

Food allergen protein families and their structural characteristics and application in component-resolved diagnosis: new data from the EuroPrevall project

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Abstract A large number of food allergens able to induce allergic symptoms in predisposed individuals, including severe, even life-threatening reactions, have been identified and characterized. However, proteins able to cause such IgE-mediated reactions can be assigned to only a limited number of protein families. Detailed knowledge about the characteristics of food allergens, their 3D structures, biological activity and stability, will help to improve diagnosis of food allergy, avoid unnecessary exclusion diets and assess the risk of cross-reactive allergies to other food sources. This review is dedicated to summarizing current knowledge about the most important food allergen protein families and to presenting data from the EuroPrevall allergen library, a proof-of-concept collection of highly purified, characterized and authenticated food allergens from animal and plant food sources to facilitate improved diagnosis of food allergies.

Keywords Food allergen · Purification · Allergen library · Cross-reactivity · Protein family

Introduction

Food allergies pose an increasing health risk for the population of industrialized countries, with around 5–6%

of children and up to 2% of adults being thought to suffer from some type of IgE-mediated food allergy, although valid and current prevalence data based on diagnostic standard procedures are scarce [1].

In the last decade great efforts have been undertaken to identify the characteristics of non-toxic food proteins which evoke IgE-mediated allergic response in predisposed individuals and many hundreds of allergens have been identified in a variety of animal and plant-derived food-stuffs. As a consequence, in the recent past a number of allergen databases have been set up to collect and curate the existing data on allergens, their physicochemical properties and their allergenic relevance. Some of these are curated collections of allergen sequences, such as AllergenOnline (<http://www.allergenonline.org/>) and the IUIS allergen database (<http://www.allergen.org/>). The IUIS database is dedicated to providing a systematic nomenclature for allergens which allows unequivocal identification of allergens, and upon evidence of IgE binding activity of the allergenic molecules being provided, the allergen nomenclature subcommittee grants official allergen designations [2].

Other allergen databases are linked to data on clinical reactivity of foods, such as InformAll (<http://foodallergens.ifr.ac.uk/>), whereas the Allergome database represents a rapidly updated non-peer-reviewed repository of information (<http://www.allergome.org/>). Another type of database is represented by Allfam, which groups allergens according to their protein family characteristics (<http://www.meduniwien.ac.at/allergens/allfam/>).

From analysis of these data it became evident that the proteins with allergenic activity are not randomly distributed among the known protein families but are restricted to a small number of protein families [3–6]. Although such structural relationships can explain IgE cross-reactivity between allergens from different food sources, no single

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feature shared by all known food allergens has been identified to date. Indeed many attempts using a variety of *in silico* approaches [7, 8] have taken collections of allergen sequences to develop an algorithm that detects protein motifs. These refined methods have proven to increase specificity and accuracy in identifying proteins with potential allergenicity once they share sequence and/or structural determinants with already known allergens. However, there is still a need to reliably predict allergenicity *per se* for novel proteins.

In addition to the intrinsic features of allergens, additional factors such as stability to processing and gastrointestinal digestion and abundance in the food may contribute to the overall allergenicity.

Food allergy: allergens to be identified

Analysis of plant food allergen sequences using postgenomic bioinformatic tools such as Pfam [9] showed that they belonged to only 27 of the then-identified 8,183 Pfam families [3]. Several protein families/superfamilies were found to predominate: the prolamin superfamily, the cupin superfamily and the Bet v 1 family, which together with the profilins accounted for more than 65% of all plant food allergens. A similar distribution was found for food allergens of animal origin [5], with three protein families, the tropomyosins, parvalbumins and caseins, dominating. Radauer et al. [6] identified a total of 29 different protein families that include more than one food allergen. Thus, the repertoire of allergenic proteins identified is small compared with the vast array of different proteins found in biology, indicating that conserved structures and biological activities play a role in determining or promoting allergenic properties of proteins. The explanations for this are lacking but may in part result from conservation of surface structures in certain families, such as the Bet v 1 and parvalbumin superfamilies, which promote IgE cross-reactivity [3, 5]. In addition, overall sequence identity is also a feature relevant for cross-reactivity. Sequence identity of 50% and above is highly significant for allergenic cross-reactivity in most cases. For example profilins from plant species share more than 60% sequence identity, while profilins from fungi and animals are less similar and cross-reactivity is not being observed [4].

In what follows the most important allergen protein families are reviewed, their structural features summarized and allergenic activity discussed (Table 1). This is illustrated using examples drawn from the EuroPrevall allergen library [10], a collection of authenticated food allergens prepared to facilitate the development of improved methods for diagnosing food allergies, notably component-resolved diagnosis [11].

The allergen library developed within the EU-funded integrated project EuroPrevall represents the blueprint of how to produce and characterize high-quality allergens suitable for diagnostic purposes as well as studies on food-processing strategies modulating allergenicity. Therefore, purification protocols for either natural food allergens or recombinant allergens were established and quality criteria of the purified allergen batches were defined before inclusion into the library. The allergenic proteins were evaluated regarding purity, authenticity and correct folding, and their allergenic activity was tested using *in vitro* human serum IgE-binding and cell-based assays. In the first phase, the library contains food allergens of animal origin, including those from cow's milk, goat's milk, hen's egg, carp, codfish and shrimp. In addition, allergens were included from the following plant food sources: apple, peach, hazelnut, peanut and celery [10]. The total of 32 purified food allergens from the first phase of the EuroPrevall allergen library comprised representatives of the most prevalent allergenic protein families. New data on their physicochemical characterization will be presented in relation to the protein families described below.

Plant food allergens: most prevalent protein families

Prolamin superfamily

This superfamily comprises several families of proteins which include the prolamin seed storage proteins (gliadins and glutenins) of cereals, the non-specific lipid transfer proteins (LTPs), α -amylase/protease inhibitors of cereals and 2S albumins. Members of this family are characterized by the presence of a conserved skeleton of eight cysteine residues, containing characteristic Cys Cys and Cys X Cys motifs, where X represents any other residue, with two additional residues being found in the α -amylase/ trypsin inhibitors. The skeleton is disrupted in the prolamin storage proteins by the insertion of a repetitive domain comprising motifs rich in proline and glutamine. Family members for which 3D structures are available (LTPs, 2S albumins and α -amylase/protease inhibitors) share a related fold consisting of bundles of four α -helices stabilized by disulfide bonds. Despite their having homologous 3D structures, sequence similarities are low between the different families.

