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Invited Review Article

Components of plant-derived food allergens: Structure, diagnostics, and immunotherapy

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A R T I C L E I N F O

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Abbreviations:
AIT, allergen-specific immunotherapy; AUC, area under the receiver operating characteristic curve; CRD, component-resolved diagnosis; FA, food allergy; GRP, gibberellin-regulated protein; ns-LTP, non-specific lipid transfer protein; PR, pathogenesis related protein; PFAS, pollen-food allergy syndrome; sIgE, specific IgE; TLP, thaumatin-like protein

A B S T R A C T

A large number of plant-derived food allergen components have been identified to date. Although these allergens are diverse, they often share common structural features such as numerous disulfide bonds or oligomeric structures. Furthermore, some plant-derived food allergen components cross-react with pollen allergens. Since the relationship between allergen components and clinical symptoms has been well characterized, measurements of specific IgE to these components have become useful for the accurate clinical diagnosis and selection of optimal treatment methods for various allergy-related conditions including allergy caused by plant-derived foods. Herein, I have described the types and structures of different plant allergen components and outlined the diagnosis as well as treatment strategies, including those reported recently, for such substances. Furthermore, I have also highlighted the contribution of allergen components to this field.

Introduction

Many plant-derived food allergen components have been identified (Table 1), wherein their allergenic activity correlates with their structural characteristics. Several allergens have high thermal stability during food processing, show resistance to digestive enzymes in humans, contain disulfide bonds, have repeated sequences, and bind to ligands (Fig. 1, 2). In some cases of food allergies (FAs) caused by plant sources, pollen allergens are the primary allergens. Recently, measurements of specific IgE (sIgE) antibody titers for plant-derived food allergen components in patient sera were shown to be useful for the accurate clinical diagnosis and selection of optimal therapy for FAs caused by plant sources. Furthermore, plant-derived food allergen components and their engineered molecules have been examined for use in FA immunotherapy.

Herein, I have comprehensively described recent reports on plant-derived food allergen components, and prospects for the diagnosis and immunotherapy of plant-derived FAs.

Plant-derived food allergen components and their structure

Plant-derived food allergen components

Plants, including peanuts, wheat, fruits, nuts, and soybean, are frequent sources of FAs.[1,2] There has been an increase in the number of patients with nut allergies worldwide over a decade.[3] More than 60% of plant-derived food allergens belong to the four protein families/superfamilies of prolamin, cupin, Bet v 1/pathogenesis related protein 10 (PR-10), and profilin (Table 1).[4] The prolamin superfamily includes 2S albumin, non-specific lipid transfer protein (ns-LTP), cereal prolamin, and α-amylase/trypsin inhibitors. The cupin superfamily contains a variety of proteins from microorganisms, plants, and animals that are supposedly derived from a common ancestral protein, molecule with

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Table 1
Plant-derived food allergen components.

