

Molecular mechanisms in allergy and clinical immunology

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Molecular and biochemical classification of plant-derived food allergens

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Molecular biology and biochemical techniques have significantly advanced the knowledge of allergens derived from plant foods. Surprisingly, many of the known plant food allergens are homologous to pathogenesis-related proteins (PRs), proteins that are induced by pathogens, wounding, or certain environmental stresses. PRs have been classified into 14 families. Examples of allergens homologous to PRs include chitinases (PR-3 family) from avocado, banana, and chestnut; antifungal proteins such as the thaumatin-like proteins (PR-5) from cherry and apple; proteins homologous to the major birch pollen allergen Bet v 1 (PR-10) from vegetables and fruits; and lipid transfer proteins (PR-14) from fruits and cereals. Allergens other than PR homologs can be allotted to other well-known protein families such as inhibitors of α -amylases and trypsin from cereal seeds, profilins from fruits and vegetables, seed storage proteins from nuts and mustard seeds, and proteases from fruits. As more clinical data and structural information on allergenic molecules becomes available, we may finally be able to answer what characteristics of a molecule are responsible for its allergenicity. (*J Allergy Clin Immunol* 2000;106:27-36.)

Key words: Food allergen, pathogenesis-related protein, cross-reactivity

According to the clinical appearance, the pattern of allergens, and the underlying immunologic mechanisms, two forms of food allergy have to be distinguished. In class 1 food allergy the sensitization process occurs in the gastrointestinal tract. One special feature of the allergens eliciting this manifestation is their particular resistance to gastric digestion.¹ This kind of food allergy is rare in adults. However, food allergy is characteristically one of the first manifestations of the atopic syndrome and

Abbreviations used

cDNA: Complementary DNA
DBPCFC: Double-blind placebo-controlled food challenge
LTP: Lipid transfer protein
OAS: Oral allergy syndrome
PR: Pathogenesis-related protein

affects young children. The most important allergens are cow's milk, hen's egg, and legumes. It can be expected in the majority of the cases that these manifestations disappear later during childhood and are replaced by other manifestations of the atopic syndrome.²

Class 2 food allergy is mainly seen in adults and develops as a consequence of an allergic sensitization to inhalant allergens. The immunologic basis for these food allergies is IgE cross-reactivity, which might be clinically manifest or irrelevant.^{3,4} This kind of food allergy found special attention during the last decade, obviously because of the steadily increasing prevalence of inhalant allergies in this period. The allergens eliciting these adverse reactions do not display obvious physicochemical characteristics. According to their behavior during the digestion process, they can cause symptoms ranging from the oral allergy syndrome (OAS) (eg, typical for the birch-apple-syndrome) to anaphylactic shock, which is not rare in the mugwort-celery-syndrome.⁵⁻⁷ The diagnostic instrumentarium is satisfactory only for class 1 food allergy. In this instance, case history, provocation tests (ideally double-blind placebo-controlled food challenge [DBPCFC]⁸) and serologic studies can provide the allergist with useful information about the specificity and quantity of the respective sensitization. In case of class 2 food allergy the situation is worse: the majority of these—typically plant-derived—proteins are difficult to extract and highly labile. Diagnostic setups using natural extracts for in vitro or in vivo assays are therefore in many cases unsatisfactory. Procedures such as DBPCFC are also hard to perform because the fresh material has to be masked and should not be swallowed immediately, as in case of OAS. One solution for this problem would be the use of standardized recombinant food allergens. A limited number of class 1 and class 2 food allergens have been cloned, sequenced, and expressed as recombinant proteins.

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Higher-order plants accumulate pathogenesis-related proteins (PRs) in response to infections by pathogens such as fungi, bacteria, or viruses; wounding; or the application of chemicals that mimic the effect of such an infection or induce similar stresses. In 1994 a unifying nomenclature for PRs was proposed on the basis of their sequence or predicted sequence of amino acid residues or enzymatic or biologic activity.⁹ Recently this list has been extended to contain 14 families.¹⁰ When the amino acid sequences of all known food allergens are analyzed and compared, it is conspicuous that the majority of plant food allergens are homologous to proteins that are included in some of the PR families. Much less variety is found among allergens of animal foods. However, also in this case, cross-reactive allergen groups such as albumins or tropomyosins are found.¹¹⁻¹³

PLANT FOOD ALLERGENS HOMOLOGOUS TO PR-TYPE PROTEINS (TABLE I)