Seed storage prolamins

The role of cereals, and especially wheat, is well documented for occupational allergy, bakers' asthma and coeliac disease. However, despite a widespread perception that cereals are important allergenic foods and the fact that

Table 1 Overview of the most important plant and animal food allergen protein families

Protein family		Function	Molecular mass (kDa)	Allergens identified from
Plant food allergens				
Prolamin superfamily	Prolamins	Seed storage	65	Cereals
	2S albumins	Seed storage	15–17	Peanut, tree nuts, mustard
	Non-specific lipid transfer proteins	Plant defence, transfer of lipids	7–9	All type of plant food, latex
	Bifunctional α -amylase/protease inhibitors	Protease/protease inhibitor	15–16	Cereals
Cupin superfamily	7/8S globulins	Seed storage	150–190	Legumes, nuts, seeds
	11S globulins	Seed storage	60	Peanut, tree nuts
Profilin		Actin binding, regulator protein	14	All type of plant food, latex
Bet v 1 related proteins (pathogenesis-related proteins family 10)		Plant defence, plant steroid carrier	17	All type of plant food
Oleosins		Stabilizing oil bodies	14–17	Legumes, nuts, seeds
Endochitinases		Plant defence, chitin hydrolysis	25–35	Banana, avocado, chestnut, latex
β -1,3-Glucanases		Plant defence	25–35	Banana, avocado, chestnut, latex
Thaumatococcus-like proteins		Plant defence	20–25	Kiwi, citrus and Rosaceae fruits, grape
Animal food allergens				
Tropomyosins		Regulate muscle contraction	36–38	Crustaceans, molluscs
EF-hand domain (parvalbumins)		Ca ²⁺ binding	12	Fish and amphibians
Caseins		Ca ²⁺ binding	20–30	Cow's, goat's and sheep's milk
Lipocalins (β -lactoglobulin)		Carrier of small molecules	18	Milk
C-type lysozyme/ α -lactalbumin family		Lactose synthesis	14	Cow's milk, hen's egg
Kazal-type protease inhibitors		Protease inhibitor	28	Hen's egg
Serpin		Protease inhibitor	44	Hen's egg
Transferrins		Iron transport	67–69	Hen's egg, cows milk

almost 50% of the protein in cereal kernels is prolamins, there are relatively few reports in the literature of their causing IgE-mediated allergies. So far, this has only been reported for wheat-induced atopic dermatitis or exercise-induced anaphylaxis [12]. Several allergens have been described as allergens, notably ω -5 gliadin [13] and other gliadin and glutenin components.

2S albumins

These seed storage proteins were identified from a vast range of dicot species and represent clinically relevant seed and nut allergens, such as Sin a 1 from yellow mustard and Ber e 1, and Jug r 1 from Brazil and English walnut, respectively. From peanut, Ara h 2 and Ara h 6 are known as important allergens.

Non-specific lipid transfer proteins

Non-specific LTPs were first described to facilitate trafficking of phospholipids between different cellular membranes in vitro. In the recent past other functions have been revealed, such as major roles in plant defence against fungal and bacterial infections. Non-specific LTPs have been identified as allergens in a range of fruits, vegetables and even nuts. They are major food allergens in fruits from the Rosaceae family with high stability against enzymatic digestion. Patients with specific IgE production tended to display severe allergic symptoms. Therefore, the production of LTP-specific IgE is regarded as a risk factor for developing severe symptoms and is being explored as a diagnostic marker in this respect. However, the overall sequence similarity among non-specific LTPs from distantly

related botanical species follows phylogeny. So far, cross-reactivity between non-specific LTPs from pollen and plant food has only been shown for Art v 3 from mugwort and Pru p 3 from peach [14].

α -Amylase/trypsin inhibitors

This group of proteins are found in cereals and sensitize individuals via the lungs, giving rise to occupational allergies such as bakers' asthma (wheat, barley and rye), or via the gastrointestinal tract, resulting in food allergies (wheat, barley and rice). The best characterized allergens are the α -amylase inhibitors of rice grain [15], with one report of a subunit with a molecular mass of approximately 15 kDa being involved in allergy to wheat [16]. Maize and beer (originating from barley) allergens with molecular masses of 16 kDa have also been characterized which appear to belong to the α -amylase inhibitor family [17, 18].

Allergens prepared from the prolamin superfamily and included in the EuroPrevall allergen library are non-specific LTPs from the Rosaceae fruits: Mal d 3 (from apple), and Pru p 3 (from peach) [19, 20]. The natural proteins were purified from fruit peel and compared with the natural and recombinant non-specific LTP from hazelnut Cor a 8 [21]. The secondary structure was assessed by circular dichroism (CD) spectroscopy and the spectra obtained indicated a native folded state of the purified proteins (Fig. 1a). The typical structure of non-specific LTPs is based on four disulfide bridges which contribute to the overall stability of these proteins against enzymatic digestion or thermal denaturation; however, the stability is pH-dependent. Under acidic conditions thermal denaturation of Pru p 3 was reversible, whereas under neutral conditions Pru p 3 was

unable to refold after cooling [20]. In addition, the 2S albumin allergens were prepared from peanut [22], including Ara h 2 and Ara h 6, with molecular masses of 17 and 15 kDa, respectively. Ara h 2 was found to comprise two isoforms (Ara h 2 and Ara h 2.02), although both proteins had N-termini from which seven and five residues had been removed, respectively, compared with published sequences. This finding can be explained by posttranslational processing *in planta*. The Ara h 6 fraction comprised three major polypeptides, one representing a single-chain unprocessed form of the protein, and the other two corresponding to the mature large and small subunits normally linked by interchain disulfide bonds, which are generally characteristic of mature 2S albumins found widely in plant seeds.

Cupin superfamily

Members of this superfamily share a β -barrel structure (*cupa* is the Latin word for “barrel”). It is thought that the different subfamilies have evolved from a common prokaryotic ancestor and member families are now identified in fungi, higher plants and animals [23]. Cupins with a single barrel domain include the germins, allergenic forms of which have been identified from bell pepper and orange [24–26]. Bicupins contain two β -barrel domains and the 7/8S and 11S seed storage globulins represent the major components of dicotyledonous plant seeds and thus are important protein sources of nutritional value. The vicilins (7S/8S globulins) are trimeric proteins (150–190 kDa) differing widely in their subunit composition based on differential proteolytic processing [27]. They include, among many others, the peanut allergen, Ara h 1, the walnut allergen, Jug r 2, and the homologue from sesame,

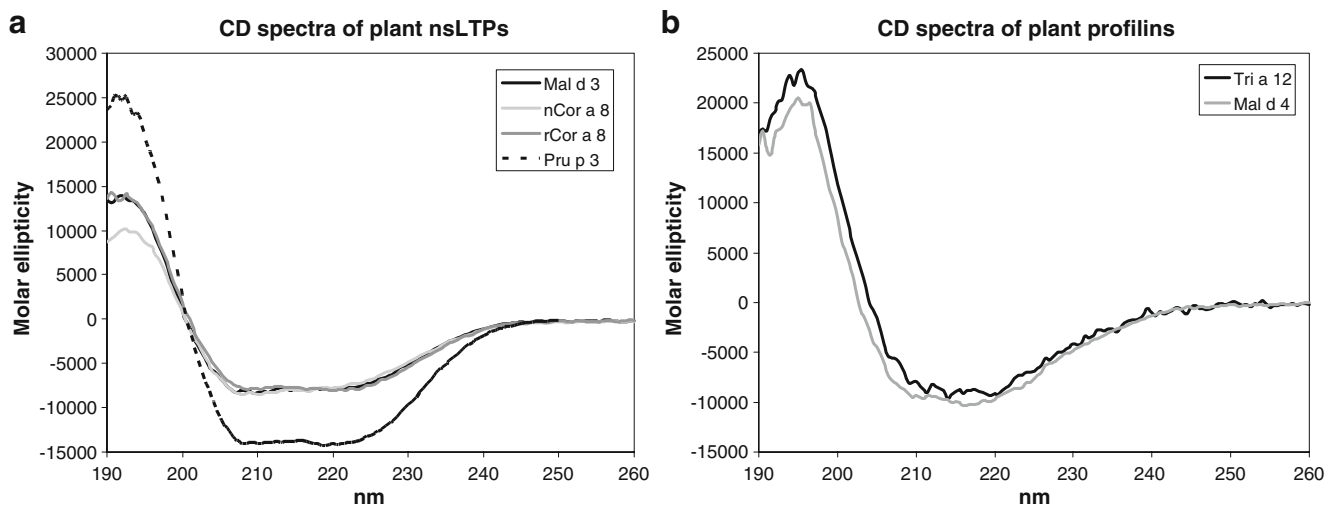


Fig. 1 Far UV-circular dichroism (CD) spectroscopy of purified allergens from the non-specific lipid transfer protein (*nsLTP*) family (**a**) including the natural proteins from apple (Mal d 3), peach (Pru p 3) and