Family/Superfamily	Prolamin				Cupin		Bet v 1/PR-10	Profilin		PR-12	PR-5	Snakin/GASA	Others
Biochemical name	2S albumin	ns-Lipid transfer protein/PR-14	Cereal prolamin	α-amylase/trypsin inhibitor	Legumin	Vicilin	PR-10	Profilin	Oleosin	Defensin/PR-12	Thaumatin-like protein/PR-5	GRP	
Peanut	Ara h 2 Ara h 6 Ara h 7	Ara h 9 Ara h 16 Ara h 17			Ara h 3	Ara h 1	Ara h 8	Ara h 5	Ara h 10 Ara h 11 Ara h 14 Ara h 15	Ara h 12 Ara h 13			Ara h 18
Walnut	Jug r 1	Jug r 3 Jug r 8			Jug r 4	Jug r 2 Jug r 6	Jug r 5	Jug r 7					
Pecan	Car i 1				Car i 4	Car i 2							
Cashew	Ana o 3				Ana o 2	Ana o 1							
Pistachio	Pis v 1				Pis v 2 Pis v 5	Pis v 3							Pis v 4
Almond		Pru du 3			Pru du 6	Pru du 8		Pru du 4					Pru du 5, Pru du 10
Hazelnut	Cor a 14	Cor a 8			Cor a 9	Cor a 11	Cor a 1	Cor a 2	Cor a 12 Cor a 13 Cor a 15				Cor a 6, Cor a 10
Macadamia					Mac i 2	Mac i 1							
Soybean	Gly m 8				Gly m 6	Gly m 5	Gly m 4	Gly m 3		Gly m 2			Gly m 1, Gly m 7
Sesame	Ses i 1 Ses i 2				Ses i 6 Ses i 7	Ses i 3			Ses i 4 Ses i 5				
Buckwheat	Fag e 2					Fag e 3 Fag e 5							Fag e 4
Wheat		Tri a 14	Tri a 19 Tri a 20 Tri a 21 Tri a 26 Tri a 36	Tri a 15 Tri a 28 Tri a 29 Tri a 30 Tri a 40				Tri a 12					Tri a 17, Tri a 18, Tri a 25, Tri a 27, Tri a 31, Tri a 32, Tri a 33, Tri a 34, Tri a 35, Tri a 37, Tri a 39, Tri a 41, Tri a 42, Tri a 43. Tri a 44, Tri a 45
Peach		Pru p 3					Pru p 1	Pru p 4			Pru p 2	Pru p 7	Pru p 9
Apple		Mal d 3					Mal d 1	Mal d 4			Mal d 2		
Banana		Mus a 3						Mus a 1			Mus a 4		Mus a 2, Mus a 5, Mus a 6
Kiwi fruit	Act d 13	Act d 10			Act d 12		Act d 8, Act d 11	Act d 9			Act d 2		Act d 1, Act d 3, Act d 4, Act d 5, Act d 6, Act d 7
Melon								Cuc m 2					Cuc m 1, Cuc m 3
Orange		Cit s 3						Cit s 2				Cit s 7	Cit s 1
Celery		Api g 2, Api g 6					Api g 1	Api g 4					Api g 3, Api g 5

The allergen components are registered in an allergen database established by the Allergen Nomenclature Subcommittee of the World Health Organization/International Federation of Immunological Societies (http://www.allergen.org/). In allergen nomenclature, the allergen name consists of the first three letters of the genus and one letter from the species, followed by Arabic numerals. It can contain information regarding isoallergens or isoforms, distinguished by the suffix of a number following the allergen number.