Allergens homologous to PR-2 type proteins: β -1,3-glucanases

Plant β -1,3-glucanases are monomers with a molecular mass in the 25 to 35 kD range. Most are endoglucanases with the potential to partially degrade fungal cell walls by hydrolysing the β -1,3-glucan fibers of the growing hyphae of filamentous fungi. Pathogen-induced acidic enzymes are mostly extracellular, whereas basic counterpart are developmentally regulated and occur in the vacuoles. A basic β -1,3-glucanase isolated from the latex of the tropical rubber tree *Hevea brasiliensis* has been characterized as Hev b 2, a relevant latex allergen.^{14,15} The association between *Hevea* latex allergy and hypersensitivity to foods, especially avocado, banana, chestnut, fig, and kiwi has been termed latex-fruit syndrome.^{16,17} Phylogenetically conserved, homologous proteins present in these fruits and vegetables are responsible for the cross-reactivity. The basic β -1,3-glucanases from *Hevea* latex were recognized by specific IgE from food-allergic patients with hypersensitivity to banana, potato, and tomato rather than to latex products.¹⁸ It has also been reported that the amount of β -1,3-glucanase expression increases significantly in banana fruit (*Musa acuminata*) during ripening.¹⁹

Allergens homologous to PR-3 type proteins: basic (class I) chitinases

Chitinases are abundant proteins found in a wide variety of seed-producing plants. Nearly all plant chitinases isolated to date are endochitinases that hydrolytically degrade chitin from within the polymer. Chitin is a major structural component of the exoskeleton of insects and the cell walls of most fungi. Chitinases therefore can function as part of the plant's defense system against pathogens. Chitinases typically exist as monomers of 25 to 35 kD. Class I chitinases are basic, possess a signal peptide that routes the protein into the endoplasmic reticulum, and contain an N-terminal so-called hevein domain of about 40 residues with putative chitin binding

properties.²⁰ As mentioned above, latex allergy is associated with sensitization to several fruits. Latex prohevein (Hev b 6.01) belongs to the group of chitin-binding proteins with a hevein domain. Hevein (Hev b 6.02), a polypeptide of 43 amino acid residues that occurs in latex cleaved from the prohevein, shares high-sequence similarities with PR-3 and PR-4 proteins and contributes to the increased prevalence of fruit allergies in individuals allergic to latex. Relevant allergens of chestnut (*Castanea sativa*) and avocado (*Persea americana*) have been identified by N-terminal amino acid sequences as class I chitinases containing such hevein domains.²¹ A 32-kD endochitinase and major allergen from avocado that was cloned and expressed in the yeast *Pichia pastoris* is now designated as Pers a 1.²² Sensitization to Pers a 1 seems to be caused by hevein in the majority of patients allergic to latex.²³ Two major IgE-binding proteins of 32 and 34 kD from banana were identified as class I chitinases with a hevein-like domain further explaining the cross-sensitization between *Hevea* latex and fruits.²⁴

Allergens homologous to PR-4 type proteins: chitinases similar to potato Win proteins

Two PR-4 type proteins, composed of two polypeptides of 13 to 15 kD each, were isolated from tobacco mosaic virus-infected leaves²⁵ and found to constitute another family of chitinases.¹⁰ Their primary structure indicates 50% sequence similarity to Win1 and Win2, proteins that are induced in potato in response to wounding. However, these tobacco PR-4 proteins lack the N-terminal hevein domain of Win and Hev b 6, a major latex allergen.²⁶ Wounding and chemical treatment of turnip (*Brassica rapa*) plants induced the expression of an 18.7-kD allergen that was recognized by IgE of natural rubber latex allergic individuals.²⁷ Peptide sequences of the turnip allergen revealed 70% identity to prohevein, the Hev b 6.01 allergen from *H brasiliensis*, and high similarities to wound-induced proteins from tomato (74%) and potato (71%). Cloning of a hevein-like protein from elderberry (*Sambucus nigra*) demonstrated the occurrence of a hybrid gene encoding a protein consisting of the N-terminal domain of PR-4 proteins and the C-terminal domain of class V chitinases.²⁸ The allergenic properties of this elderberry protein remain to be investigated.

Allergens homologous to PR-5 type proteins: thaumatin-like proteins

The family of PR-5 proteins comprises unique proteins with diverse functions including antifungal activity. Because of the sequence homologies between PR-5 proteins and thaumatin, an intensely sweet-tasting protein isolated from the fruits of the West African shrub *Thaumatococcus daniellii*, members of this family of proteins are referred to as thaumatin-like proteins. A 31-kD major apple allergen whose amino-terminal sequence shared 46% identity with PR-5 proteins was the first thaumatin-like protein described as an allergen.²⁹ The full complementary DNA (cDNA) sequence has been obtained and the allergen received the designation Mal d 2 from the