hazelnut (nCor a 8) and the recombinant Cor a 8. From the profilin protein family (**b**), purified recombinant wheat profilin (Tri a 12) and recombinant apple profilin (Mal d 4) were compared. (Based on [19–21])

Ses i 3 [27]. The hexameric allergenic globulins (11S globulin) were identified from peanut, Ara h 3, soybean glycinin, from Brazil nut, Ber e 2, and from buckwheat, Fag e 1 [27].

Cupin allergens included in the EuroPrevall library included both, 7S and 11S seed storage globulins. Examples of the 7S globulins entered into the EuroPrevall library are Ara h 1, Cor a 11 [22, 28] and the homologous proteins from soybean, Jug r 2 and Ses i 3. Fourier transform IR (FT-IR) spectral analysis of the purified proteins essentially confirmed the similar fold of these allergens with the peak at around $1,656\text{ cm}^{-1}$ characteristic for β -sheet-rich proteins, such as seed storage proteins (Fig. 2a). Despite coming from different plant species, even from non-legumes, such as hazelnut, the FT-IR spectra of 7S globulins are almost superimposable. The homologous 3D structures of the 7S and 11S seed storage globulins are further emphasized by the similarity in their FT-IR spectra, despite low levels of sequence homology (Fig. 2) [22, 28].

Profilins

Profilins are present in all eukaryotic cells. These small (12–15 kDa) proteins are located in the cytosol and act as actin binding proteins [29]. They may play a key role in regulating intracellular transport processes and cell morphogenesis and division. Profilins encoding genes form a small gene family as shown for apple profilin [30]. Sequence similarity from profilins from lower eukaryotes, plants and animals is low, whereas profilins from higher plants share 75% and more of their sequence. All profilins have a compact globular structure consisting of a central seven-stranded antiparallel β -sheet enclosed by the N- and C-terminal α -helices on one side and one or two helices on the other side [31]. The plant homologues are somewhat divergent, notably with a slightly longer solvent-exposed loop between the N-terminal α -helix and the first β -strand, which is more variable and represents part of an IgE epitope in the allergenic profilin from birch pollen, Bet v 2 [32].

It is generally assumed that 10–20% of tree pollen allergic patients display allergic symptoms to profilin. The high sequence and structural similarity of profilins from even distantly related plants accounts for IgE cross-reactivity. Although the observed IgE cross-reactivity does not correlate with clinical symptoms, profilin sensitization is regarded as a potential risk for developing multiple pollen-associated food allergies.

In a group of celery allergic patients, 23% had specific IgE reactivity to celery profilin [33], and in a group of patients with carrot allergy, 33% displayed positive IgE reactivity to carrot profilin [34]. Asero et al. [35] investigated a group of patients with fruit and vegetable allergy and found an association between profilin sensitization and clinical allergy to melon, citrus fruits, banana and tomato.

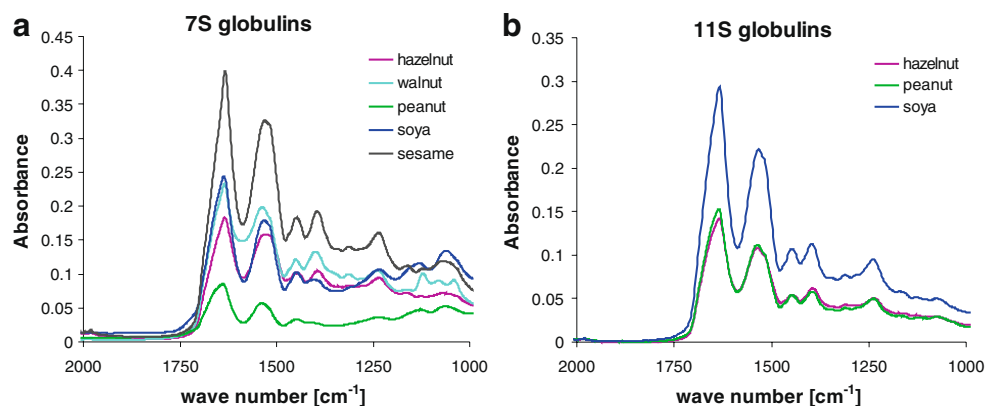
This finding was corroborated for orange and melon, since Crespo et al. [26] and Lopez-Torreon et al. [36, 37] verified positive reaction to profilin in skin-prick tests and histamine release and ELISA for orange and melon allergic patients from Spain.

In vitro experiments with purified profilins classified the proteins of intermediate stability. When subjected to heat treatment, irradiation or ultrahigh pressure, for profilin from celery, IgE binding activity was not affected. However, when the pH is lowered and gastric fluid assays are performed, profilins are readily degraded and display no further allergenic activity, as has been shown for Mal d 4 and Cuc m 2 [36, 38].

Profilins are minor allergens, yet are highly cross reactive and pose a risk for developing multiple pollen-food allergies not always linked with clinical symptoms. In cases where clinical symptoms were identified, the symptoms ranged from mild to severe.

Profilins from various botanical species such as apple, hazelnut, peanut, celery and wheat were included in the EuroPrevall allergen library [19–21, 39]. The overall fold of the purified proteins was analysed and compared by CD spectroscopy, revealing spectra (Fig 1b), again emphasizing

Fig. 2 Fourier transform infrared spectroscopy of purified 7S seed storage globulin allergens from hazelnut (Cor a 11), walnut (Jug r 2), peanut (Ara h 1), soya and sesame (Ses i 3) (a) and of purified 11S seed storage globulins from hazelnut (Cor a 9), peanut (Ara h 3) and soybean (b). Data were collected using methods described in [22, 28] for Cor a 9, Cor a 11, Ara h 1 and Ara h 3



how the 3D structures of proteins belonging to the profilin family are conserved across different plant species ranging from Rosaceae to Poaceae.

Bet v 1 related proteins

Bet v 1-related proteins are widely spread in vascular plants and comprise eight subfamilies [40], among them pathogenesis-related proteins family 10 (PR-10) [41], major latex proteins, and proteins involved in alkaloid biosynthesis.

The first allergen identified from this group was the major birch pollen allergen, Bet v 1, which induces allergic symptoms in more than 90% of birch pollen allergic patients [42].

