

Review

Food allergens of plant origin—their molecular and evolutionary relationships

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Along with other forms of allergic disease, food allergies appear to be on the increase, with childhood allergies to foods such as peanuts being of particular concern. Around 7–10 foods are responsible for the majority of allergies, including several of plant origin, notably peanut. Allergies are usually triggered by the protein components in a food, which are also known as allergens. However, not all the proteins in an allergenic food like peanut are allergens. Why should this be? This question has been addressed by an EU-funded inter-disciplinary network of clinicians, food chemists and plant biochemists called Protall. From the groups considerations it is clear that, whilst the abundance of a protein in a food is one factor involved in determining its allergenic potential, this is not sufficient on its own to predict its allergenicity. Through an analysis of common properties of plant food allergens that trigger a reaction via the

gastrointestinal tract it has become evident that they belong almost exclusively to three structurally related protein superfamilies—the prolamin superfamily (which includes the prolamin storage proteins of cereals, non specific lipid transfer proteins, α -amylase inhibitors, and 2S albumins), the cupin superfamily (specifically the 11S and 7S globulin storage proteins) and the cysteine proteases. Whilst we cannot as yet predict the allergenicity of a given protein, such an understanding of the structural attributes of proteins that predispose them to becoming allergens is important if we are to understand what makes some foods more allergenic than others. It is also highly relevant to developing more reliable means of assessing the allergenic risks posed by novel foods, developing effective processing strategies for reducing allergen loads in foods and developing novel therapeutic options as an alternative to dietary exclusion.

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Introduction

It has been estimated that around 1–2% of the population, and up to 8% of children suffer from some type of food allergy (Helm & Burks, 2000). This is in contrast to the public perception that food allergy is a common condition, often believed to affect up to 1 in 3 of the population (Sloan & Powers, 1986). Both the number of incriminated foods and the frequency of severe reactions appear to be rising rapidly. Of these, peanut allergy has become of particular concern since it affects children, frequently producing severe reactions (sometimes fatal), which are usually life-long. Only around eight types of foods are responsible for causing the majority of food allergies, including a number of foods of plant origin such as peanuts, tree nuts, wheat and soya (Bush & Hefle, 1996). What makes certain plant foods more allergenic than others? The answer to this question needs to be understood more fully if we are to develop knowledge-based means of assessing the allergenic potential of novel foods and processing strategies to reduce allergen loads. A consideration of secondary structural elements has shown no apparent relationship between such elements and allergenic properties but did not address molecular and evolutionary properties that might be shared by food allergens (Alberse, 2000). Through Protall, an EU-funded network of clinicians, immunologists, food scientists and plant biologists,

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these issues have been considered in relation to plant food allergens. Over the last 3 years, a series of workshops was held to discuss three-dimensional structures and physicochemical properties of plant food allergens and the potential these might play in determining allergenicity. This article sets out to summarise the progress made through the network regarding these issues.

What is an allergy?

There is some confusion regarding what constitutes a food allergy as opposed to food intolerance. A generally accepted definition of allergy is that it constitutes a hypersensitivity reaction where symptoms appear rapidly and are caused by exposure to exogenous macromolecules, also known as antigens or allergens. This reaction results in a marked increase in reactivity and responsiveness to that antigen/allergen on subsequent exposure, resulting in adverse health effects and is most commonly mediated by a specific class of antibody, known as immunoglobulin E (IgE). In contrast, food intolerances are reproducible non-immune-mediated reactions whose symptoms can sometimes take days to manifest themselves, an example of which is the gluten intolerance syndrome called Coeliac disease. This review is restricted to the former hypersensitivity reactions, which are generally IgE-mediated food allergies. Such allergies have two phases—(1) sensitization and (2) subsequent reaction on re-exposure to an allergen, both stages being triggered by antigens (almost always proteins) known as allergens.

Allergic sensitization

To be able to elicit an immune response antigen has to be presented to immune competent cells, T-lymphocytes (Fig. 1). This presentation is done by cells (macrophages, dendritic cells and B-lymphocytes) that are able to take up the antigen, process it within the cell and present it on the cell surface bound to a receptor known as a major histocompatibility complex (MHC) type II. As well as MHC type II molecules these cell types also express important costimulatory adhesion molecules and therefore are called professional antigen-presenting cells (APCs). After taking up exogenous antigens these cells process them in the endocytic pathway, following which peptide fragments become selectively bound in the polymorphic groove of class II molecules and transported to the cell surface. Mucosal macrophages, progenitor B-cells and dendritic cells (such as those found in the gut) then migrate to the regional lymph nodes where antigen presentation takes place. Here the complex of 'foreign peptide plus self MHC molecule' on the surface of the APCs is recognised by the T-cell receptors of CD4⁺ T helper cells (Brantzaeg, 2001). This triggers many other events, including maturation of B-cells into antibody secreting cells. In predisposed

individuals, the resulting immune response may take the form of a so-called Th2 response leading to specific IgE production, rather than the 'normal' IgG response. Even in predisposed individuals most protein antigens do not induce an IgE response. It is not known which structural properties of the allergen (if any) drive the response in the direction of an allergic (Th2) response.

IgE epitopes

The sites on a protein which are recognized by antibodies are called epitopes. Two different forms of epitope have been identified, linear and conformational. For the former only the primary sequence of a polypeptide is involved in antibody binding, but conformational epitopes are formed from a number of segments of the polypeptide chain (which may be quite distant in the amino acid sequence of a protein) which are brought together spatially by the protein's three-dimensional structure (van Regenmortel, 1992). Most epitopes are thought to be conformational in nature and are particularly difficult to define in relation to food allergens where processing can have such a disruptive effect on native protein structure.

Eliciting an allergic reaction

In the IgE-dependent allergic reaction IgE molecules bind through Fc receptors to the surface of tissue mast cells and blood basophils (Fig. 2). When the allergen binds to a number of IgE molecules on the basophil or mast cell surface (an event known as cross-linking), it triggers an intracellular response which results in degranulation, with release of preformed pharmacological mediators. These mediators include vasoactive amines such as histamine, which causes increased vascular permeability, vasodilation, bronchial spasm and mucus secretion. Secondary mediators released include leucotrienes, prostaglandin D₂, and chemotactic mediators which attract blood cells, particularly eosinophils and basophils, to the site of the reaction. After some hours the activated mast cells produces various cytokines, IL-3, IL-5 and GM-CSF, which produce pro-inflammatory activity. A similar range of cytokines is produced by Th2 lymphocytes, which are simultaneously activated by the allergen. When the reaction moves into the late phase, cellular infiltration and the release of numerous mediators are involved (Centner & de Weck, 1995; Cruse & Lewis, 1999).

Routes of exposure in sensitization to food allergens

It is generally assumed that sensitization to the classical food allergens such as milk, egg, peanut and fish occur via the gastro-intestinal tract. In infants, immaturity of the gut is supposed to be an important factor for sensitization (Strobel, 1997). In support of this it has been shown that the annual incidence rate (new cases/

year) of sensitization to food allergens (egg, milk, soy, wheat) decreases from 10% at 1 year of age to 3% at 6 years of age whereas the incidence of sensitization to inhalant allergens increases with age from 1.5% at 1 year to 8% at 6 years (Kulig *et al.*, 1999). Adult onset food allergy may be caused by inhalation, as illustrated by the bird–egg syndrome where sensitization to egg α -livetin is caused by inhalation of cross-reacting bird serum albumin from feathers (Ortolani *et al.*, 1998).