TABLE I. Plant food allergens homologous to PR-type proteins

Allergen homologous to	Protein classification	Allergen source/allergen
PR-2 type proteins	β -1,3-Glucanases	Fruits, vegetables
PR-3 type proteins	Basic class I chitinases	Avocado (Pers a 1), chestnut, banana
PR-4 type proteins	Chitinases similar to potato Win proteins	Turnip, elderberry
PR-5 type proteins	Thaumatococcus-like proteins	Cherry (Pru av 2), apple (Mal d 2), bell pepper (P23)
PR-10 type proteins	Bet v 1-homologous proteins	Apple (Mal d 1), cherry (Pru av 1), apricot (Pru ar 1), pear (Pyr c 1), celery (Api g 1), carrot (Dau c 1), parsley (pcPR), potato (pSTH)
PR-14 type proteins	Lipid transfer proteins	Peach (Pru p 3), apple (Mal d 3), soybean (Gly m 1),* barley

*Another allergen from the same plant can be found with an identical designation in the literature.

international nomenclature committee (Krebitz, unpublished data). In sweet cherry (*Prunus avium*) a 23.3-kd thaumatin-like protein was identified as a major allergen, designated as Pru av 2 and its cDNA was cloned.³⁰ The N-terminal sequence of a 23-kd bell pepper allergen was found to be identical to a corresponding portion of the osmotin-like protein P23 from tomatoes.³¹

Allergens homologous to PR-10 type proteins: Bet v 1 homologous proteins

The most frequent clinical syndrome caused by cross-reactive IgE antibodies is the OAS, an association of food allergies to fruits, nuts, and vegetables in patients with pollen allergy. In the majority of the cases, OAS in individuals allergic to tree pollens is caused by IgE cross-reactivity of Bet v 1 (the major allergen of birch) and its homologous proteins. Bet v 1 is a member of the PR-10 family, and its homologs form a ubiquitous group of proteins that have been identified in a wide range of flowering plants (Fig 1).^{32,33} Mal d 1, the major allergen from apple, was the first Bet v 1 homologous fruit allergen cloned and produced as a recombinant allergen by Vanek-Krebitz et al.³⁴ Other cloned Bet v 1 homologous allergen sequences from fruits include the cDNAs encoding Pru av 1 from sweet cherry,³⁵ Pru ar 1 from apricot (Pühringer H, EMBL/GenBank accession No. AF020784; Mbeguie-A-Mbeguie D, AF134731), and Pyr c 1 from pear (Karamloo F, AF057030). Recombinant proteins have been produced for the Bet v 1 homologous allergens Api g 1 from celery³⁶ and Dau c 1 from carrot.³⁷ Additional Bet v 1-homologous proteins capable of binding anti-Bet v 1 IgE were described as pcPR1 and pcPR2 from parsley and pSTH-2 and pSTH-21 from potato.³⁸ The N-terminus of the major hazelnut allergen was determined and found to be 72% identical with that of the major hazel pollen allergen Cor a 1, another Bet v 1 homolog.³⁹ It is of interest to note that the majority of Bet v 1-homologous food allergens have been found in the fruits of Rosaceae species (eg, apple, pear, cherry, apricot) or somatic tissues, "vegetables," of Apiaceae species (eg, celery, carrot, parsley). Sequence identities among the fruit or the vegetable allergens are significantly higher than with Bet v 1, reflecting the close taxonomic rela-

tionship of the species of each of the two botanical families (Fig 1). In addition, the described Bet v 1-homologous allergens from the Apiaceae are by 5 amino acid residues shorter at their N-terminus than the known respective allergens from the Rosaceae (Fig 1).

Allergens homologous to PR-14 type proteins: lipid transfer proteins

Plant lipid transfer proteins (LTPs), named for their ability to transfer phospholipids from liposomes to mitochondria, form a family of 9 kd (90 to 95 amino acids) polypeptides that is widely distributed throughout the plant kingdom. LTPs can take part in plant defense because some LTPs have potent antifungal and antibacterial activities.⁴⁰ LTPs are generally located in the outer cell layer of plant organs. Most of these molecules contain 8 conserved cysteines forming 4 disulfide bridges, which makes them highly resistant to harsh temperature and pH changes.⁴¹ LTPs are the most important allergens of the Prunoideae, such as peach, apricot, plum, and cherry, when no pollinosis is involved.⁴² An 8- to 10-kd protein doublet exclusive to peach skin extracts, which largely accounted for the allergenicity of that fruit, was tentatively named Pru p 1.⁴³ The LTP of peach was granted the designation Pru p 3,^{44,45} and the LTP of apple the name Mal d 3⁴⁶ by the international allergen nomenclature committee. The complete amino acid sequence of Pru p 3 (in that publication called Pru p 1) has been determined.⁴⁷ IgE-mediated allergy to beer can be caused by the LTP from barley, which survives the malting and brewing process, is found intact in the beer, and is involved in beer foam formation.⁴⁸