Proteins belonging to this family are 154–160 amino acid residue polypeptides which share high sequence similarity and are found throughout the plant kingdom. In general, these proteins are encoded by a number of genes forming a multigene family [43]. Some of the genes contain one intron inserted at a conserved position [44]. In the case of the apple PR-10, at least 18 different genes coding for Mal d 1 were identified and their loci mapped on different chromosomes [43].

The Bet v 1 homologous proteins contain a nucleotide binding motif, GXGXXG, which can be reduced to GXG in some proteins and forms the phosphate-binding loop. This motif is frequently found in protein kinases and nucleotide binding proteins [45]. Experimentally, the nucleotide binding function was determined in Bet v 1.

X-ray-based structure analyses are available from Bet v 1, Pru av 1 from cherry, Api g 1 from celery and Dau c 1 from carrot. They all share a characteristic overall fold formed by seven stranded antiparallel β -sheets which curve around a long C-terminal helix and two additional short helices connecting β 1 with β 2 [46, 47]. This structure provides a large y-shaped hydrophobic pocket which seems to bind plant steroids [46]. For other members of this family ribonuclease activity was observed [48].

Recently, the ubiquitous distribution of Bet v 1 related proteins was confirmed, identifying members of this superfamily from eukaryotes as well as prokaryotes and archaea. It seems that during evolution this protein diversified into numerous families low in sequence similarity but sharing a common fold, which specialized to bind to a range of different classes of ligands [40].

Bet v 1 homologous proteins are developmentally regulated and constitutively expressed in certain tissues such as mature pollen (birch), ripe fruits, (apple, pear, cherry and other members of the Rosaceae family), roots, and bulbs (carrot and celery), and in old leaves. In addition, the expression can be upregulated upon pathogen attack and/or abiotic stress [49].

Bet v 1 related proteins are known as potent inhalant allergens from pollen of the early flowering trees from the

botanical order Fagales. After primary sensitization to the pollen allergen, the majority of patients tend to develop allergic symptoms after ingestion of the Bet v 1 related proteins present in a range of fruits (apple, pear, cherry, kiwi, jackfruit), nuts (hazelnut) and vegetables (carrot, celery, parsley).

The allergic symptoms are usually rather mild and restricted to the oral cavity, summed up as the oral allergy syndrome. However, severe allergic reactions have also been ascribed to the Bet v 1 homologue from soy, Gly m 4. The high sequence similarity among the Bet v 1 related proteins even among rather distantly related species and the similar 3D structures may account for the IgE cross-reactivity leading also to the clinically relevant pollen-food syndrome.

In general, the Bet v 1 related proteins are labile to pH changes and of intermediate resistance towards heat treatment. This may explain why in the majority of cases of consumption of Bet v 1 related proteins in fruits and vegetables only mild local symptoms are induced. However, in cases where the pH of the matrix remains around neutral and does not drop to acidic, heat treatment does not affect the structure of the Bet v 1 related proteins, as has been shown for the peach allergen, Pru p 1 [20]. This may account for cases where severe allergic reactions were reported and points at the relevance of the food matrix for the allergenicity of individual proteins.

Members of the Bet v 1 family from apple, peach, celery, hazelnut and peanut were produced as recombinant proteins for the EuroPrevall allergen library [19–22, 39]. Stability of the Bet v 1 homologues was tested by subjecting the proteins to different pH conditions. Peach Bet v 1, Pru p 1, displayed the CD spectrum typical for Bet v 1 proteins at pH 7.5 (Fig. 3). However, when the pH was lowered to 3.0, even at 25°C the protein was rapidly denatured and unable to refold (Fig. 3).

Other plant food allergen families

A number of other allergens have been identified belonging to less widespread allergen families. A few selected protein families are presented in the following.

Oleosins are considered to contribute to stabilizing plant lipid storage bodies and represent the proteinaceous component. Recently, oleosins with allergenic activity were identified from legumes, nuts and seeds [50–52].

Pathogenesis-related proteins are upregulated within plants upon pathogen attack or exposure to abiotic stress factors [41]. Surprisingly, a considerable number of food allergens have been identified from various families of pathogenesis-related proteins, such as the β -1,3-glucanases,

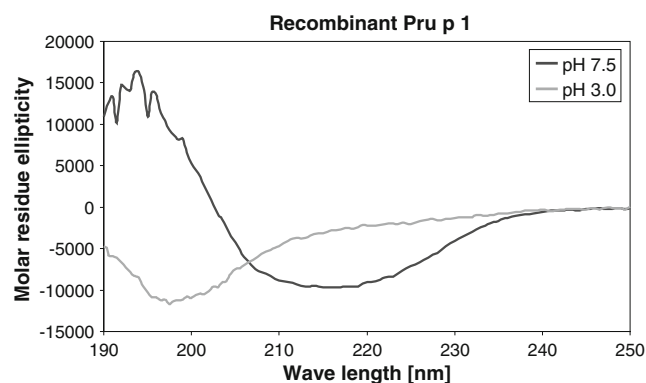


Fig. 3 Far-UV-CD spectroscopy of purified peach Bet v 1, Pru p 1, under neutral and acidic pH conditions. Data were collected and proteins were prepared as described in [20]

various types of chitinases and the thaumatin-like proteins [53].

Plant (class 1) endochitinases are able to hydrolyse chitin of fungal cell walls and the exoskeleton of hexapods and can thus play a role in plant protection against pathogen and pest attack. These glycoside hydrolases (usually 25–35 kDa) contain a domain binding to chitin subunits which is called the “hevein-like domain”. This domain, characteristic for this protein family, is rich in cysteine residues, comprises 40 amino acid residues and is located at the N-terminus. The enzymatic activity of chitinases is determined by a catalytic domain of 220–230 amino acid residues [54]. The hevein domain has been identified as a cross-reactive determinant between hevein, from *Hevea brasiliensis* latex, and food allergens from avocado, Pers a 1, chestnut, Cas s 5, grape, Vit v 5, and banana and accounts for the latex-fruit syndrome [55]. The catalytic domain of these proteins displays rather low IgE binding capacity [56].

Another type of plant defence is conducted by β -1,3 glucanases. These glycosyl hydrolases share an $(\alpha\beta)_8$ TIM barrel structure and are usually 25–35 kDa in size. They catalyse hydrolysis of 1,3- β -D-glucosidic linkages in β -1,3-glucans, the abundant component of plant cell walls. Therefore, these enzymes are thought to play a role in response of plants to microbial pathogens, but they are also implicated in a number of other physiological and developmental processes in uninfected plants, such as pollen germination and tube growth, fertilization, fruit ripening, seed germination and mobilization of storage products in the endosperm of cereal grains [57] and, in addition, they represent a response to wounding, cold, ozone and UV-B radiation. Allergens from this protein family have been identified from avocado, banana, chestnut, fig and kiwi [55, 58], and inhalant allergens are known from olive pollen [59] and latex [60] contributing to the latex fruit syndrome.