In addition to sensitization via the gastrointestinal tract, it is clear that certain types of food allergy occur as a consequence of sensitization to inhaled allergens

such as pollen. Thus, it is clinically well described that many patients allergic to birch pollen get symptoms such as oral itching, lip swelling, labial angioedema, and glottis oedema (oral allergy syndrome) when they eat fresh fruit (mainly apples), nuts (hazelnuts), and raw vegetables (celery, carrot). Systemic symptoms such as urticaria, angioedema, asthma, or anaphylactic shock may also occur. These patients have specific IgE to Bet v 1 or Bet v 2 (profilin), the major birch pollen allergens. Concomitant sensitization to mugwort and/or grass pollen is also often present (Wüthrich & Strauman, 1997) and usually precedes food allergy, leading to the

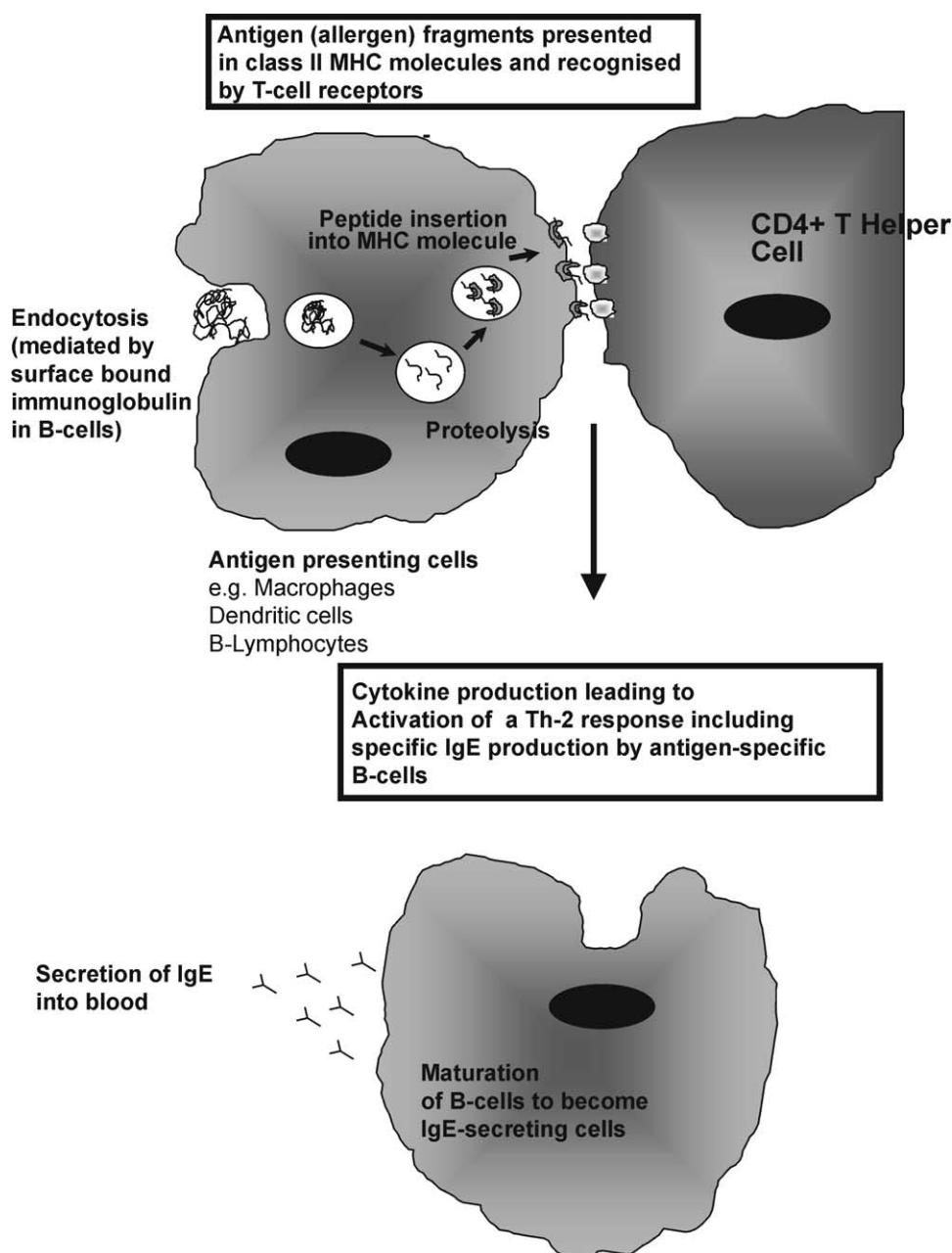


Fig. 1. Allergic sensitization—the role of antigen presentation.

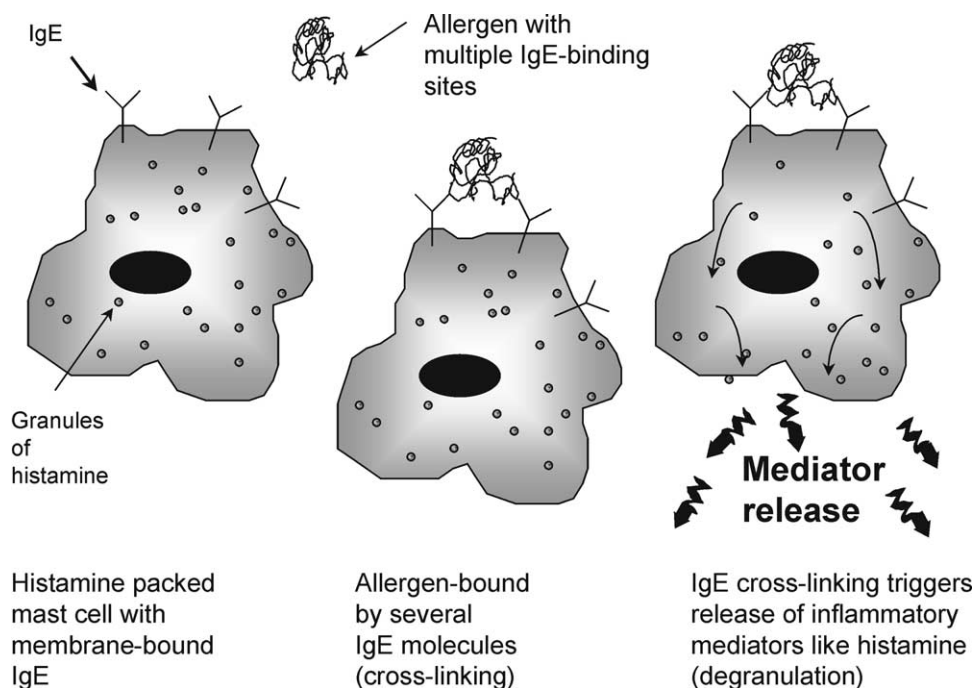


Fig. 2. Eliciting allergic reactions—IgE binding and mast cell degranulation.

hypothesis that the original sensitization was via the respiratory system rather than the gut. However, the presence in the patient's blood of specific cross-reacting antibodies to birch pollen and to fruit and vegetables does not indicate which came first: the pollen allergy or the food allergy. Kazemi-Shirazi *et al.* (1999) investigated this using sera from 71 patients with oral allergy syndrome, on the premise that the primary sensitizing molecule would carry most, if not all, of the relevant IgE-reactive epitopes, whereas the secondary cross-reactive allergen would contain fewer epitopes. Pre-incubation of sera with recombinant (rBet v 1 and rBet v 2) and natural pollen (birch and timothy grass) allergens led to an almost complete inhibition of IgE binding to plant food allergens in immunoblots, as well as in RAST inhibition experiments. In contrast, recombinant plant food allergens from apple, rMal d 1, celery, (rApi g 1), and carrot, (rDau c 1), poorly inhibited IgE binding to Bet v 1. These data support the case for respiratory, rather than gut, sensitization in patients with pollen allergy and oral allergy syndrome to food.