A low-molecular-weight allergen (about 8 kd) localized in soybean hulls was identified as the main protein responsible for several asthma outbreaks in Spain.⁴⁹ The allergen Gly m 1 consists of the two isoforms 1A and 1B. Both isoallergens are glycoproteins. The N-terminal amino acid sequences have been determined.⁵⁰ Gly m 1B is a derivative of form 1A lacking the N-terminal tripeptide sequence. Gly m 1 is identical with the hydrophobic protein from soybean seed (HPS) described previously.⁵¹ HPS is a member of a new family of cysteine-rich proteins with a pattern of eight cysteine residues that form disulfide bridges similar to several nonspecific LTPs.⁵²

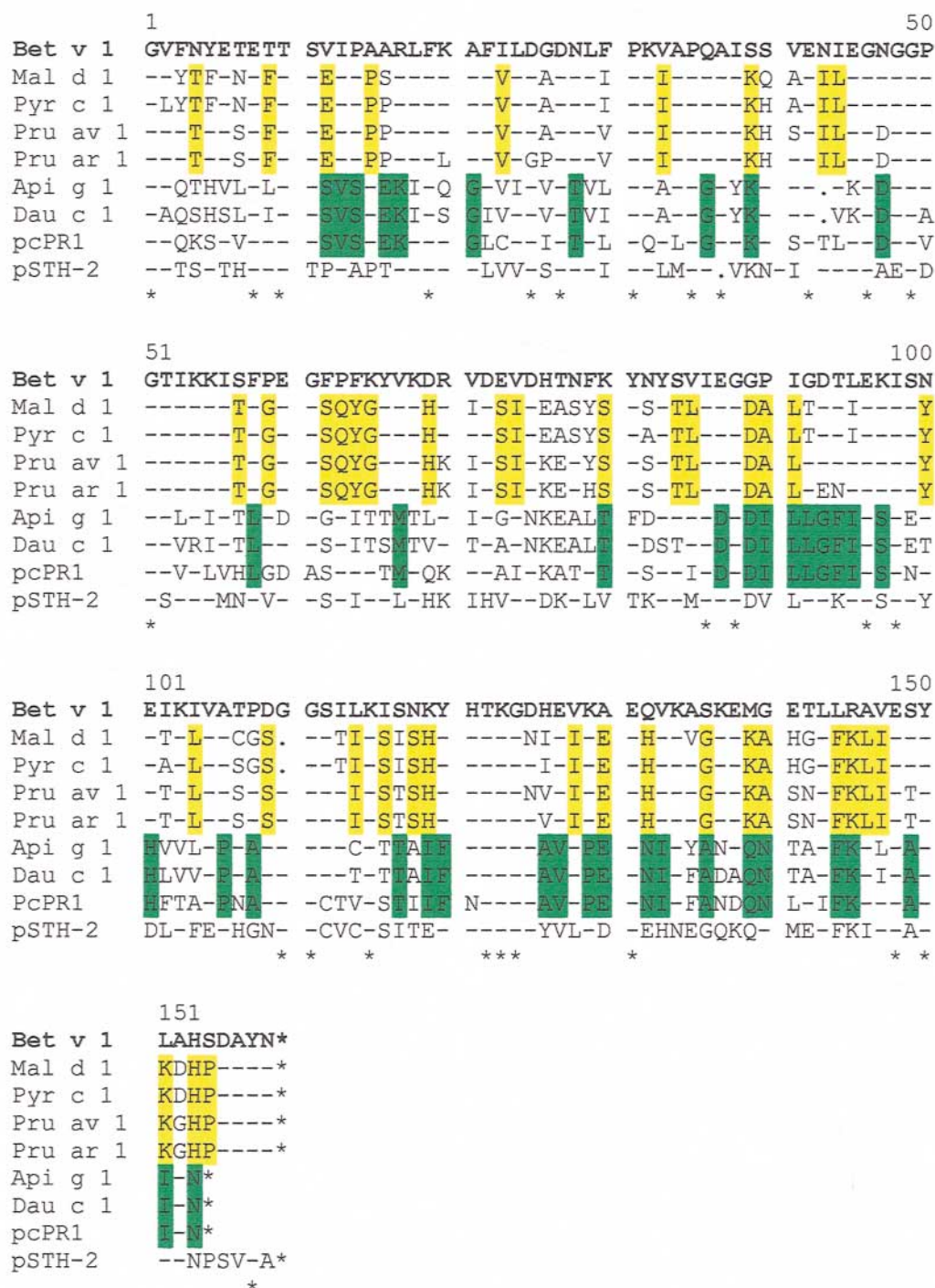


FIG 1. Comparison of amino acid sequences of Bet v 1 homologous plant food allergens. Amino acid sequences of plant food allergens from apple (Mal d 1, EMBL/GenBank accession No. P43211), pear (Pyr c 1, AF057030), sweet cherry (Pru av 1, U66076), apricot (Pru ar 1, AF020784), celery (Api g 1, P49372), carrot (Dau c 1, Z84376), parsley (PcPR1, P19417), and potato (pSTH-2, P17624) that are homologous to PR-10 type proteins were aligned. Because the major birch pollen allergen Bet v 1 (P15494) is assumed act as the sensitizing agent, its sequence was used as the reference. In each case the sequence of only one representative variant of the allergen was used. *Dashes*, Identical residues; *dots*, gaps in sequences. *Asterisks*, Identical residues present in all sequences aligned or denoting the end of a sequence. Residues that are identical between fruit allergens are colored in yellow, those between vegetable allergens in green.