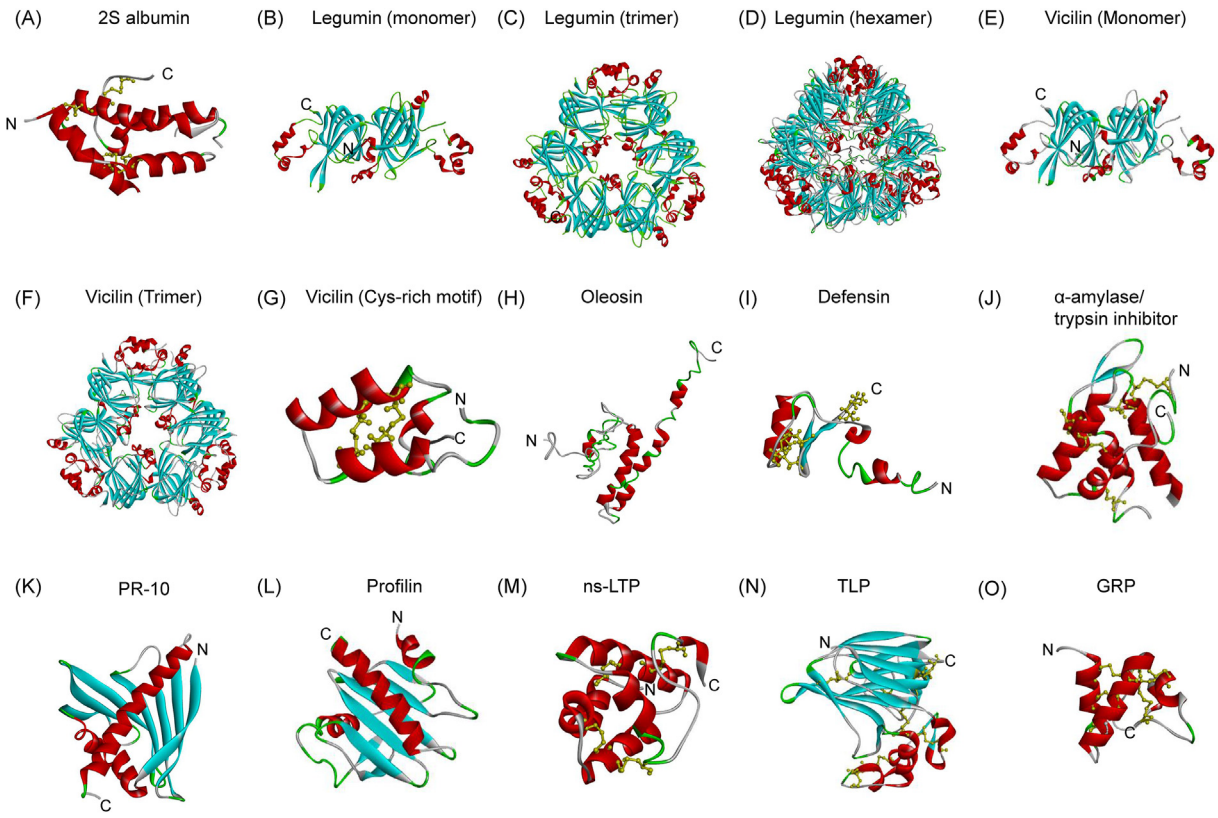

Fig. 1. Three-dimensional structures of plant-derived allergen components. **(A)** Peanut 2S albumin (Ara h 2, PDB ID 3OB4), **(B)** peanut legumin monomer (Ara h 3, PDB ID 3C3V), **(C)** peanut legumin trimer (Ara h 3), **(D)** peanut legumin hexamer (Ara h 3), **(E)** peanut vicilin monomer (Ara h 1, PDB ID 3SMH), **(F)** peanut vicilin trimer (Ara h 1), **(G)** Cys-rich motif present in the N-terminal extension region of vicilin (homology model), **(H)** peanut oleosin (Ara h 10, homology model), **(I)** peanut defensin (Ara h 12, homology model), **(J)** wheat α-amylase/trypsin inhibitor (PDB ID 1HSS), **(K)** soybean PR-10 (Gly m 4, PDB ID 2K7H), **(L)** melon profilin (Cuc m 2, PDB ID 6MBX), **(M)** peach ns-LTP (Pru p 3, PDB ID 2B5S), **(N)** banana TLP (Mus a 4, PDB ID 1Z3Q), and **(O)** peach GRP (Pru p 7, homology model). PDB indicates Protein Data Bank. Conserved disulfide bonds are shown using ball and stick models. Cyan and red indicate β-strands and α-helices, respectively.

a conserved motif. Allergens such as vicilin (7S globulin) and legumin (11S globulin) belong to the cupin superfamily. The Bet v 1/PR-10 and profilin families include major allergens associated with fruit allergies associated with cross-reactive pollen allergens. Oleosins, involved in the formation of oil bodies, are components of plant seeds.[5] Plant defensins belonging to PR-12 family are associated with the innate immune system and have antifungal and antibacterial properties; they have been identified as peanut allergens.[6] Thaumatin-like proteins (TLPs)[7] and gibberellin-regulated proteins (GRPs)[8] are classified as part of PR-5 and snakin/GASA families, respectively.