Thaumatococcus-like proteins were named after the intensely sweet tasting protein thaumatococcus, originating from the

African shrub *Thaumatococcus daniellii*, described as long ago as 1852 [61], although none of the other proteins have been described to have a sweet taste. In plants they are synthesized upon biotic and abiotic stress and in certain developmental stages, particularly during fruit ripening [62]. Thaumatococcus-like proteins have a molecular mass around 20 kDa, and form antiparallel β -sheets and are stabilized via eight disulfide bonds [63]. Owing to their rigid structure formed by the disulfide bridges, they are resistant to heat treatment and proteolytic degradation [64]. Food allergens of the pathogenesis-related proteins family 5 group have been found in several fruits, such as cherry (Pru av 2), apple (Mal d 2), kiwi (Act d 2—green kiwi, Act c 2—gold kiwi), orange, grape and bell pepper (Cup a 1) [64]. From pollen of mountain cedar (Jun a 3) and cypress (Cup a 3), thaumatococcus-like proteins were identified as inhalant allergens [64].

Within the EuroPrevall allergen library, TLP was purified from apple fruit [19]. The protein is abundant in the fruit flesh, depending on ripeness. Purified apple TLP migrates anomalously in sodium dodecyl sulfate polyacrylamide gel electrophoresis experiments, as is known from other members of this protein family, and this is ascribed to the high number of disulfide bridges. While the upper band in the sodium dodecyl sulfate polyacrylamide gel electrophoresis experiment represents the glycosylated form, the lower band consists of non-glycosylated TLP. In our studies more than 70% of sera from apple allergic patients displayed IgE binding to the glycosylated form, with only 15% having specific IgE directed to the non-glycosylated form [19]. In general, the presence of IgE specific to ubiquitous plant glycan structures is regarded as an immune response of low clinical significance, in contrast to IgE specific for protein determinants. Having both forms, Mal d 2 glycosylated and Mal d 2 non-glycosylated, available in the library will allow in vitro diagnostics to be developed able to discriminate between IgE reactivity of clinical relevance and without clinical implications and thus will refine the diagnostic sensitivity.

Animal food allergen: most prevalent protein families

Tropomyosin

Tropomyosins are highly conserved proteins present in all eukaryotic cells. They are α -helical proteins forming a coiled-coil structure of two parallel helices with two sets of seven actin binding sites. In striated muscle cells they facilitate interactions between the troponin and actin complex, thus regulating muscle contraction [65]. Tropomyosins relevant as food allergens were identified from crustaceans, molluscs and the fish parasite *Anisakis simplex*

and as inhalant allergens in mites and cockroaches [66]. So far, only non-vertebrate tropomyosins have been identified with allergenic activity but they are highly cross reactive. Although tropomyosins represent major food allergens, they are only minor inhalant allergens in most societies (Europe, Australia and the USA). Owing to their structure, their IgE binding capacity is preserved even after heat treatment or enzymatic digestions.

Within the EuroPrevall library, the shrimp tropomyosin, Pen a 1, was produced as a recombinant protein and purification protocols were optimized [67]. The highly purified allergen displayed a secondary structure typical for a flexible α -helical protein and storage at room temperature for at least 2 weeks affected neither stability nor IgE binding activity [67].

EF-hand domain

The EF-hand domain is characteristic for different types of Ca^{2+} -binding proteins. They all share a conserved domain of a Ca^{2+} -binding residue loop of 12 amino acid residues in length and two neighbouring α -helices of 12 amino acids (EF-hand). They function as Ca^{2+} -binding proteins and are involved in either signalling or Ca^{2+} buffering and/or transport. Calmodulin and troponin C are involved in signalling and undergo a conformational change upon Ca^{2+} binding. Parvalbumins are Ca^{2+} -binding proteins of 12 kDa from vertebrates which bind Ca^{2+} during muscle relaxation and thus are abundantly expressed in muscle tissue. Although parvalbumins from fish are major food allergens and cross-react with parvalbumins from amphibians, no allergenic parvalbumins from higher vertebrates have been identified so far [68]. Also troponin C, another Ca^{2+} -binding subgroup from cockroaches, has been identified as an inhalant allergen [69]. The IgE binding capacity of parvalbumins is dependent on structural determinants also involved in the Ca^{2+} binding [70].

For the EuroPrevall allergen library two parvalbumins, from carp and codfish, were purified and characterized [71]. Carp and cod parvalbumin are highly similar in sequence and secondary structure and IgE cross-reactivity was observed. However, cross-inhibition experiments indicated that species-specific B-cell epitopes are also present on the two proteins. Carp and cod parvalbumin are typical α -helical proteins according to the CD spectra and display protein stability even when heated up to 95 °C [71]. However, under acidic conditions the parvalbumins are found to be unstable. Thus, most of the allergenic activity of these major fish allergens is abolished during gastric enzymatic degradation. However, impairing the physiological digestion conditions may preserve these allergens and consequently increase the allergenic risk [72].

Caseins

Caseins are mammalian proteins present in milk and bind calcium via a cluster of phosphoserine and phosphothreonine residues of α_{s1} -casein, α_{s2} -casein and β -casein. These type of caseins form clusters around amorphous calcium phosphate, increasing the calcium level in milk. κ -Casein stabilizes these nanostructures [73]. α_{s1} -casein and α_{s2} -casein seem to be the most important allergens, followed by β -casein. The caseins display a random-coil structure. According to the literature, IgE cross-reactivity between cow's milk caseins and caseins from goat and sheep has been demonstrated, whereas mare's milk caseins seems to represent a lower risk of IgE cross-reactivity [74].

Since the caseins are all highly phosphorylated and heterogenous, the naturally derived casein fraction from a single genotyped cow was included in the EuroPrevall allergen library and was further characterized [75]. In parallel, casein from goat's milk was purified and the two fractions were subsequently compared regarding folding characteristics and composition [75]. Purified cow's milk casein consisted primarily of α_{s1} -casein and β -casein and only low amounts of α_{s2} -casein and κ -casein. In contrast, the purified goat's milk casein fraction contained primarily β -casein and to a much lower extent variants of α_{s1} -casein and only traces of κ -casein and α_{s2} -casein. When a range of ten milk allergic patients were tested, some of those showed IgE cross-reactivity to both caseins, whereas other sera showed monosensitization to goat's milk casein [75]. Both casein fractions were potent at even very low concentrations (1–10 ng/ml) in the basophil degranulation assay. This approach, using casein fractions from a single animal, proved useful for obtaining naturally derived protein batches consisting of a restricted number of variants, which reflects the heterogeneity of caseins, but also allows reproducibility and traceability of casein fractions [75].

Other animal food allergen families

A number of other allergens were identified belonging to less widespread allergen families. For example, another milk allergen, β -lactoglobulin (Bos d 5), belongs to the lipocalin protein family. Members of this protein family share a conserved 3D structure but the overall sequence similarity is low. They serve as carriers for a range of small molecules such as lipids, steroids, hormones, bilins and retinoids. Besides the food allergen from milk, inhalant allergens are known from animal dander and saliva from mammals as well as from insects and cockroaches [76].

α -Lactalbumin, Bos d 4, is a member of the C-type lysozyme/ α -lactalbumin family. It is able to bind calcium and is involved in lactose synthesis in cow's milk and has a superimposable 3D structure with hen egg lysozyme. Other

minor allergens families include the Kazal-type protease inhibitors, which are represented by the hen's egg white allergen ovomucoid, Gal d 1, a major allergen from egg white [77, 78]. This protein is extensively glycosylated, which may act to stabilize the protein against proteolysis [79]. Another inhibitor family represented in the animal food allergens is the serpin serine protease inhibitor, ovalbumin, Gal d 2 [77]. Lastly, transferrins, sulfur-rich iron-binding glycoproteins, have been identified as minor allergens in milk, lactoferrin [80], and in egg, ovotransferrin, Gal d 3 [81].