OTHER PLANT FOOD ALLERGENS WITH KNOWN BIOLOGIC FUNCTIONS (TABLE II)

Inhibitors of proteases and α -amylases

Soybeans are members of the legume family and are among the five major foods that cause type I allergy in children. The 20-kd Kunitz soybean trypsin inhibitor bound IgE of 20% of soy challenge-positive patients, indicating that this protein is a minor allergen.⁵³ However, the Kunitz soybean trypsin inhibitor was also reported to induce food anaphylaxis.⁵⁴

Inhibitors of proteases and α -amylases are also found in high concentrations in plant storage organs such as seeds or tubers. They have the capacity to inhibit proteolytic enzymes of invading microorganisms and insects. The best characterized allergens of cereal flours have molecular masses of 12 to 15 kd, possess inhibitory activities against heterologous α -amylases or trypsin, and belong to a single protein family present in many cereals.⁵⁵ These allergens can cause inhalant allergies as well as food allergy. A 14.5-kd protein from barley endosperm that is a major allergen associated with baker's asthma was identified as glycosylated monomeric inhibitor of insect α -amylases.⁵⁶ Its cDNA coded for the 132 amino acid residues of the allergen BMAI-1, designated as Hor v 1 by the international allergen nomenclature committee. Most of the allergens of barley and wheat characterized so far belong to the family of α -amylase/trypsin inhibitors. The ones with the highest allergenicity are the glycosylated subunits of the tetrameric inhibitors from wheat (CM16*), the homologous glycoprotein from barley (CMB*), and BMAI-1.^{57,58} A 13.5-kd rye flour protein, designated by the authors as Sec c 1, and its homologous protein from barley (barley dimeric protein) were isolated.⁵⁹ The N-terminal sequences showed significant similarities to several members of the cereal α -amylase/trypsin inhibitor family but did not show inhibitory activities against these enzymes. Three members of the α -amylase/trypsin inhibitor family from rye flour, Sec c 1, RDAI-1, and RDAI-3, were also described as important allergens in baker's asthma.⁶⁰

A cDNA encoding the major allergenic protein from rice seeds was isolated from an expression library. The mature protein (rice allergenic protein, RAP) of a molecular mass of 14.8 kd is another food allergen that is a member of the α -amylase/trypsin inhibitor family, with considerable similarity to barley trypsin inhibitor and wheat α -amylase inhibitor.⁶¹ Similar to the rice allergenic protein, the 15-kd wheat α -amylase inhibitor is capable of sensitizing susceptible atopic patients by ingestion or by inhalation.⁶²

Peroxidases

Peroxidases use hydrogen peroxide for a series of oxidative reactions. Peroxidases have been thoroughly researched in higher plants where their activities are correlated with a large number of growth, developmental, and defense processes. Specific lignin-forming peroxi-

TABLE II. Other plant food allergens with known biologic functions

Protein classification	Allergen source/allergen
Inhibitors of proteases and α -amylases	Soybean: Kunitz trypsin inhibitor family; cereals: trypsin/ α -amylase inhibitors; barley: Hor v 1/BMAI-1, CMB, BDP; wheat: CM16; rye: Sec c 1,* RDAI-1, RDAI-3; rice: RAP
Peroxidases	Wheat, barley
Profilins	Peanut: Ara h 5; soybean: Gly m 3; celery: Api g 4; pear: Pyr c 4; hazelnut, apple, carrot, lychee, tomato, pumpkin seeds
Seed storage proteins	
2S albumins	Yellow mustard: Sin a 1; oriental mustard: Bra j 1; oilseed rape: BnIII; Brazil nut: Ber e 1; English walnut: Jug r 1
Vicilins	Peanut: Ara h 1; English walnut: Jug r 2
Conglutins	Peanut: Ara h 2, Ara h 6, Ara h 7
Glycinins	Peanut: Ara h 3, Ara h 4; soybean
Beta-conglycinins	Soybean
Thiol-proteases	Papaya: papain; fig: ficin; pineapple: bromelain; kiwi: actinidin/Act c 1; soybean: Gly m 1*
Lectins	Peanut: agglutinin

*Signifies that another allergen from the same plant can be found with an identical designation in the literature.