Legumes and nuts

2S albumin

The 2S albumin is a storage protein present in many plant seeds. It is an important allergen in peanuts, soybean, nuts, sesame, and buckwheat (Table 1).[9] The 2S albumin is synthesized as an 18–21 kDa proteins. The overall structure includes five α helices and a hypervariable region between two α helices (Fig. 1A). In most plant species, the 2S albumin polypeptide undergoes cleavage, resulting in two chains (small and large subunits) (Fig. 2A). It contains eight-cysteine motifs (C-X[11–12 residues] –C…C–C-X[9residues]-C-X-C-X[29-42residues]-C-X[6-7residues]C) and inter-subunit disulfide bonds.[9] The sequences of 2S albumin from peanuts, nuts, and sesame seeds showed low similarity (<20–60%), but those from walnut (Jug r 1) and pecan (Car i 1), members of the Juglandaceae family, showed exceptionally high identity (88%).[10] The identities of Ara h 2, Ara h 6, and Ara h 7 are not high (46–55%) despite being isoforms. Although 2S albumins of cashew nut, pistachio, hazelnut, soybean, sesame, and buckwheat are allergens, the presence of 2S albumin in almond is still unclear.

Legumin

Legumins, which are storage proteins present in high amounts in various seeds, have been identified as allergens in many plant species (Table 1). Similar to vicilin monomers, legumin monomers are composed of repeated structures of a β barrel and an α helix in a domain (Fig. 1B),[11] but do not contain any carbohydrate moiety. The molecular mass of the monomer is 50–60 kDa, and it is stable in a trimeric proform (Fig. 1C). Following cleavage by proteases in the seeds, the polypeptide chains are divided into acidic and basic chains with different isoelectric points, resulting in the formation of hexamers (Fig. 1D). Legumin contains highly conserved intra-subunit disulfide bonds between the acidic and basic chains (Fig. 2B). Legumins of different plant species show a high sequence identity (32–95%).[11]

Vicilin

Vicilins, like 2S albumin and legumin, are storage proteins. The monomeric form of vicilin has a molecular mass of ~50–70 kDa. The core region, which is highly conserved among plant species, consists of a repeated domain composed of a β barrel and an α helix (Fig. 1E)[11]; the monomers form a stable trimeric structure (Fig. 1F).

