate-tolerant corn	Dekalb Genetics Corp.	1996
	Glufosinate-tolerant corn	AgrEvo Inc.	1995
	Glyphosate-tolerant/ insect-protected corn	Monsanto Co.	1996
	Insect-protected corn	Dekalb Genetics Corp.	1997
	Insect-protected corn	Monsanto Co.	1996
	Insect-protected corn	Northrup King	1996
	Insect-protected corn	Ciba-Geigy Corp.	1995
	Lepidopteran-resistant corn	Plant Genetics System	Ongoing
	High-oleic oil corn	Dupont	Ongoing
	Male sterile corn	Pioneer Hi-Bred	Ongoing
	Male sterile corn	Plant Genetic Systems	1996
Tomato	Cherry tomato with lowered ethylene content	DNA Plant Technology	Ongoing
	Tomato with lowered ethylene content	DNA Plant Technology	Ongoing
	Modified fruit ripening tomato	Agritope Inc.	1996
	Flavr Savr tomato	Calgene Inc.	1994
	Improved ripening tomato	DNA Plant Technology	1994
	Delayed softening tomato	Zeneca Plant Science	1994
	Improved ripening tomato	Monsanto Co.	1994
Cotton	Bromoxynil-tolerant cotton	Calgene Inc.	Ongoing
	Bromoxynil-tolerant cotton	Calgene Inc.	1994
	Sulfonylurea-tolerant cotton	Dupont	1996
	Glyphosate-tolerant cotton	Monsanto Co.	1995
	Insect-protected cotton	Monsanto Co.	1996
Canola	Canola with altered phytase activity	BASF	Ongoing
	Glufosinate-tolerant canola	AgrEvo Inc.	1997
	Glufosinate-tolerant canola	AgrEvo Inc.	1995
	Glyphosate-tolerant canola	Monsanto Co.	1995
	Laurate canola	Calgene Inc.	1995
Potato	Disease-resistant, increased potato	Frito Lay	Ongoing
	Colorado potato beetle-resistant potato	Monsanto Co.	Ongoing
	Insect-protected potato	Monsanto Co.	1996
	Insect-protected potato	Monsanto Co.	1994
Soybean	Glyphosate-tolerant soybean	Monsanto Co.	1994
	High-oleic, low-linoleic oil soybean	Dupont	Ongoing
	High oleic acid soybean	Dupont	1997

Table 4.3 (*continued*)

Plant	Product	Company	Year of FDA evaluation
Pepper	Sweet pepper with improved texture	DNA Plant Technology	Ongoing
	Pepper with increased sweetness	DNA Plant Technology	Ongoing
Squash	Virus-resistant squash	Seminis Vegetable Seeds	1997
	Virus-resistant squash	Asgro Seed Co.	1994
Miscellaneous	High-protein lupin	Resource Seeds Inc.	Ongoing
	Pea with increased sweetness	DNA Plant Technology	Ongoing
	Glufosinate-tolerant sugar beet	AgrEvo Inc.	Ongoing
	Phosphinothricin-tolerant rice	AgrEvo Inc.	Ongoing
	Sulfonylurea-tolerant flax	Univ. of Saskatchewan	1997
	Male sterile radicchio rosso	Bejo Zaden BV	1997
	Virus-resistant papaya	Univ. Hawaii & Cornell Univ.	1997
	Male sterile/fertility restorer oilseed rape	Plant Genetic Systems	1996

Modified from Astwood *et al.* (1997).

extracts yielded substantial inhibition of the wild-type RAST. Both inhibition curves, analysed by logit-log transformation and linear regression were identical. Immunoblot analysis showed no significant differences in the number of bands or the intensity of the IgE antibody reactivities. These studies indicate that increased oleic acid content did not substantially affect soy allergens either quantitatively or qualitatively and that such transgenic soybeans do not pose increased risk of allergy to consumers.