Not all allergies to fruits can be explained by cross-reactions to pollen. More recently non specific lipid-transfer proteins (LTP) in apple and peach have been described as clinically relevant allergens with no cross-reactions to pollen (Pastorello, Incorvaia, *et al.*, 1998; Sánchez-Monge, Lombardero, García-Sellés, Barber, & Salcedo, 1999). The LTPs are more stable than the Bet v 1 cross-reacting allergens, making it more likely that the LTPs may sensitize via the gastrointestinal tract.

Plant food protein allergen families

Plant tissues, including those consumed by humankind, contain immense numbers of proteins, many of which are present in small amounts or transiently. For example, molecular analyses indicate that between 4500 and 8000 genes are expressed in the developing wheat grain but only about 1700 proteins were identified by two-dimensional electrophoresis (Clarke, Hobbs, Skylas, & Appels, 2000). The sheer complexity of this mixture makes it difficult to isolate and characterize any but a small number of components (usually those present in large amounts or with important biological or functional properties). However, the past few years have seen an explosion in the amount of information available, derived from large scale genome sequencing (of the 'model' cruciferous species *Arabidopsis*) and more focused sequencing of individual mRNAs (called expressed sequence tags or ESTs) from specific tissues. The development of powerful computer-based methods to compare the amino acid sequences and structures of proteins has allowed us to exploit this information to determine the structural and evolutionary relationships of plant proteins, including major allergens.

Plant food allergen database

In order to support the studies of the Protall partnership, a database of information about plant food allergens was constructed. Allergens were included that satisfied the following criteria:

- A plant allergen was defined as an antigen which produces an IgE-mediated response

- A major allergen was defined as a protein towards which 50% (or more) of a group of at least 10 allergic individuals have developed an IgE-response

It was also agreed that the minimum criteria for inclusion of an allergen in the catalogue was

- Knowledge of its molecular weight
- Evidence of an associated allergic reaction in at least three patients, preferably established by double blind placebo controlled challenge with the food, or a convincing description of an anaphylactic reaction to the food in question.

Table 1 summarizes some of the major plant food allergens identified to date which have been included in the database.

Protein allergens sensitizing via the gastrointestinal tract

Such comparisons show that the vast majority of plant food allergens that sensitize via the gastrointestinal tract belong to only three major groups of plant proteins: the prolamin, cupin and cysteine protease superfamilies.

The prolamin superfamily was initially defined in 1985 and contains the alcohol-soluble (prolamin) storage proteins of cereals: the major prolamins of wheat, barley, rye and oats and minor groups of prolamins in maize, sorghum and millets. Although allergic prolamins have been reported in wheat, the most important allergens in this superfamily are not prolamins but three related groups of proteins: 2S storage albumins, non-specific lipid transfer proteins (nsLTP) and cereal inhibitors of α -amylase and/or trypsin. 2S storage albumins occur in many dicotyledonous seeds and major allergenic components occur in oriental and yellow mustards (Bra j 1, Sin a 1), oilseed rape (the napin Bra n 1), castor bean (Ric c 1, 3), walnut (Jug r 1), sunflower, sesame (Ses i 1), Brazil nut (Ber e 1), peanut (Ara h 2, 6, 7) and cottonseed.

The nsLTPs have an even wider distribution than 2S albumins, with over 100 sequences being available from seeds, fruit and vegetative tissues, and major allergenic forms in peach (Pru p 1), apple (Mal d 3) and maize (Zea m 14). Although the major route of sensitization is probably via the GI tract, the presence of related allergens in pollen (Duro *et al.*, 1996) indicates that sensitization may also occur via the lungs.

Similarly, the cereal α -amylase inhibitors may sensitize via the lungs to give bakers' asthma (wheat, barley and rye) or via the gastrointestinal tract (wheat, barley and rice). The best characterized allergens of this group are the α -amylase inhibitors of rice grain (Nakase *et al.*, 1996).

Although the 2S albumins, nsLTP and cereal inhibitors have limited similarity in their sequences, all are small molecules (M_r about 7000–16,000) which are tightly folded with a conserved pattern of inter-chain disulphide bonds. They also have similar three-dimensional structures, and are defined in the SCOP structural database (Murzin Brenner, Hubbard, & Chothia, 1995) as the 'all-alpha' proteins class with a "common fold at four α -helices, folded leaf, right-handed superhelix". This similarity is apparent in the three structures shown in Fig. 3. All three groups of proteins also have defensive roles against pests and pathogens, at least in some tissues and species.

The cupins are an immensely diverse superfamily of proteins, many of which originate from bacteria and plants, which share two short conserved sequence motifs (Dunwell, 1998). They also have a common basic structural feature, a double stranded α -helix domain, which led to the name 'cupin' based on the latin for a small barrel or cask. The most widely studied cupins are the 7S and 11S globulin storage proteins which are widespread in the seeds of monocotyledonous and dicotyledonous plants. Both of these protein groups appear to have arisen from the duplication of an ancestral cupin domain and hence are termed bicupins. An example of this type of protein structural motif is shown in Fig. 4, for Jack bean canavalin.

Typical 7S globulins are trimeric complexes of three subunits of M_r about 50,000, while the 11S globulins are typically hexameric with M_r 60,000 subunits which are proteolytically cleaved to give acidic and basic chains but remain associated by a single disulphide bond (Casey, 1999). Although the subunits of the two types of globulins have no obvious sequence similarity, they do have remarkably similar three-dimensional structures (Adachi, Takenaka, Gidamis, Mikami, & Utsumi, 2001). Major allergens include the 7S globulins of soybean (β -conglycinin), peanut (conarachin, Ara h 1), walnut (Jug r 2) and lentil and 11S globulins of peanut (arachin, Ara h 3), soybean (glycinin) and hazelnut, with coconut having been only implicated as an allergen. Other members of the cupin superfamily do not appear to be allergenic, with the exception of one germin (germination-specific protein) from pepper (Leitner *et al.*, 1998).

The 7S/11S globulins differ greatly from the 2S albumins/nsLTP/cereal inhibitors in having higher masses and lacking intra-chain disulphide bonds (with the exception of the single bond in 11S globulin subunits). Similarly, all of the prolamin superfamily allergens either bind or associate with lipids while the 7S globulin subunits may be glycosylated. However, all of these proteins show unusually high stability to denaturation and proteolysis. This property may relate to their allergenicity.