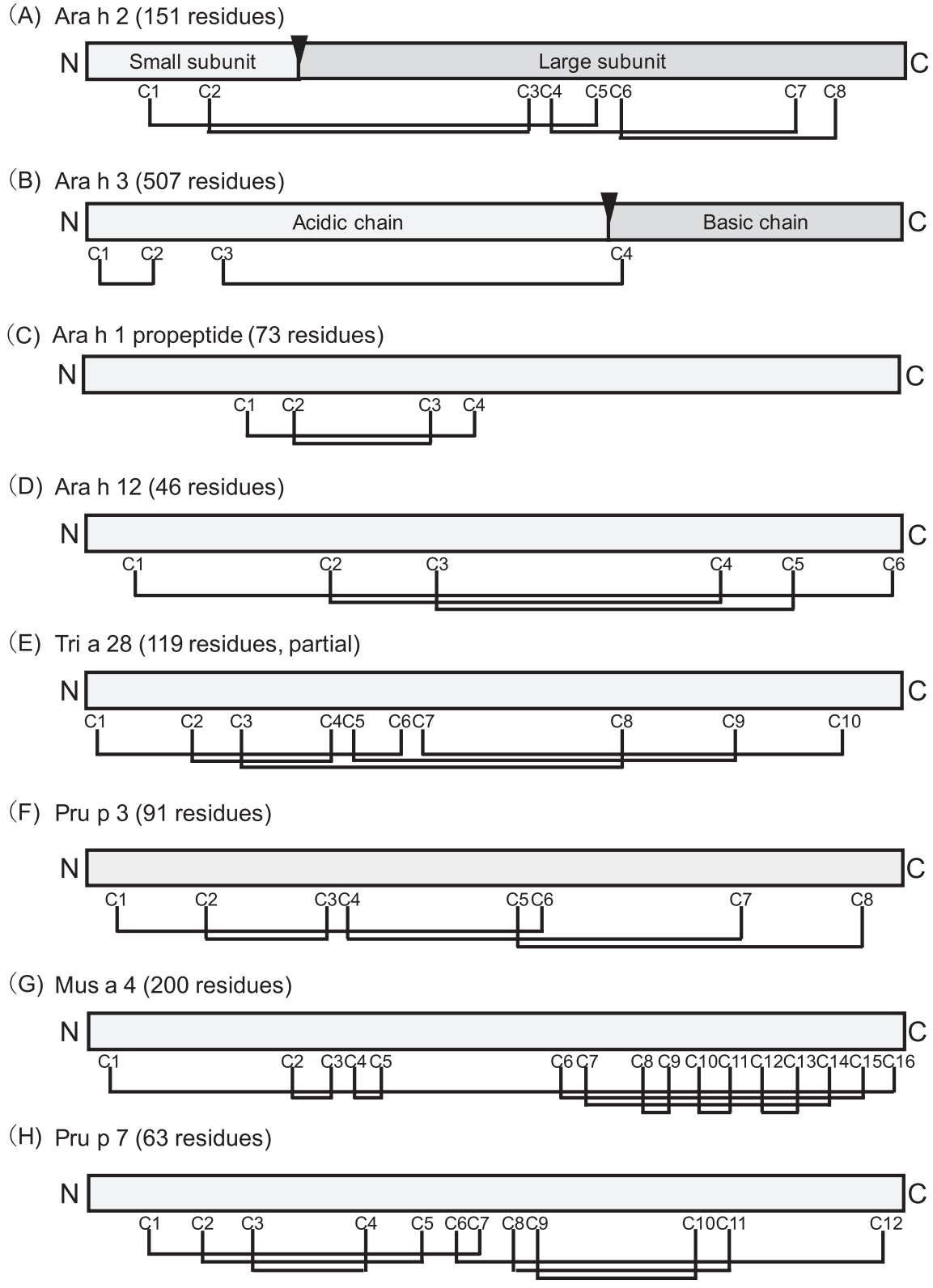

Fig. 2. Schematic structures of plant-derived food allergen components and their conserved disulfide bonds. **(A)** Peanut 2S albumin of prolamin superfamily, **(B)** peanut legumin of cupin superfamily, **(C)** N-terminal propeptide of peanut vicilin of cupin superfamily, **(D)** peanut defensin of PR-12 family, **(E)** wheat α-amylase/trypsin inhibitor of prolamin superfamily, **(F)** peach ns-LTP of prolamin superfamily, **(G)** banana TLP of PR-5 family, and **(H)** peach GRP of snakin/GASA family. Conserved disulfide bonds and cysteine residues are shown as lines and C, respectively. Arrow heads in **(A)** and **(B)** indicate processing sites.

Unlike 2S albumin and legumin, vicilins often contain a carbohydrate moiety in the core region. In some plant species, vicilins contain a hydrophilic domain at the N-terminus, called the extension region. Sequence identity of the extension region among various plant species is relatively low compared to that of the core region. The extension region may harbor a motif composed of cysteine residues (C-X-X-X-C-X[10–12 residues]-C-X-X-X-C) depending on the plant species (Fig. 2C).[12] The extension region of vicilin in various nuts including almonds, macadamia nuts, and walnuts undergoes processing within the seeds, and the derived fragments accumulate. The cysteine-containing motif forms a helix-turn-helix structure (Fig. 1G), thereby affecting the peptide's antimicrobial activity, which possibly plays a crucial role in plant defense.

Oleosin

Neutral lipids in plant seeds are stored in lipid droplets and are utilized as a source of energy and carbon during seed growth.[13] Lipid droplets have a core surrounded by a monolayer of phospholipids and proteins. The major protein present in the monolayer of these lipid droplets is oleosin. Oleosins have been identified as allergenic molecules in peanut (Ara h 10, Ara h 11, Ara h 14 and Ara h 15), sesame (Ses i 4 and Ses i 5), and hazelnut (Cor a 12, Cor a 13, and Cor a 15) (Table 1). Their molecular mass ranges between 14 and 17.5 kDa. Oleosin have a central hydrophobic hairpin adjacent to the N- and C-terminal domains that can penetrate the phospholipid monolayer and contact the hydrophobic core of the oil droplet (Fig. 1H).