For the EuroPrevall allergen library, natural Bos d 4 and Bos d 5 were purified from cow's milk and subsequently characterized for their purity and secondary structure [75]. Finally, relevant allergens from hen's egg were purified from natural sources and their spectrum of isoforms was analysed [82]. The spectrum of the avian food allergens comprised ovomucoid, (Gal d 1) ovalbumin, (Gal d 2) ovotransferrin (Gal d 3), C-type lysozyme (Gal d 4), and α -livet in (Gal d 5), an egg yolk allergen. For these highly heterogenous protein fractions, purification protocols were optimized and their individual range of posttranslational modifications and isoforms was investigated. Compared with previously described egg allergen panels, the present protein fractions displayed improved characteristics, such as higher purity, evidence of folded proteins and coverage of ranges of isoallergens. All these features contributed to specific IgE binding activity with less cross-contamination, as shown by in vitro assays [82].

Component-resolved diagnosis

At present, total protein extracts from various food sources are used in routine clinical diagnosis. Up to now these extracts have lacked standardization, and they show batch-to-batch inconsistency [83, 84]. Important allergens may be present in low amounts or may be absent, and enzymatic processes can further reduce the content of allergens [85]. Therefore, the concept of using well-defined allergen batches termed "component-resolved diagnosis" has gained increasing interest in the past [86–88]. This approach will not only improve the performance of the diagnostic test, but will additionally provide information on sensitization patterns in patient groups, e.g. due to geographical differences or dietary habits. It will also be feasible to discriminate between patients at risk of developing severe food allergic symptoms and patients experiencing rather mild symptoms and to relate this to certain marker allergens. In the case of hazelnut and Rosaceae fruit allergy (cherry, apple and peach), IgE antibodies directed against the non-specific LTPs could be related to severe food allergic symptoms [89–91]. The latex-fruit syndrome described is another example which is explained at least

in part by the cross-reactivity of chitinases, glucanases and patatin present in the various fruit- and plant-derived material [92].

The component-resolved approach is considered to increase the sensitivity of the diagnostic test and to provide patient-tailored results. However, the specificity of the assay has to be investigated as well and it will be challenging to discriminate between IgE reactivity related to clinical significance and IgE reactivity without observed symptoms. For example, IgE cross-reactivity to a vast range of plant-derived profilins accounts for only a restricted number of fruits and vegetables, such as melon, citrus fruits, banana and melon, causing food allergic symptoms. The allergen library developed in the EuroPrevall project will help to address relevant questions such as the identification of potential marker allergens linked with either symptom severity or clinical cross-reactivity patterns.

Concluding remarks

Food allergens seem to be restricted to a small number of protein families. They are dominated by proteins with certain biological functions such as hydrolysis of proteins and polysaccharides, binding, transport and storage of ligands, and cytoskeleton association [6].

In general, IgE cross-reactivity, and thus the risk of developing allergic symptoms to similar proteins, is due to shared 3D structures. Specific surface-exposed areas of proteins give rise to the production of specific IgE antibodies in the allergic individual. However, individual amino acid residues critical for antibody recognition have been identified in a number of proteins. Exchanges of these residues can affect the IgE binding activity significantly, as has been shown for group 2 mite allergens [93] and the Bet v 1 homologues [94].

Other factors influencing allergenicity are different food matrices and their impact on stability of proteins and food-processing techniques, which can either upregulate or downregulate allergenic activity of individual allergens. It will be a challenge in the next few years to investigate these factors modulating allergenicity in more detail. In vitro studies using highly pure and well defined allergens are a prerequisite to study these effects. As a proof of concept, a food allergen library was established, including a range of important food allergens, of high quality and well characterized according to established quality criteria such as folding, authenticity and allergenic activity.

Collecting data on the performance of individual food allergen families (proteins) will help to fine-tune food-processing strategies aiming at controlled/reduced risk of allergen exposure and to advise the food allergic patients regarding dietary recommendations and to improve diagnosis of food allergy, by using the component-resolved approach.