The cysteine protease, also known as the C1, or papain-like, family was originally identified as having

conserved Gln, Cys, His and Asn residues at the active site and includes many peptidases with exo- and/or endo-peptidase activities (Rawlings & Barrett, 1993). Found in both prokaryotes and eukaryotes, C1 proteases are generally lysosomal or secreted, being synthesized as a pro-protein, proteolytic cleavage of the propeptide being required for enzyme activation. They participate in a number of processes in plants including

senescence, digestion of storage proteins during seed germination as well as having a role in plant protection against pests and pathogens. Cysteine protease food allergens have been identified in kiwi fruit (actinidin, Act c 1), (Pastorello, Conti, *et al.*, 1998) and the soybean 34kD so-called oil body-associated protein, known variously as Gly m Bd 30K, Gly m 1, or P34. (Ogawa *et al.*, 1993). Gly m Bd 30K has lost its protease activity, a

Table 1. Properties of major plant food allergens

Allergen	Allergen designation	Molecular weight	Function and stability
Cross-reactive food allergens (sensitisation by inhalant allergens)			
Homologues of birch pollen allergen <i>Bet v 1</i>	Apple (Mal d 1); apricot (Pru ar 1); cherry (Pru av 1); Pear (Pyr c 1); carrot (Dau c 1); celery (Api g 1); hazel nut (Cor a 1.0401)	16,000–17,700	Function unknown; member of PR-10 anti-pathogen proteins
Profilins	Cherry (Pru av 4); pear (Pyr c 4); Peanut (Ara h 5); soybean (Gly m 3); celery (Api g 4)	14,300–15,000	Actin binding protein
Thaumatococin-like proteins	Apple (Mal d 2); cherry (Pru av 2)	33,500	Causes cell lysis through binding membrane proteins; member of PR-5 anti-pathogen proteins
Isoflavone reductase	Pear (Pyr c 5)	32,000	Enzyme involved in plant secondary metabolism
Cysteine protease	Kiwi (Act c 1)	30–34,000	Aspartyl protease
Class II chitinase	Avocado (<i>Prs a 1</i>); banana (<i>Mus p1.1</i> <i>Mus p1.2</i>); turnip (<i>Bra r 2</i>); Chestnut (<i>Cas s 1</i>)		Enzyme that degrades chitin in fungal cell walls and belongs to the PR-3 group of anti-pathogen proteins
Allergens that sensitise via the GI tract: Prolamin superfamily:			
Cereal prolamins	α - and γ -Gliadin; LMW glutenin	30–55,000	Seed storage proteins
α -Amylase inhibitors	Barley (<i>Hor v 1</i> CMb); rice (RAP); rye (<i>Sec c 1</i>); wheat	15–16,000	Inhibitor of fungal and insect α -amylases and hence have a role in protection of plants against pathogen attack
Non-specific lipid transfer proteins (ns LTPs)	Apple (Mal d 3); apricot (Pru ar 3); plum (Pru d 3); peach (Pru p 3); maize (<i>Zea m 14</i>)	9000	Thought to be involved in the transport of suberin monomers during cutin biosynthesis
2S albumins	Brazil nut (<i>Ber e 1</i>); Peanut (<i>Ara h 2,6,7</i>); walnut (<i>Jug r 1</i>); Castor bean (<i>Ric c 1</i>); yellow mustard (<i>Sin a 1</i>); oriental mustard (<i>Bra j 1</i>); sesame (<i>Ses i 1</i>)	9000–17,000 (precursor frequently processed into a small 3000 and a larger >9000 subunit)	Seed storage protein
<i>Cupin</i> superfamily 11S Globulins	Peanut arachin (<i>Ara h 3,4</i>); soybean glycinin	~50–60,00 precursor cleaved post-translationally into disulphide-linked 20,000 and 30–40,000 subunits assembled in to hexamers	Seed storage protein
7S Globulins	Peanut (<i>Ara h 1</i>); walnut (<i>Jug r 2</i>); soybean β -conglycinin; lentil	~50–70,000 subunits, often glycosylated, assembled into trimers	Seed storage protein

glycine having replaced the cysteine residue in the active site and it has now been shown to be associated with the storage vacuoles of soybean (Kalinski, Weisemann, Matthews, & Herman, 1990).

Food allergens arising from cross-reactive inhalant allergens

A more diverse range of food proteins may be allergenic due to sensitization inhalation. These may be broadly divided into two groups, based on whether the sensitization is via pollen or latex.

The major Bet v 1, is related to a class of putative plant defence proteins (PR10) and results in dietary reaction to proteins in a range of fruit (including Mal d 1 of apple and Pru av 1 of cherry) and vegetables (including Api g 1 of celery, Dau c 1 of carrot). In contrast, the second major pollen allergen, Bet v 2, is a profilin, proteins which bind to the actin cytoskeleton of cells. Sensitization results in allergic reactions to proteins in various plant foods including hazelnut, peanut (Ara h 5), soybean (Gly m 3), celery (Api g 4) and pear (Pyr c 4).

Although the biological role of PR10 proteins has not been established, they are presumed to be defensive, as they form part of a broad spectrum of proteins (called pathogenesis-related or PR proteins), which are synthesized in plants in response to infection or damage (Van Loon & Van Strien, 1999). Inhalation of two other PR-type proteins from latex similarly results in dietary allergy to related proteins in fruit and vegetables. These are PR-2/Hev b 2 (β 1,3-glucanase) and PR-3/Hev b 6 (class II chitinases) which result in cross-reactions with avocado, chestnut, banana, fig, kiwi and other foods. A further type of PR protein (PR-5) which is related to the sweet protein thaumatin, is a major allergen in apple (Mal d 2), cherry (Pru cv 2) and some other foods.

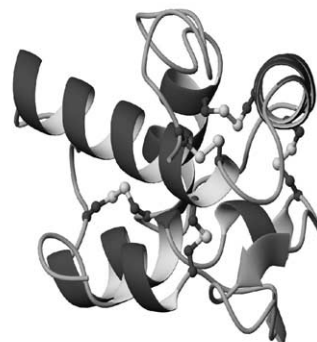
It is not possible to describe the full range of plant food allergens in this short article but the vast majority fall into the classes discussed above. These include the cupin, prolamin and cysteine protease superfamilies, which have storage and/or protective functions and predominantly sensitize via the GI tract, and the pollen/fruit latex/fruit allergens which predominantly sensitize via inhalation and are mostly related to defence (PR) proteins. It is notable that no major allergens appear to correspond to the enzymes and structural proteins responsible for the day-to-day running of the cell. Although protein abundance may be a factor in determining allergenicity, this is clearly not the only one, as the most abundant protein in the world, the photosynthetic enzyme ribulose biphosphate carboxylase/oxygenase (rubisco) has never been identified as an allergen. It is probable, therefore, that a range of other factors also contribute to allergenicity including stability to low pH and proteolysis and other activities such as the ability to bind to lipids and other molecules (starch, other carbohydrates, proteins).

How does this knowledge help?