dases, induced by pathogens and involved in plant defense against pathogens, have been designated PR-9.⁹ A prominent 36-kd allergen was isolated from wheat flour and identified by N-terminal and internal amino acid sequences as a peroxidase.⁶³

Profilins

The actin cytoskeleton is composed of a network of actin filaments whose precise organization is regulated by a number of actin-binding proteins. One of them is profilin, a 12- to 15-kd monomeric actin-binding protein. Profilin was first identified as an allergen in birch pollen and named Bet v 2. Profilins are now recognized as ubiquitous cross-reactive plant allergens.⁶⁴ Patients sensitized to pollen profilins characteristically react to a broad range of inhalant and nutritive allergen sources. Profilin is involved in the celery-mugwort-spice syndrome.^{7,65} In addition, it was demonstrated that grass pollen profilin-allergic patients also reacted to homologs in celery and carrots.⁶⁶ A hazelnut profilin was shown to be the relevant IgE-binding protein in the minority of pollen-nut-allergic individuals.⁶⁷ IgE of food-hypersensitive tree pollen-allergic individuals cross-reacted with Bet v 2 homologous proteins from apple, pear, carrot, celery, and potato.⁴ An anaphylactic reaction to lychee fruit was mediated by a profilin.⁶⁸ Furthermore, profilins were identified as causing allergic reactions to tomato⁶⁸ and to

pumpkin seeds.⁶⁹ The profilins from the legumes peanut⁷⁰ and soybean⁷¹ were characterized and produced as recombinant allergens and designated as Ara h 5 and Gly m 3, respectively. cDNA sequences have also been obtained for the profilin from celery, Api g 4 (Scheurer, unpublished data, EMBL/GenBank database entry No. AF129423), and for the profilin from pear, Pyr c 4 (Scheurer, unpublished data, EMBL/GenBank database entry No. AF129424).

Seed storage proteins

With the exception of cereals, albumins and globulins make up the seed storage proteins of angiosperms. Albumins are water soluble at low salt concentrations and globulins at high salt concentrations. In addition to their solubility, seed storage proteins are characterized by sedimentation constants. The 2S albumins are small globular proteins rich in arginine, glutamine, asparagine, and often cysteine. These proteins are subjected to modifications after their synthesis. Most of the 2S albumins are cleaved into a large and small subunit held together by a disulfide bond.

The major allergens of yellow mustard (*Sinapis alba*), Sin a 1,⁷² and oriental mustard (*Brassica juncea*), Bra j 1,⁷³ which can elicit allergic reactions when ingested by sensitized atopic subjects, are found in the 2S albumin fraction. Sin a 1 consists of two polypeptide chains linked by a disulfide bridge and was expressed in *Escherichia coli*.⁷⁴ Similar to the natural Sin a 1, the recombinant allergen was highly resistant to digestion by trypsin. The seeds of oilseed rape (*Brassica napus*) contain two types of storage proteins, 2S albumins (also called napins) and 12S globulins. The most abundant isoform of the allergenic napins, BnIII, consists of two different chains of 9.5 and 4.5 kd. IgE cross-reactivity between rapeseed and mustard allergens was demonstrated.⁷⁵

The 2S albumins are also found abundantly in the seeds of the Brazil nut (*Bertholletia excelsa*). The amino acid composition and sequences of various isoforms of Brazil nut 2S albumins have been determined.⁷⁶ The major allergenic isoform, designated as Ber e 1, has a methionine content of 18%. For these reasons, the Brazil nut 2S albumins were for some time interesting candidates for the correction of methionine-deficient crop plants by recombinant DNA technology. Soybeans transgenic for Ber e 1 became the paradigm for the experimental transfer of an allergen into a crop plant.⁷⁷ Jug r 1, a major food allergen from the English walnut (*Juglans regia*) was identified as a 2S albumin seed storage protein with 46% identity with Ber e 1.⁷⁸ Another walnut allergen, Jug r 2, was identified as belonging to the vicilin group of seed storage proteins.⁷⁹

Allergy to peanut (*Arachis hypogaea*) is a significant health problem because of the high frequency of systemic allergic reactions. It is the most common cause of fatal and near-fatal food-induced anaphylaxis. Three of the major allergens, Ara h 1, Ara h 2, and Ara h 3, are abundant proteins in the peanut. Ara h 1, a 64.5-kd glycoprotein, belongs to the vicilin family of seed storage