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References

- Rona RJ, Keil T, Summers C, Gislason D, Zuidmeer L, Sodergren E, Sigurdardottir ST, Lindner T, Goldhahn K, Dahlstrom J, McBride D, Madsen C (2007) *J Allergy Clin Immunol* 120:638–646
- Chapman MD, Pomes A, Breiteneder H, Ferreira F (2007) *J Allergy Clin Immunol* 119:414–420
- Jenkins JA, Griffiths-Jones S, Shewry PR, Breiteneder H, Mills EN (2005) *J Allergy Clin Immunol* 115:163–170
- Radauer C, Breiteneder H (2006) *J Allergy Clin Immunol* 117:141–147
- Jenkins JA, Breiteneder H, Mills EN (2007) *J Allergy Clin Immunol* 120:1399–1405
- Radauer C, Bublin M, Wagner S, Mari A, Breiteneder H (2008) *J Allergy Clin Immunol* 121:847–852
- Stadler MB, Stadler BM (2003) *FASEB J* 17:1141–1143
- Soeria-Atmadja D, Lundell T, Gustafsson MG, Hammerling U (2006) *Nucleic Acids Res* 34:3779–3793
- Bateman A, Coin L, Durbin R, Finn RD, Hollich V, Griffiths-Jones S, Khanna A, Marshall M, Moxon S, Sonnhammer EL, Studholme DJ, Yeats C, Eddy SR (2004) *Nucleic Acids Res* 32: D138–D141
- Hoffmann-Sommergruber K, Mills EN, Vieths S (2008) *Mol Nutr Food Res* 52(Suppl 2):S159–S165
- Asero R, Ballmer-Weber BK, Beyer K, Conti A, Dubakiene R, Fernandez-Rivas M, Hoffmann-Sommergruber K, Lidholm J, Mustakov T, Oude Elberink JN, Pumphrey RS, Stahl Skov P, van Ree R, Vlieg-Boerstra BJ, Hiller R, Hourihane JO, Kowalski M, Papadopoulos NG, Wal JM, Mills EN, Vieths S (2007) *Mol Nutr Food Res* 51:135–147
- Sandiford CP, Tatham AS, Fido R, Welch JA, Jones MG, Tee RD, Shewry PR, Newman Taylor AJ (1997) *Clin Exp Allergy* 27:1120–1129
- Palosuo K, Alenius H, Varjonen E, Koivuluhta M, Mikkola J, Keskinen H, Kalkkinen N, Reunala T (1999) *J Allergy Clin Immunol* 103:912–917
- Lombardero M, Garcia-Selles FJ, Polo F, Jimeno L, Chamorro MJ, Garcia-Casado G, Sanchez-Monge R, Diaz-Perales A, Salcedo G, Barber D (2004) *Clin Exp Allergy* 34:1415–1421
- Nakase M, Hotta H, Adachi T, Aoki N, Nakamura R, Masumura T, Tanaka K, Matsuda T (1996) *Gene* 170:223–226
- James JM, Sixbey JP, Helm RM, Bannon GA, Burks AW (1997) *J Allergy Clin Immunol* 99:239–244
- Curioni A, Santucci B, Cristaudo A, Canistraci C, Pietravalle M, Simonato B, Giannattasio M (1999) *Clin Exp Allergy* 29:407–413
- Pastorello EA, Pompei C, Pravettoni V, Farioli L, Calamari AM, Scibilia J, Robino AM, Conti A, Iametti S, Fortunato D, Bonomi S, Ortolani C (2003) *J Allergy Clin Immunol* 112:775–783
- Oberhuber C, Ma Y, Marsh J, Rigby N, Smole U, Radauer C, Alessandri S, Briza P, Zuidmeer L, Maderegger B, Himly M, Sancho AI, van Ree R, Knulst A, Ebner C, Shewry P, Mills EN, Wellner K, Breiteneder H, Hoffmann-Sommergruber K, Bublin M (2008) *Mol Nutr Food Res* 52(Suppl 2):S208–S219
- Gaier S, Marsh J, Oberhuber C, Rigby NM, Lovegrove A, Alessandri S, Briza P, Radauer C, Zuidmeer L, van Ree R, Hemmer W, Sancho AI, Mills C, Hoffmann-Sommergruber K, Shewry PR (2008) *Mol Nutr Food Res* 52(Suppl 2):S220–S229
- Lauer I, Alessandri S, Pokoj S, Reuter A, Conti A, Vieths S, Scheurer S (2008) *Mol Nutr Food Res* 52(Suppl 2):S262–S271
- Marsh J, Rigby N, Wellner K, Reese G, Knulst A, Akkerdaas J, van Ree R, Radauer C, Lovegrove A, Sancho A, Mills C, Vieths S, Hoffmann-Sommergruber K, Shewry PR (2008) *Mol Nutr Food Res* 52(Suppl 2):S272–S285
- Dunwell JM, Purvis A, Khuri S (2004) *Phytochemistry* 65:7–17
- Leitner A, Jensen-Jarolim E, Grimm R, Wuthrich B, Ebner H, Scheiner O, Kraft D, Ebner C (1998) *Allergy* 53:36–41
- Ahrazem O, Ibanez MD, Lopez-Torrejon G, Sanchez-Monge R, Sastre J, Lombardero M, Barber D, Salcedo G (2006) *Int Arch Allergy Immunol* 139:96–103
- Crespo JF, Retzek M, Foetisch K, Sierra-Maestro E, Cid-Sanchez AB, Pascual CY, Conti A, Feliu A, Rodriguez J, Vieths S, Scheurer S (2006) *Mol Nutr Food Res* 50:282–290
- Mills EN, Jenkins JA, Alcocer MJ, Shewry PR (2004) *Crit Rev Food Sci Nutr* 44:379–407
- Rigby NM, Marsh J, Sancho AI, Wellner K, Akkerdaas J, van Ree R, Knulst A, Fernandez-Rivas M, Brettlova V, Schilte PP, Summer C, Pumphrey R, Shewry PR, Mills EN (2008) *Mol Nutr Food Res* 52(Suppl 2):S251–S261
- Witke W (2004) *Trends Cell Biol* 14:461–469
- Gao ZS, Van de Weg WE, Schaart JG, Van Arkel G, Breiteneder H, Hoffmann-Sommergruber K, Gilissen LJWJ (2005) *Theor Appl Genet* 6:1–11
- Radauer C, Hoffmann-Sommergruber K (2004) In: Mills E, Shewry P (eds) *Plant food allergens*. Blackwell, Oxford, pp 105–124
- Fedorov AA, Ball T, Mahoney NM, Valenta R, Almo SC (1997) *Structure* 5:33–45
- Luttkopf D, Ballmer-Weber BK, Wuthrich B, Vieths S (2000) *J Allergy Clin Immunol* 106:390–399
- Ballmer-Weber BK, Wangorsch A, Bohle B, Kaul S, Kundig T, Fotisch K, van Ree R, Vieths S (2005) *Clin Exp Allergy* 35:970–978
- Asero R, Mistrello G, Roncarolo D, Amato S, Zanoni D, Barocci F, Caldiroli G (2003) *J Allergy Clin Immunol* 112:427–432
- Lopez-Torrejon G, Crespo JF, Sanchez-Monge R, Sanchez-Jimenez M, Alvarez J, Rodriguez J, Salcedo G (2005) *Clin Exp Allergy* 35:1065–1072
- Lopez-Torrejon G, Ibanez MD, Ahrazem O, Sanchez-Monge R, Sastre J, Lombardero M, Barber D, Salcedo G (2005) *Allergy* 60:1424–1429
- Ma Y, Zuidmeer L, Bohle B, Bolhaar ST, Gadermaier G, Gonzalez-Mancebo E, Fernandez-Rivas M, Knulst AC, Himly M, Asero R, Ebner C, van Ree R, Ferreira F, Breiteneder H, Hoffmann-Sommergruber K (2006) *Clin Exp Allergy* 36:1087–1096
- Bublin M, Lauer I, Oberhuber C, Alessandri S, Briza P, Radauer C, Himly M, Breiteneder H, Vieths S, Hoffmann-Sommergruber K (2008) *Mol Nutr Food Res* 52(Suppl 2):S241–S250
- Radauer C, Lackner P, Breiteneder H (2008) *BMC Evol Biol* 8:286
- van Loon LC, van Strien EA (1999) *Phys Mol Plant Pathol* 55:85–87
- Jarolim E, Rumpold H, Endler AT, Ebner H, Breitenbach M, Scheiner O, Kraft D (1989) *Allergy* 44:385–395
- Gao ZS, van de Weg WE, Schaart JG, Schouten HJ, Tran DH, Kodde LP, van der Meer IM, van der Geest AH, Kodde J, Breiteneder H, Hoffmann-Sommergruber K, Bosch D, Gilissen LJ (2005) *Theor Appl Genet* 111:171–183
- Hoffmann-Sommergruber K, Vanek-Krebitz M, Radauer C, Wen J, Ferreira F, Scheiner O, Breiteneder H (1997) *Gene* 197:91–100
- Saraste M, Sibbald PR, Wittinghofer A (1990) *Trends Biochem Sci* 15:430–434
- Markovic-Housley Z, Degano M, Lamba D, von Roepenack-Lahaye E, Clemens S, Susani M, Ferreira F, Scheiner O, Breiteneder H (2003) *J Mol Biol* 325:123–133