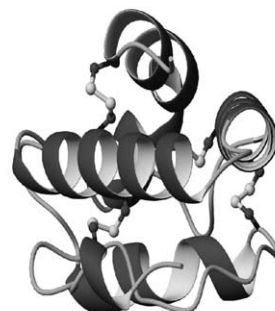
Improved safety assessment

One of the problems that beset the assessment of allergenic risks posed by novel foods, (including GMOs) and food produced using novel processing technologies, is our lack of adequate animal models for food allergenicity. This process of prediction is further hampered by our incomplete understanding of the molecular basis

Wheat 0.19 Amylase Inhibitor



Maize n-LTP



Napin model 1

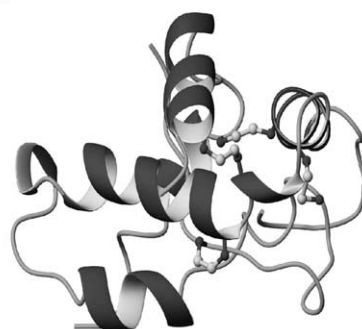


Fig. 3. Ribbon representations of the three-dimensional structures of representatives of three major classes of allergen belonging to the prolamin superfamily: cereal α -amylase/trypsin inhibitors (wheat 0.19 α -amylase inhibitor), nsLTP (maize) and 2S albumin (oilseed rape napin). (A) A ribbon representation of maize non-specific lipid transfer protein (Shin, Lee, Hwang, Kinm, & Suh, 1995). The conserved disulphides on the second-helix are at the top right. (B) A ribbon representation of wheat 0.19 α -amylase inhibitor (Oda, Matsunaga, Fukuyama, Miyazaki, & Morimoto, 1997). (C) A ribbon representation of oilseed rape napin (Rico, Bruix, Gonzalez, Monsalve, & Rodriguez, 1996).

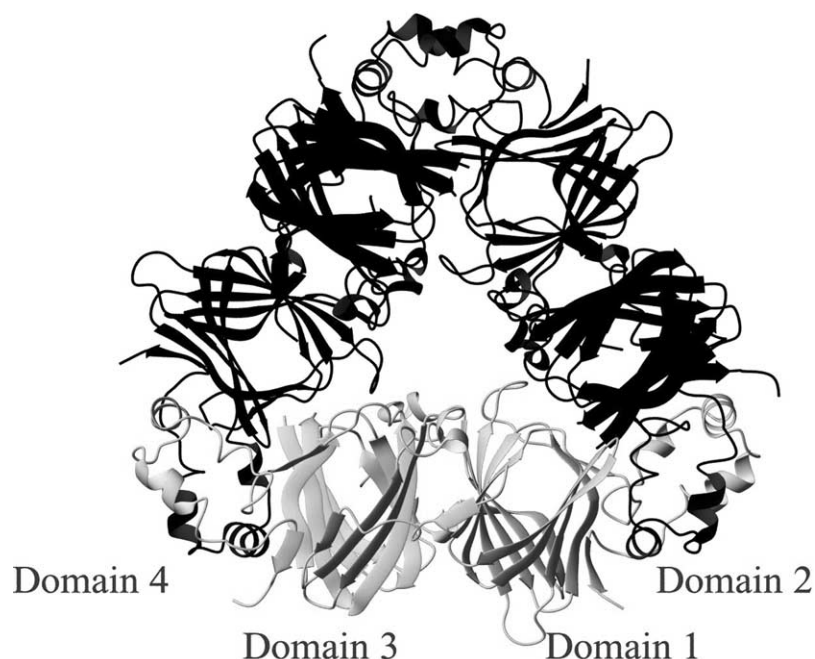


Fig. 4. Ribbon representation of the structure of canavalin (a 7S globulin belonging to the cupin superfamily) from seeds of jack bean (*Canavalia ensiformis*). Ribbon diagram viewed down the molecular three fold axis (coordinates from PDB code 1DGW (Ko, Ng, & McPherson, 1993) and the trimer generated by the PQS server (Henrick & Thornton, 1998). The ribbons from one molecule are lighter shaded while the other two molecules are dark. Domains 1 and 3 are the standard cupin barrels formed by coiling a beta-hairpin into a double beta-helix. Domains 2 and 4 mediate trimer formation and are formed from alpha-helices of two molecules.

for the development of the aberrant IgE responses in food allergy. As a consequence, a decision-tree approach has been proposed for assessing allergenic risks by a joint FAO/WHO (2001) Expert Consultation on Foods Derived from Biotechnology held in 2001. As, currently, we do not know the sum of the properties that makes a protein into a food allergen, and we cannot simply take the structure of an unknown protein and predict its allergenicity, the decision tree is based primarily on direct and indirect comparisons of allergen structure. Subsequently in March 2002, the third session of the FAO-WHO (2002) Codex Alimentarius Commission *Ad Hoc* Intergovernmental task force on Foods Derived from Biotechnology (ALINORM 03/34) decided against elaborating the decision tree proposed by the Expert Consultation. Instead they have recommended an integrated step-wise case-by-case approach which takes account of the evidence derived from several types of information and data, as no single criterion is sufficiently predictive of allergenicity. However, it seems likely that for practical point reasons the decision tree approach will still have a value in planning the research likely to be required to support a risk assessment.

In the first instance the WHO/FAO tree proposes that the sequence of a 'new' protein should be compared with those of all known allergens, cross-reactivity with a known allergen being likely if a match showing $\geq 35\%$ homology is found. There are no generally accepted

criteria for defining significant matches and, hence, the results of such sequence comparisons must be combined with other considerations, such as the source of the protein (has it come from a source that is known to cause allergic reactions in man?), and its stability to digestion and food processing. Any putative allergens identified by this process must then be verified using suitable antibodies (from human or animal source) to substantiate the potential for cross-reactivity. When no relationship with an existing allergen is demonstrated, the WHO/FAO decision tree suggests that the novel proteins must then be tested for reactivity against panels of well-defined human allergic sera. This would determine whether the novel protein has IgE-epitopes identical to those present in related inhalant or food allergens. Again, because of our current lack of knowledge regarding the mechanisms of food allergenicity, the positive predictability of the targeted serum screen is uncertain, making a risk assessment difficult. As a consequence of this lack of knowledge it has been suggested that if a protein is negative in the targeted serum screen it should be tested in an animal model (despite the lack of well validated animal models for oral sensitization) and its pepsin resistance evaluated.

An issue that has to be decided early in this assessment process is 'can the source of the gene or a novel protein known to cause an allergic reaction?' Whilst the ability of some foods to elicit allergic reactions is commonly known, there are many reports in the literature

of allergens being described for all types of foods, including fruits like mango and grains such as buckwheat. As part of the network activities the Protall partnership have put together a searchable database containing biochemical and clinical information about plant food allergens involved in classical IgE-induced hypersensitivity reactions to assist in this identification. It contains information about 77 allergens from 48 plant species, and can be found at www.ifr.ac.uk/Protall.