proteins.⁸⁰ Immunodominant linear IgE epitopes on Ara h 1 were determined and mutated to significantly reduce the IgE-binding capacity of the recombinant allergen.⁸¹ Ara h 2, a 17.5-kd glycoprotein, is a member of the conglutin family of seed storage proteins.⁸² Mutational analysis of the immunodominant IgE epitopes of Ara h 2 indicated that single amino acid exchanges in the center of each epitope resulted in the loss of the IgE-binding capacity of the allergen.⁸³ Ara h 3 was identified as a 14-kd allergen with high-sequence similarities to glycinins. Also for this molecule, mutational analysis of IgE epitopes was performed.⁸⁴ Glycinins are synthesized as ~60-kd precursor proteins that are cleaved into 40- and 20-kd proteins. The 14-kd Ara h 3 represents the N-terminal end of the 57-kd peanut glycinin. The 35.9-kd allergen Ara h 4 is another member of the glycinin family that represents storage proteins. The 14.5-kd Ara h 6 and the 15.8-kd Ara h 7 allergens both show similarities to sequences of proteins from the conglutin family related to the 2S superfamily of storage proteins.⁷⁰

Soybeans (*Glycine max*) and soybean proteins are used in an increasing number of foods and food products, therefore increasing the potential for atopic subjects to become sensitized. Approximately 90% of the soybean seed storage proteins are salt-soluble globulins, with the bulk of the remainder being water-soluble albumins. The two principal soybean globulins are β -conglycinin and glycinin. β -Conglycinin, a glycoprotein, has a molecular mass of about 180 kd. It is a trimer composed of three major subunits, α' , α , and β , which have molecular masses of 76, 72, and 53 kd, respectively. Glycinin has a molecular mass of 320 kd and is made up of 6 subunits. Each subunit is composed of one acidic and one basic polypeptide linked by a disulfide bond. Both the acidic and basic polypeptides are heterogeneous and possess molecular masses ranging from 34 to 45 kd and 19 to 22 kd, respectively. Both glycinin and β -conglycinin are major allergenic soybean proteins that are involved in food hypersensitivity reactions.⁸⁵ In a later study one of the major allergenic proteins in the soybean 7S-globulin fraction, which was recognized by sera of about 25% of soybean-sensitive patients with atopic dermatitis, was identified as the α -subunit of β -conglycinin.⁸⁶ The N-terminal sequence of the first 20 amino acids of the 8-kd soybean allergen Gly m 2 showed 71% similarity with the corresponding region of a storage protein from the cotyledons of *Vigna radiata* (cowpea) and 64% similarity with a disease-response protein from the pea *Pisum sativum*.⁸⁷

Proteases

The class of thiol proteases includes several proteolytic plant enzymes such as papain from papaya, ficin from fig, bromelain from pineapple, and actinidin from kiwi. Patients with allergic reactions to kiwi fruit also have IgE reactive to bromelain and papain.⁸⁸ The presence of cross-reactive allergens from fig and papaya was shown by cross-inhibition experiments including ficin and papain.⁸⁹ Actinidin, a thiol protease of the kiwi (*Actinidia chinensis*), was identified as its major allergen and designated Act