47. Schirmer T, Hoffmann-Sommergrube K, Susani M, Breiteneder H, Markovic-Housley Z (2005) *J Mol Biol* 351:1101–1109
48. Park CJ, Kim KJ, Shin R, Park JM, Shin YC, Paek KH (2004) *Plant J* 37:186–198
49. Hoffmann-Sommergruber K, Radauer C (2004) In: Mills E, Shewry P (eds) *Plant food allergens*. Blackwell, Oxford, pp 125–140
50. Pons L, Chery C, Romano A, Namour F, Artesani MC, Gueant JL (2002) *Allergy* 57(Suppl 72):88–93
51. Akkerdaas JH, Schocker F, Vieths S, Versteeg S, Zuidmeer L, Hefle SL, Aalberse RC, Richter K, Ferreira F, van Ree R (2006) *Mol Nutr Food Res* 50:18–23
52. Leduc V, Moneret-Vautrin DA, Tzen JT, Morisset M, Guerin L, Kanny G (2006) *Allergy* 61:349–356
53. Hoffmann-Sommergruber K (2000) *Int Arch Allergy Immunol* 122:155–166
54. Mauch F, Staehelin LA (1989) *Plant Cell* 1:447–457
55. Alenius H, Kalkkinen N, Reunala T, Turjanmaa K, Palosuo T (1996) *J Immunol* 156:1618–1625
56. Diaz-Perales A, Sanchez-Monge R, Blanco C, Lombardero M, Carrillo T, Salcedo G (2002) *Clin Exp Allergy* 32:448–454
57. Leubner-Metzger G, Meins F Jr (1999) In: Datta SK, Muthukrishnan S (eds) *Pathogenesis-related proteins in plants*. CRC, Boca Raton, pp 49–76
58. Blanco C, Carrillo T, Castillo R, Quiralte J, Cuevas M (1994) *Allergy* 49:454–459
59. Palomares O, Villalba M, Quiralte J, Rodriguez R (2006) *Ann Allergy Asthma Immunol* 97:61–65
60. Yagami T, Osuna H, Kouno M, Haishima Y, Nakamura A, Ikezawa Z (2002) *Int Arch Allergy Immunol* 129:27–37
61. Daniell W (1852) *Pharm J* 11:445
62. Fils-Lycaon BR, Wiersma PA, Eastwell KC, Sautiere P (1996) *Plant Physiol* 111:269–273
63. Leone P, Menu-Bouaouiche L, Peumans WJ, Payan F, Barre A, Roussel A, Van Damme EJ, Rouge P (2006) *Biochimie* 88:45–52
64. Breiteneder H (2004) *Allergy* 59:479–481
65. Brown JH, Cohen C (2005) *Adv Protein Chem* 71:121–159
66. Jeong KY, Hong CS, Yong TS (2006) *Prot Peptide Letters* 13:835–845
67. Albrecht M, Alessandri S, Conti A, Reuter A, Lauer I, Vieths S, Reese G (2008) *Mol Nutr Food Res* 52(Suppl 2):S186–S195
68. Wild LG, Lehrer SB (2005) *Curr Allergy Asthma Rep* 5:74–79
69. Hindley J, Wunschmann S, Satinover SM, Woodfolk JA, Chew FT, Chapman MD, Pomes A (2006) *J Allergy Clin Immunol* 117:1389–1395
70. Swoboda I, Bugajska-Schretter A, Linhart B, Verdino P, Keller W, Schulmeister U, Sperr WR, Valent P, Peltre G, Quirce S, Douladiris N, Papadopoulos NG, Valenta R, Spitzauer S (2007) *J Immunol* 178:6290–6296
71. Ma Y, Griesmeier U, Susani M, Radauer C, Briza P, Erler A, Bublin M, Alessandri S, Himly M, Vazquez-Cortes S, de Arellano IR, Vassilopoulou E, Saxoni-Papageorgiou P, Knulst AC, Fernandez-Rivas M, Hoffmann-Sommergruber K, Breiteneder H (2008) *Mol Nutr Food Res* 52(Suppl 2):S196–S207
72. Untersmayr E, Poulsen LK, Platzner MH, Pedersen MH, Boltz-Nitulescu G, Skov PS, Jensen-Jarolim E (2005) *J Allergy Clin Immunol* 115:377–382
73. Tuinier R, Rolin C, de Kruif CG (2002) *Biomacromolecules* 3:632–638
74. Spuergerin P, Walter M, Schiltz E, Deichmann K, Forster J, Mueller H (1997) *Allergy* 52:293–298
75. Blanc F, Bernard H, Alessandri S, Bublin M, Paty E, Leung SA, Patient KA, Wal JM (2008) *Mol Nutr Food Res* 52(Suppl 2):S166–S175
76. Chapman MD, Wood RA (2001) *J Allergy Clin Immunol* 107: S414–S421
77. Bernhisel-Broadbent J, Dintzis HM, Dintzis RZ, Sampson HA (1994) *J Allergy Clin Immunol* 93:1047–1059
78. Bernhisel-Broadbent J, Yolken RH, Sampson HA (1991) *Pediatrics* 87:208–214
79. Cooke SK, Sampson HA (1997) *J Immunol* 159:2026–2032
80. Wal JM (2002) *Ann Allergy Asthma Immunol* 89:3–10
81. Holen E, Elsayed S (1990) *Int Arch Allergy Appl Immunol* 91:136–141
82. Jacobsen B, Hoffmann-Sommergruber K, Have TT, Foss N, Briza P, Oberhuber C, Radauer C, Alessandri S, Knulst AC, Fernandez-Rivas M, Barkholt V (2008) *Mol Nutr Food Res* 52(Suppl 2):S176–S185
83. Vieths S, Scheurer S, Reindl J, Luttkopf D, Wangorsch A, Kastner M, Haase T, Hausteiner D (2001) *Allergy* 56(Suppl 67):78–82
84. Akkerdaas JH, Wensing M, Knulst AC, Aalberse RC, Hefle SL, van Ree R (2003) *Arb Paul Ehrlich Inst Bundesamt Sera Impfst Frankf* 87–95; discussion 6
85. Garcia A, Wichers JH, Wichers HJ (2007) *Food Chem* 103:94–100
86. Lidholm J, Ballmer-Weber BK, Mari A, Vieths S (2006) *Curr Opin Allergy Clin Immunol* 6:234–240
87. Valenta R, Twaroch T, Swoboda I (2007) *J Invest Allergol Clin Immunol* 17(Suppl 1):36–40
88. Steckelbroeck S, Ballmer-Weber BK, Vieths S (2008) *J Allergy Clin Immunol* 121:1323–1330
89. Ballmer-Weber BK, Scheurer S, Fritsche P, Enrique E, Cistero-Bahima A, Haase T, Wuthrich B (2002) *J Allergy Clin Immunol* 110:167–173
90. Fernandez-Rivas M, Bolhaar S, Gonzalez-Mancebo E, Asero R, van Leeuwen A, Bohle B, Ma Y, Ebner C, Rigby N, Sancho AI, Miles S, Zuidmeer L, Knulst A, Breiteneder H, Mills C, Hoffmann-Sommergruber K, van Ree R (2006) *J Allergy Clin Immunol* 118:481–488
91. Flinterman AE, Akkerdaas JH, den Hartog Jager CF, Rigby NM, Fernandez-Rivas M, Hoekstra MO, Bruijnzeel-Koomen CA, Knulst AC, van Ree R, Pasmans SG (2008) *J Allergy Clin Immunol* 121:423–428
92. Wagner S, Breiteneder H (2002) *Biochem Soc Trans* 30:935–940
93. Smith AM, Benjamin DC, Hozic N, Derewenda U, Smith WA, Thomas WR, Gafvelin G, van Hage-Hamsten M, Chapman MD (2001) *J Allergy Clin Immunol* 107:977–984
94. Ma Y, Gadermaier G, Bohle B, Bolhaar S, Knulst A, Markovic-Housley Z, Breiteneder H, Briza P, Hoffmann-Sommergruber K, Ferreira F (2006) *Int Arch Allergy Immunol* 139:53–62