Another important resource for performing the homology checks involved in the early stages of novel foods allergenic risk assessments are the allergen sequence databases. However, the coverage of some of the sequence databases, such as the ExPASy list of allergen sequences, is not as complete as that published on the Food Allergy Research and Resource Programme (FARRP) website. Using homology searching strategies based on the sequences available in these databases, it is possible to identify allergens which demonstrate

1. *High levels of homology WITH conservation of allergenicity*—The major apple allergen Mal d 1 has a sequence homology with the major birch pollen allergen (Bet v 1) of 56% and with the major hazel pollen allergen of 54% (Son Scheurer, Hoffman, Haustein, & Vieths, 1999). Homology searching is highly effective at identifying cross-reactive allergens such as these.
2. *High levels of homology WITHOUT conservation of allergenicity*—Tropomyosin from chicken has 60% sequence homology with tropomyosin from shrimp, the major shrimp allergen, but there is no IgE cross-reactivity between shellfish and vertebrate tropomyosins, which are not known as food allergens (Leung *et al.*, 1996). Homology searching gives false positive results in such instances.
3. *Low levels of homology WITH conservation of allergenicity*—Members of the cupin superfamily of allergens show a low level of sequence homology (<35%) but have conserved structures and function, and are NOT detected using this approach (Lawrence, Izard, Beuchat, Blagrove, & Colman, 1994).

The relationships between plant protein families are highly relevant to demonstrating the lack of similarity between a 'novel' protein and known allergens necessary for a protein to be acceptable for consumption. We suggest that this would be a valuable approach to include in the risk assessment process which would be complementary to direct homology searching. Thus, a novel protein would also be evaluated in terms of its membership of plant protein families that include a high proportion of food allergens, such as the cupin and prolamin superfamilies. Such an approach would

address the problems presented by proteins that have low levels of homology but have conserved three-dimensional structures and functions, such as members of the cupin superfamily, which are poorly detected using homology searching alone. It could also be extended to other protein families to which a high proportion of allergens belong, such as the lipocalin superfamily, to which a number of inhalant allergens (including animal dander and urinary proteins) and the cows milk allergen, β -lactoglobulin, all belong (Mantylarvi, Rautiainen, & Virtanen, 2000).

Reduced allergen foods

Food technology aims to reduce allergenicity by either irreversible removal of allergens, or by modifying the allergen structure in such a way that the allergenic epitopes are no longer recognized by the immune system. The harshness of processing methodology is limited because of 'processing side effects' that cause unacceptable sensory losses (e.g. in colour or taste), or a loss of desired processing properties (e.g. swelling behaviour, baking properties of flour, etc.). The most suitable technology will, therefore, depend on a thorough evaluation of raw material characteristics, and product specifications. Until now, the technological approach to decrease allergenicity has largely been empirical. The main reason for this is a lack of detailed knowledge on integral allergen and epitope structure and their genetic background, which hampers the design of more rational, generic strategies for processing (Soler-Rivas & Wichers 2001; Besler, Steinhart, & Paschke, 2001).

Thermal processing effects on allergenicity

Processing-induced changes in allergen conformation

Whilst a large number of food allergens, such as the ns LTPs and globulin storage proteins, are highly thermostable and require temperatures in excess of 75°C to begin to unfold, other allergens, particularly those associated with the pollen-fruit/vegetable cross-reactive allergy syndromes, are more thermolabile. They can be effectively denatured and their allergenic activity destroyed by thermal processing. However, this thermolability is not equivalent in different members of the Bet v 1 family of allergens. Thus, the major apple allergen, Mal d 1, is rather easily destroyed upon heating, as is the homologue from celery, Api g 1 (Wigotzki, 2001). However, the equivalent allergens from carrot (Dau c 1) and peach (Pru p 1), are much more thermostable, retaining their allergenicity after heating for 40 min at 100°C or even when autoclaved at 121°C (Wigotzki, 2001).

Processing-induced chemical modification of allergens

The IgE-binding capacity of the peanut allergens Ara h 1, Ara h 2 and Ara h 3 is reduced in fried and boiled nuts, compared with dry roasted peanuts (Beyer *et al.*,

2001). It seems likely that the formation of Maillard reaction-related neoallergens plays a role in these differences (Davis, Smales, & James, 2001). Peanut allergy is much more widespread in the USA than countries in China, and it is intriguing to speculate that this is linked to different patterns of consumption, dry roasted peanuts being the predominant form consumed in the United States, whereas in China boiled nuts are more widely consumed. If this apparent relationship can be further substantiated, it would open the way to reduce the allergenic potential of nuts through the application of specific processing methods.

Enzymatic processing

Biochemical (enzymatic) processing of raw materials may also aid the removal of allergenicity. Prerequisites are sufficient contact between the allergen, or epitope, and the enzyme, and sufficient control of undesired side effects that may affect sensory quality or processing properties. In some cases, proteolytic processing is insufficient to reduce allergenicity (Brenna *et al.*, 2000; Maleki *et al.*, 2000). This may arise because of poor contact between proteases and allergens in the food matrix, or lack of specific proteases. However, the allergenicity of wheat flour prolamins can be decreased by treatment with the protease bromelain, which cleaves the prolamins IgE-epitope Gln-Gln-Gln-Pro-Pro near the proline residues, or by treatment with a non-food grade bacterial collagenase. This was achieved without a loss of functional performance, the hypoallergenic flour still being able to produce a muffin (Tanabe, Arai, & Watanabe, 1996; Watanabe, Tanabe, Suzuki, Ikezawa, & Arai, 1995). Another example of successful enzymatic processing is the production of hypoallergenic rice by a two-stage process, employing actinase, the treated rice grains retaining an acceptable texture (Watanabe, 1993; Watanabe *et al.*, 1990). In addition to proteolytic processing, oxidising enzymes have been used to reduce allergen reactivity in certain foods. This has been demonstrated for apple, where it is thought that oxidases reduce IgE-reactivity by linking natural fruit phenolic compounds to the allergen (Wigotzki, 2001).

Novel processing technology and food allergenicity

There has been some interest in exploring new technologies, particularly high pressure processing, as an alternative to thermal processing to alter allergen conformation and hence decrease allergenic activity. A rice product with a lower allergenic potential is on the market in Japan, based on the preferential release of allergenic proteins by high pressure treatment. When polished rice grains were immersed in water and pressurized at 100–400 MPa, a considerable amount of protein was released. The major proteins released were identified as a 16 kDa albumin, α -globulin and 33 kDa globulin, all of which are major rice allergens. The pro-

posed mechanism is that the partial destruction of endosperm cells by pressurization enhances the permeation of the surrounding solution into the rice grains. The removal of allergens by pressurization alone was insufficient to reduce allergenic activity, which was almost completely eliminated by pressurization in the presence of proteolytic enzymes (Kato, Katayama, Matsuura, Omi, & Matsuda, 2000). However, high pressure treatment (600 MPa, 20°C) was not sufficient to destroy the IgE-binding capacity of the Bet v 1-like allergen, Api g 1, from celery, nor pulsed electric field processing at 10 kV at 50 Hz or γ -irradiation (10 kGy) (Jankiewicz *et al.*, 1997).

Future directions

Our improved understanding of the structural attributes common to plant food allergens will allow us to investigate whether these shared properties play a role in determining allergenic potential in the future. Investigations are needed to determine whether the structural attributes and properties of allergens have played a role in determining their allergenicity. They could effect this either by maximizing immune system exposure to allergens (or allergen fragments), or an intrinsic ability of allergens to preferentially elicit an IgE response. Such knowledge will, in turn, lead to improved methods and approaches for assessing the allergenic risks posed by novel foods. It will also improve our ability to use food processing to reduce the allergenic properties of foods. Another area likely to benefit from our improved understanding of allergen structure and how that affects interactions with the immune system, in the development of allergic disease are diagnosis and treatment strategies. At present, *in vitro* diagnostic methods are unable to give a clear unequivocal diagnosis of food allergy, leaving double blind placebo controlled food challenges as the 'gold standard' of food allergy diagnosis. Time-consuming and difficult to perform, such an unequivocal diagnosis of food allergy is available in only a limited number of centres across Europe. Improvements in the stability of the allergen preparations used for diagnosis, and an understanding of how both food processing and subsequent digestion may alter the presentation of potential allergens to the immune system during the early stages of sensitization, will undoubtedly lead to the development of a new generation of diagnostic aids. These will have the potential to deliver a faster, more effective diagnosis of food allergies to a wider population.