A much more difficult problem is evaluation of the allergenic potential of foods engineered using proteins from sources of undetermined allergenicity. Predicting potential allergenicity is a major challenge since there is no single predictive assay to assess the potential allergenicity of any proteins. An initial step, evaluation of amino acid sequence homology with known allergens, may be useful in predicting allergenicity of a transgenic protein. If sequence homologies are observed, particularly with regions containing IgE-binding epitopes, it is essential that *in vitro* testing with RAST or ELISA should be performed to assess IgE reactivity. If no IgE antibody reactivity is detected, the second step should be to compare the physicochemical and biological characteristics, including molecular size, stability, solubility and isoelectric point of these proteins with major food allergens.

Contamination and hidden allergens

As of today, avoidance is the only accepted form of food allergen therapy (Taylor *et al.*, 1986; Taylor, 1989) as even minute amounts of allergen-containing foods can cause anaphylactic reactions. Avoidance is therefore the primary means to prevent actual incidence of acute episodes. However, hidden allergens and contaminations can pose a serious risk for highly allergic individuals. Food preparation at home, restaurants and commercial food processing plants can result in prepared or processed foods that contain allergens. This may be due to shared utensils or equipment used to prepare and process foods, or equipment that was not adequately cleaned between runs. Another source of hidden allergens is undeclared or insufficiently declared ingredients in processed food products when certain ingredients make up only a small percentage and do not have to be indexed separately on the ingredient list. For example, small amounts of peanut do not have to be declared and spices used do not have to be identified individually. Another possibility for unexpected exposure is ingredients that are sometimes, but not always, contaminated. For example, an anaphylactic reaction to beignets, a form of doughnuts, was traced to a contamination of the beignet mix with house dust mites, a potent inhalent allergen (Erben *et al.*, 1993).

Conclusion and future directions

In comparison with inhalant allergens such as grass pollen, house dust mite allergens and hymenoptera allergens such as bee venom allergens, food allergens are not well characterised. Of the hundreds of foods which can cause allergy, only a small number have been characterised; the primary structure of only a few food allergens is known, and very little information about allergenic epitopes is available even though some of the most advanced epitope studies on allergens have been performed on codfish and peanut allergens. The route of exposure to food allergens is different from other allergens: food allergens are normally ingested; the gut-associated lymphoid tissue (GALT) may respond differently compared with peripheral lymphoid tissue in spleen and lymph nodes from which most of our information concerning the immune response has been obtained. Food antigens, in such an environment, may therefore have different properties as compared with other non-food allergens.

Features that make proteins resistant to digestion, heat and acid denaturation and preserve the immunological integrity must be studied since these properties have been linked to protein allergenicity. The clinical relevance of *in vitro* cross-reactivity must be further explored. Is it the structure of cross-reacting proteins that reduces the IgE antibody affinity and thus prevents the mediator release from mast cells, or is the number of epitopes reduced? These questions concerning the structural features responsible for the allergenicity of a protein need to be studied in detail.

Even though the characterisation of food allergens lags behind that of inhalant allergens, studying food allergens may provide a unique opportunity to understand the contribution of structure to the allergenicity of proteins. The studies of peanut allergens Ara h 1 and Ara h 2 show that a single amino substitution may render a given IgE-binding epitope non-reactive, although it remains to be seen whether it is possible to reduce or abolish the IgE reactivity of the entire allergen molecule. Another interesting food allergen is tropomyosin, the major shrimp allergen. Since tropomyosin is ubiquitous and structurally a well-characterised muscle protein, and tropomyosins of meats including poultry, pork and beef are not allergenic, sequence comparison, systematic amino acid substitutions and IgE binding and T-cell activation assays of modified epitopes may help to understand protein allergenicity. Using genetically modified allergens that do not bind IgE, but are still able to interact with T cells inducing T-cell tolerance/anergy may be developed into new concepts of allergen-specific and safer immunotherapy. If the basis of allergenicity is better defined, genetically modified plants may be developed with reduced allergenic potential. Furthermore, it is important to develop better methods to assess genetically engineered varieties as the introduced traits may result from the expression of proteins that are not necessarily part of the species' original genome.

In conclusion, more information is needed about the primary and tertiary structures of food allergens and allergenic epitopes in order to understand the relationship between protein structure and allergenicity, to provide insights into the basic mechanisms of food allergy, to develop an approach to the immunotherapy of food allergy, to use this knowledge to address the immunological safety of transgenic crops and to develop new, less-allergenic varieties.

Acknowledgements

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