Defensin

Plant defensins are small cysteine-rich peptides (~8 kDa) with antifungal and antibacterial activity. Peanut defensins (Ara h 12 and Ara h 13) have been extracted using chloroform/methanol.[6] Soybean defensin (Gly m 2) is also identified as an allergen (Table 1). Defensins have conserved cysteine residues (Fig. 1I, 2D). The sequence of mugwort pollen allergen (Art v 1) shares a low identity with that of Ara h 12, Ara h 13, and Gly m 2, suggesting a low probability of cross-reactivity.

Wheat allergen component

Cereal prolamin

The major seed allergen components of wheat are gliadin and glutenin, which constitute gluten. Gliadins are classified as α-, γ-, and ω-gliadins, and glutenins as low and high-molecular-weight glutenins.[14] Based on the sensitization profiles of major wheat allergens, α- and γ-gliadin (α-gliadin for Tri a 21; γ-gliadin for Tri a 20), and low-molecular-weight glutenin (Tri a 36) are the major allergens associated with wheat allergy in children (Table 1). Furthermore, wheat is a frequent cause of food-dependent exercise-induced anaphylaxis. In this case, ω5-gliadin (Tri a 19) and high-molecular-weight glutenin (Tri a 26) are the major allergens involved (Table 1).[15] Repeated motifs containing proline and glutamine residues are common to both molecules. Epitopes are present in repeated sequences, and these features are thought to contribute to the symptoms of allergy.

α-Amylase/trypsin inhibitor

The wheat α-amylase/trypsin inhibitor includes major components responsible for baker's asthma and are classified into three types based on their assembly, with all members being immunoreactive and listed in the database (monomeric, Tri a 15; dimeric, Tri a 28; tetrameric, Tri a 29, Tri a 30, Tri a 40) (Table 1).[14,16] The family comprises proteins that are extracted using chloroform/methanol and have molecular masses of 12–16 kDa. These proteins have a common fold (4–5 α-helices and a short antiparallel β-sheet) and include 4–5 intrachain disulfide bridges (Fig. 1J, 2E).

Fruits and vegetables

Bet v 1/PR-10

Many fruits and vegetables cause FA symptoms due to cross-reactivity with pollen, called pollen-food allergy syndrome (PFAS).[17] The main allergens responsible for this cross-reactivity belong to the Bet v 1/PR-10 family molecules (Table 1). Bet v 1 of birch pollen serves as the primary antigen and causes allergic symptoms by cross-reactivity. Its structure is composed of seven anti-parallel β strands and three α helices surrounding a large hydrophobic cavity (Fig. 1K),[18] wherein this cavity serves as a ligand for plant hormones. PR-10 proteins are also found in seeds, such as peanuts and soybeans (Table 1). In particular, patients allergic to soybean milk show high sIgE antibody titers to Gly m 4, which is a major cause of soybean allergy in adults.[19]

Profilin

Profilins are actin monomer–binding proteins, with molecular masses of 12–16 kDa.[20] The first allergenic profilin identified from birch pollen was Bet v 2; several profilins have been identified in various foods since then (Table 1). The profilin family proteins share highly conserved amino acid sequences and have been associated with PFAS. Most of them are composed of seven β strand and three to four α helices (Fig. 1L).[21]

ns-LTP

ns-LTP, a defense protein belonging to the PR-14 family, is an important allergen in seeds and fruits (Table 1). In the Mediterranean region of Europe, ns-LTP is frequently associated with peach and apple allergies, and induces systemic symptoms.[22] Rosaceae fruits and vegetables, walnuts, hazelnuts, and peanuts contain ns-LTP (Table 1). Although its relationship with pollen is not clear, ns-LTP has been reported to be a major allergen in mugwort pollen-related allergies in China.[23] The three-dimensional structure of peach ns-LTP contains α-helices with short loops (Fig. 1M). It has a characteristic globular structure stabilized by four disulfide bonds (Fig. 2F)[24] and hydrophobic cavities that serve as ligand-binding sites for lipid and other hydrophobic molecules.