However, currently the only therapy available to food allergic individuals is dietary exclusion, a poor option, fraught with difficulties for the severely allergic. Social costs are difficult to quantify, but include a need for the severely allergic and their families to be constantly vigilant when shopping, eating out in restaurants or visiting friends, to avoid inadvertent consumption of a problem

food. It also results in the need to label allergenic foods and for the food industry to demonstrate effective hazard control procedures and monitoring for cross-contact allergens in foods, which do not normally contain allergenic ingredients, such as peanuts. The greatest challenge in food allergy research undoubtedly lies in the search for effective therapies to ameliorate the condition, particularly to reduce the severity of the symptoms experienced by some sufferers. The search for food allergy therapeutics depends on our understanding of the molecular nature of food allergens and those structures which are important for effector cell activation. This is especially true for the design of allergen derivatives for use in immunotherapy with a reduced risk of inducing adverse reactions, such as anaphylaxis (Valenta & Kraft, 2001).

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References

- Adachi, M., Takenaka, Y., Gidamis, A. B., Mikami, B., & Utsumi, S. (2001). Crystal structure of soybean proglycinin A1aB1b homotrimer. *Journal of Molecular Biology*, 305, 291–305.
- Alberse, R. C. (2000). Structural biology of allergens. *Journal of Allergy and Clinical Immunology*, 106, 228–238.
- Besler, M., Steinhart, H., & Paschke, A. (2001). Stability of food allergens and allergenicity of processed foods. *Journal of Chromatography, B* 756, 207–228.
- Beyer, K., Morrow, E., Li, X.-M., Bardina, L., Bannon, G. A., Burks, A. W., & Sampson, H. A. (2001). Effects of cooking methods on peanut allergenicity. *Journal of Allergy and Clinical Immunology*, 107, 1077–1081.
- Brantzaeg, P. (2001). Nature and function of gastrointestinal antigen-presenting cells. *Allergy*, 56(Suppl. 67), 16–20.
- Brenna, O., Pompei, C., Ortolani, C., Pravettoni, V., Farioli, L., & Pastorello, E. A. (2000). Technological processes to decrease the allergenicity of peach juice and nectar. *Journal of Agricultural and Food Chemistry*, 48, 493–497.
- Bush, R. K., & Hefle, S. L. (1996). Food allergens. *CRC Critical Reviews in Food Science and Nutrition*, 36(Suppl.), S119.
- Centner, J., & de Weck, A. L. (1995). *Atlas of immuno-allergy*. Seattle: Hogrefe & Huber.
- Casey, R. (1999). Distribution and some properties of seed globulins. In P. R. Shewry, & R. Casey (Eds.), *Seed Proteins* (pp. 159–169). Netherlands: Kluwer.
- Clarke, B. C., Hobbs, M., Skylas, D., & Appels, R. (2000). Genes active in developing wheat endosperm. *Functional and Integrative Genomics*, 1, 44–55.
- Crusie, J. M., & Lewis, R. E. (1999). *Atlas of immunology*. Boca Raton: CRC Press.
- Davis, P. J., Smales, C. M., & James, D. C. (2001). How can thermal processing modify the antigenicity of proteins? *Allergy*, 56(Suppl. 67), 56–60.
- Dunwell, J. M. (1998). Cupins: a new superfamily of functionally diverse proteins that include germins and plant storage proteins. *Biotechnology Genetic Engineering Reviews*, 15, 1–32.
- Duro, G., Colombo, P., Costa, M. A., Izzo, V., Porcasi, R., Di Fiore, R., Locorotondo, G., Mirisola, M. G., Cocchiara, R., & Geraci, D. (1996). cDNA cloning, sequence analysis and allergological characterization of Par j 2.0101, a new major allergen of the *Parietaria judaica* pollen. *FEBS Letters*, 399, 295–298.
- FAO-WHO. (2001). *Evaluation of allergenicity of genetically modified foods. Report of the joint FAO/WHO expert consultation on allergenicity of foods derived from biotechnology* (pp. 1–26) 22–25 January 2001
- FAO-WHO. (2002). *Report of the third session of the Codex Ad Hoc Intergovernmental Task Force on Foods derived from Biotechnology* (pp. 1–73), 4–8 March 2002. Available: ftp://ftp.fao.org/codex/alnorm03/Al03_34e.pdf.
- Helm, R. M., & Burks, A. W. (2000). Mechanisms of food allergy. *Current Opinions in Immunology*, 12, 647–653.
- Henrick, K., & Thornton, J. M. (1998). PQS: a protein quaternary structure file server. *Trends in Biochemical Sciences*, 23, 358–361.
- Jankiewicz, A., Baltes, W., Bögl, K. W., Dehne, L. I., Jamin, A., Hoffmann, A., Haustein, D., & Vieths, S. (1997). Influence of food processing on the immunochemical stability of celery allergens. *Journal of the Science of Food and Agriculture*, 75, 359–370.
- Kalinski, A., Weisemann, J. M., Matthews, B. F., & Herman, E. M. (1990). Molecular cloning of a protein associated with soybean seed oil bodies that is similar to thiol proteases of the papain family. *Journal of Biological Chemistry*, 265, 13843–13848.
- Kazemi-Shirazi, L., Pauli, G., Purohit, A., Spitzauer, S., Fröschl, R., Hoffmann-Sommergruber, K., Breitender, H., Scheiner, O., Kraft, D., & Valenta, R. (1999). Quantitative IgE inhibition experiments with purified recombinant allergens indicate pollen-derived allergens as the sensitizing agents responsible for many forms of plant food allergens. *Journal of Allergy and Clinical Immunology*, 105, 116–125.
- Kato, T., Katayama, E., Matsubara, S., Omi, Y., & Matsuda, T. (2000). Release of allergenic proteins from rice grains induced by high hydrostatic pressure. *Journal of Agricultural and Food Chemistry*, 48, 3124–3129.
- Ko, T. P., Ng, J. D., & McPherson, A. (1993). The three-dimensional structure of canavalin from jack bean (*Canavalia ensiformis*). *Plant Physiology*, 101, 729–744.
- Kulig, M., Bergmann, R., Klettke, U., Wahn, V., Tacke, U., & Wahn, U. (1999). Natural course of sensitization to food and inhalant allergens during the first 6 years of life. *Journal of Allergy and Clinical Immunology*, 103, 1173–1179.
- Leung, P. S., Chow, W. K., Duffey, S., Kwan, H. S., Gershwin, M. E., & Chu, K. H. (1996). IgE reactivity against cross-reactive allergen in crustacea and mollusca: evidence for tropomyosin as the common allergen. *Journal of Allergy and Clinical Immunology*, 5(pt 1), 483–487.
- Lawrence, M. C., Izard, T., Beuchat, M., Blagrove, R. J., & Colman, P. M. (1994). Structure of phaseolin at 2.2 Å resolution. Implications for a common vicilin/legumin structure and the genetic engineering of seed storage proteins. *Journal of Molecular Biology*, 238, 748–776.
- Leitner, A., Jensen-Jarolim, E., Grimm, R., Wuthrich, B., Ebner, H., Scheiner, O., Kraft, D., & Ebner, C. (1998). Allergens in pepper and paprika. Immunologic investigation of the celery-birch-mugwort-spice syndrome. *Allergy*, 53, 36–41.
- Maleki, S. J., Kopper, R. A., Shin, D. S., Park, C.-W., Compadre, C. M., Sampson, H., Burks, A. W., & Bannon, G. A. (2000). Structure of the major peanut allergen Ara h 1 may protect IgE-binding epitopes from degradation. *Journal of Immunology*, 164, 5844–5849.
- Mantylä, R., Rautiainen, J., & Virtanen, T. (2000). Lipocalins as allergens. *Biochimica Biophysica Acta*, 1482, 308–317.
- Murzin, A. G., Brenner, S. E., Hubbard, T., & Chothia, C. (1995). SCOP: a structural classification of proteins database for the investigation of sequences and structures. *Journal of Molecular Biology*, 247, 536–540.
- Nakase, M., Adachi, T., Urisu, A., Miyashita, T., Alvarez, A. M., Nagasaka, S., Aoki, N., Nakamura, R., & Matsuda, T. (1996). Rice (*Oryza sativa* L.) α -amylase inhibitors of 14–16 kDa are potential