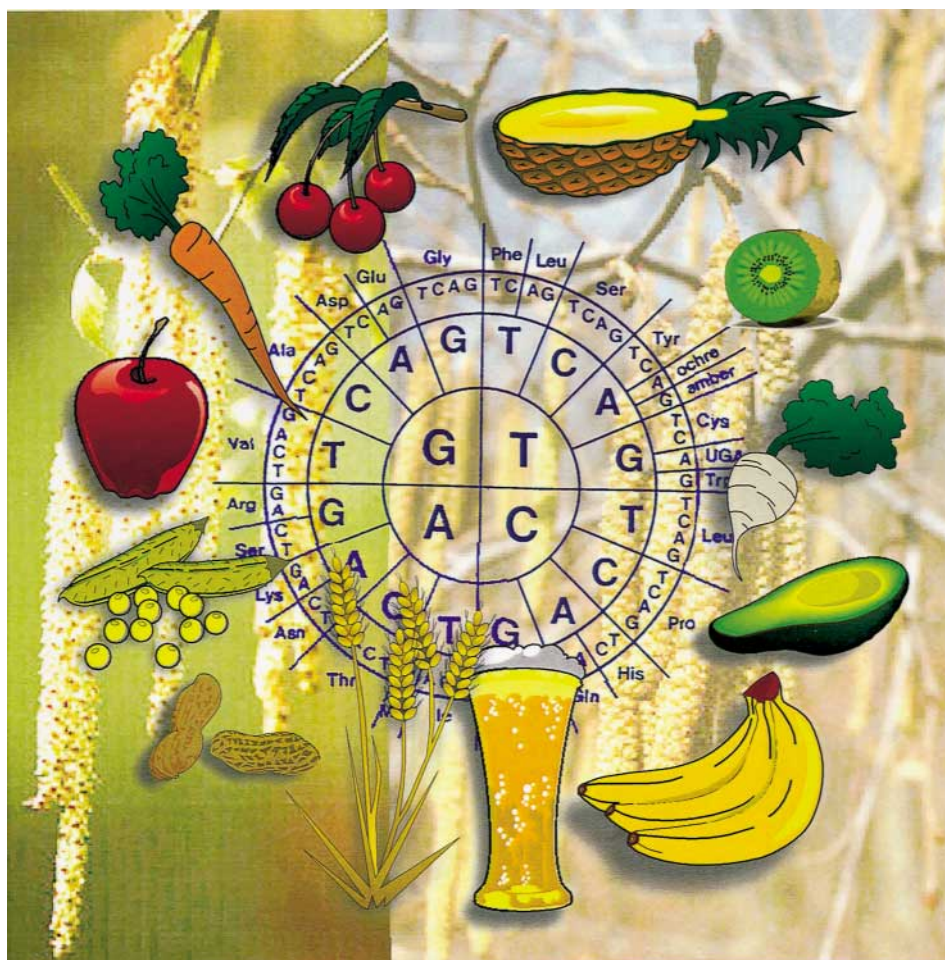


FIG 2. Plant food allergen classes and cross-reactivity: it's all in the genes. The genetic code stands for homologous DNA sequences that encode such cross-reacting allergens. Allergy to certain plant foods such as apple, carrot, or cherry can be induced by pollen allergens that are homologous to PR-10 type proteins and that are represented by the background of hazel and birch inflorescences. In addition, the antifungal thaumatin-like proteins (PR-5) of apple and cherry have been identified as allergens. Pineapple and kiwi fruit are examples of sources of thiol proteases that can act as allergens. Other proteins that are part of the plant defense system and have been identified as allergens include a prohevein-like protein from turnip (PR-4), chitinases from avocado and banana (PR-3), and LTPs (PR-14) from fruits and seeds. The LTP of barley that survives the malting and brewing process is involved in beer foam formation. Plant food allergens have also been found among inhibitors of proteases and α -amylases from cereals or among a variety of seed storage proteins from peanut or soybean.

c 1.⁹⁰ Actinidin accounts for about 50% of the soluble protein of the fruit. Murine mAbs specific for the allergens Der p 1 and Der f 1 from house dust mites, which also belong to the family of thiol proteases, cross-reacted with the plant enzymes papain, bromelain, and ficin.⁹¹ However, no clinical association between house dust mite allergy and food allergy to tropical fruits could be observed. IgE from more than 60% of soy-allergic patients reacted with a 30-kd protein in soybean extracts. This allergen was designated Gly m 1 by the authors⁹² and should not be confused with another soybean allergen also designated Gly m 1.⁵⁰ The amino acid sequence showed that it is identical to the 34-kd soybean seed vacuolar protein P34, which has been identified as a cysteine/thiol protease, a member of the papain superfamily of proteins.⁹³

Lectins

Lectins, also known as plant agglutinins, are a class of proteins that bind to specific sequences of sugar determinants on glycoproteins. Lectins are found in seeds, especially those of legumes. Some lectins react unspecifically with the carbohydrate moieties of IgE, induce histamine-release, and can thus induce allergy-like symptoms.⁹⁴ The 31-kd peanut agglutinin was identified as a lectin that is specifically recognized by IgE from a minority of peanut-allergic patients.⁵³

CONCLUSION

Plant food allergens described to date are either homologous to PR-type proteins or they belong to a

small number of classes of proteins (Fig 2). In fruits and vegetables with a high water content, such as cherries, papayas, bananas, or avocados, that are prone to fungal attack, the chitinases, proteases, and antifungal proteins are the predominant allergens. The allergens in hard and dry seeds such as wheat berries, rice, mustard seeds, or soybeans fall into two classes: the seed storage proteins and the enzyme inhibitors. They prevent the endosperm from being digested by insects or fungi. Parts of whole plants such as carrot roots, celery, or potato tubers contain allergens that are homologous to certain PR families, especially to the PR-10 family. The characteristics of plant allergens may indicate that the allergenicity of at least some of these allergens depends on their biochemical functions. In the future, allergies may become defined by sensitization to a group of allergens with identical biochemical functions. For example, a person with allergy to avocado, banana, chestnut, and latex may better be described as being allergic to chitinases. A fruit-allergic pollinosis patient may therefore be classified as having an allergy to PR-10 type proteins, such as Bet v 1 or to profilins. Allergy to plant proteins may then be diagnosed with a small panel of cross-reactive allergens in recombinant form, replacing a large amount of protein extracts from different plant sources. From the diagnostic and therapeutic point of view, it makes sense to define allergen clusters in which cross-sensitization possibly allows "cross-therapy." Moreover, the information for patients with a characterized cluster of hypersensitivities will be more precise. An important task for the future will be the correlation of the increasing scientific data with clinical information. For this purpose, studies executing the necessary food-provocation tests in serologically well-defined patients are indispensable.

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