- allergens and products of a multigene family. *Journal of Agricultural and Food Chemistry*, 44, 2624–2628.
- Oda, Y., Matsunaga, T., Fukuyama, K., Miyazaki, T., & Morimoto, T. (1997). Tertiary and quaternary structures of 0.19 alpha-amylase inhibitor from wheat kernel determined by X-ray analysis at 2.06 angstrom resolution. *Biochemistry*, 36, 13503–13511.
- Ogawa, T., Tsuji, H., Bando, N., Kitamura, K., Zhu, Y.-L., Hirano, H., & Nishikawa, K. (1993). Identification of the soyabean allergenic proteins, Gly m Bd 30K, with the soybean seed 34kDa oil-body-associated protein. *Bioscience Biotechnology and Biochemistry*, 57, 1030–1033.
- Ortolani, C., Ispano, M., Ansaloni, R., Rotondo, F., Incorvaia, C., & Pastorello, E. A. (1998). Diagnostic problems due to cross-reactions in food allergy. *Allergy*, 53(Suppl. 46), 58–61.
- Pastorello, E. A., Incorvaia, C., Pravettoni, V., Farioli, L., Conti, A., Viganò, G., Rivolta, F., Ispano, M., Rotondo, F., & Ortolani, C. (1998). New allergens in fruit and vegetables. *Allergy*, 53(Suppl. 46), 48–51.
- Pastorello, E. A., Conti, A., Pravettoni, V., Farioli, L., Rivolta, F., Ansaloni, R., Ispano, M., Incorvaia, C., Giuffrida, M. G., & Ortolani, C. (1998). Identification of actinidin as the major allergen of kiwi fruit. *Journal of Allergy and Clinical Immunology*, 101(4 Pt 1), 531–537.
- Rawlings, N. D., & Barrett, A. J. (1993). Evolutionary families of peptidases. *Biochemical Journal*, 290, 205–218.
- Rico, M., Bruix, M., Gonzalez, C., Monsalve, R. I., & Rodriguez, R. (1996). H-1 NMR assignment and global fold of napin Bn1b, a representative 2S albumin seed protein. *Biochemistry*, 35, 5672–5682.
- Sánchez-Monge, R., Lombardero, M., Garcia-Sellés, F. J., Barber, D., & Salcedo, G. (1999). Lipid-transfer proteins are relevant proteins in fruit allergy. *Journal of Allergy and Clinical Immunology*, 103, 514–519.
- Shin, D. H., Lee, J. Y., Hwang, K. Y., Kim, K. K., & Suh, S. W. (1995). High-resolution crystal-structure of the nonspecific lipid-transfer protein from maize seedlings. *Structure*, 3, 189–199.
- Sloan, A. E., & Powers, M. E. (1986). A perspective on popular perceptions of adverse reactions to foods. *Journal of Allergy and Clinical Immunology*, 78(1 Pt 2), 127–133.
- Soler-Rivas, C., & Wichers, H. J. (2001). Impact of (bio)chemical and physical procedures on food allergen stability. *Allergy*, 53(Suppl. 67), 52–55.
- Son, D. Y., Scheurer, S., Hoffman, A., Hausteiner, D., & Vieths, S. (1999). Pollen-related food allergy: cloning and immunological analysis of isoforms and mutants of Mal d 1, the major apple allergen, and Bet v 1, the major birch pollen allergen. *European Journal of Nutrition*, 38, 201–215.
- Strobel, S. (1997). Oral tolerance: Immune responses to food antigens. In D. D. Metcalfe, H. A. Sampson, & R. Simon (Eds.), *Food allergy. Adverse reactions to food and food additives* (pp. 107–135). USA: Blackwell Science MA.
- Tanabe, S., Arai, S., & Watanabe, M. (1996). Modification of wheat flour with bromelain and baking hypoallergenic bread with added ingredients. *Bioscience, Biotechnology and Biochemistry*, 60, 1269–1272.
- Valenta, R. (2001). & Kraft, D Recombinant allergen molecules: tools to study effector cell activation. *Immunology Reviews*, 179, 119–127.
- Van Loon, L. C., & Van Strien, E. A. (1999). The families of pathogenesis-related proteins, their activities and comparative analysis of PR-1 type proteins. *Physiology and Molecular Plant Pathology*, 55, 85–97.
- van Regenmortel, M. H. V. (1992). Molecular dissection of protein antigens. In M. H. V. Van Regenmortel (Ed.), *Structure of antigens Vol. 1* (pp. 1–27). USA: CRC Press.
- Watanabe, M. (1993). Hypoallergenic rice as a physiological functional food. *Trends in Food Science and Technology*, 4, 125–128.
- Watanabe, M., Tanabe, S., Suzuki, T., Ikezawa, Z., & Arai, S. (1995). Primary structure of an allergenic peptide occurring in the chymotryptic hydrolysate of gluten. *Bioscience, Biotechnology and Biochemistry*, 59, 1596–1597.
- Watanabe, M., Yoshizawa, T., Miyakawa, J., Ikezawa, Z., Abe, K., Yanagisawa, T., & Arai, S. (1990). Quality improvement and evaluation of hypoallergenic rice grains. *Journal of Food Science*, 55, 1105–1107.
- Wigotzki, M. (2001). *Einfluß der technologischen Verarbeitung auf das allergene Potential der birkenpollenassozierten Lebensmittel Haselnuß, Apfel und Karotte*. Thesis, University of Hamburg, Germany.
- Wüthrich, B., & Strauman, F. (1997). Pollen cross-reactivity. Can we establish a link between the *in vitro* results and the clinical situation? *Allergy*, 52, 1187–1193.

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