TLP

Sequences of the family 5 of PRs show homologies with those of thaumatin, a sweet-tasting protein isolated from the fruits of the west African rain forest shrub *Thaumatococcus daniellii*.[7] Therefore, the PR-5 family proteins are referred to as TLPs. Mal d 2, Pru p 2, Act d 2, Mus a 4, and others are allergic TLPs (Table 1). The molecular mass of these proteins is ~23 kDa. The three-dimensional structure of TLPs is composed of three distinct domains (Fig. 1N).[25] The core domain consists of a β sandwich of two β sheets. The other two domains contain an extended α helix with three shorter α helices and two short strands of β sheet linked to an extended loop. These are stabilized by eight disulfide bonds conserved in TLPs from different plant species (Fig. 2G).

GRP

GRPs have been classified as members of the snakin/GASA family, which consist of plant antimicrobial peptides (Table 1).[26] GRPs are small molecular weight proteins (5–6 kDa) that contain disulfide bonds conserved in the snakin/GASA family consisting of stable proteins (Fig. 1O, 2H). Therefore, their physicochemical properties differ from those of the PR-10 and profilin molecules, which are easily degraded by digestive enzymes. GRPs of peach, Japanese apricot, and citrus fruits induce FAs.[8] Peach GRP (Pru p 7)

is associated with systemic symptoms of peach allergy in patients from France and other countries.[27–29] A cross-reactivity with a protein homologous to GRP present in cedar pollens probably causes fruit allergies related to GRP sensitization.[28–31]

Component-resolved diagnosis (CRD) for FA caused by plant sources

Skin prick tests and serum-sIgE antibody tests of crude extracts are routinely used for the diagnosis of FAs. Although these tests can identify foods to which the patient is sensitized, it is often difficult to determine whether the foods are causing the symptoms. Therefore, the measurement of sIgE antibody titers to allergen components has been termined.[1,32,33] This reduces the risk to the patient by providing an alternative to the oral food challenge test, which is associated with a risk of anaphylaxis. Furthermore, it helps to identify allergic symptoms due to cross-reactivity by determining the primary antigen. In allergic reactions caused by plant sources, there are allergen components for which measurement of sIgE antibody titers is useful for clinical diagnosis.[1,32,33] The recent reports are summarized in Table 2.

2S albumin

Peanut, sesame, and soybean

The clinical performance of 2S albumin in seeds of many plant species has been evaluated, and the efficacy of sIgE antibody titers against Ara h 2 (peanut),[32–34] Ses i 1 (sesame)[35,36] and Gly m 8 (soybean)[37–40] in clinical diagnosis has also been shown (Tables 1, 2, Fig. 1A).

Walnut

A high frequency of allergic reactions to walnut was reported in the Mediterranean region of Europe.[41] A survey of 177 patients with a probable walnut allergy, encompassing twelve European cities, was conducted to analyze Jug r 1–Jug r 7 sensitization profiles.[42] The distribution of sensitization to walnut components was similar to that of other tree nuts, with sensitization to Jug r 5 observed in northern and central Europe, Jug r 7 across Europe, and Jug r 3 in the Mediterranean region. Jug r 5 and Jug r 7 were involved in mild to moderate symptoms, but the storage proteins did not show a clear correlation with the severity of the condition (Table 1).[42] Conversely, in the study of walnut-allergic subjects from Switzerland and other countries, sIgE antibody titers to Jug r 1, Jug r 4, and vicilin fractions were associated with systemic reactions to walnut (allergic, n = 91; walnut tolerance, n = 24) (Table 1, 2).[43] In a retrospective study in the UK, Jug r 1 showed higher specificity (75.9%) and an area under the receiver operating characteristic curve (AUC) of (0.86) as a predictor of walnut allergy (n = 16, allergic; n = 29, tolerant) (Table 2).[44] Data collected in Japan indicated that a positive oral challenge test correlate with sensitization to Jug r 1 (AUC = 0.843) in patients with suspected walnut allergy (n = 108; allergic, n = 60, tolerant, n = 48) (Table 2).[45] In a report from Korea, sensitization to Jug r 1 was found in most patients allergic to walnut (31 out of 32 patients) (Table 2).[46] A report from Israel also showed that measuring Jug r 1 titer was useful in the diagnosis of walnut allergy (n = 76; allergic, n = 61, sensitized, n = 15) (AUC = 0.85) (Table 2).[47]

Hazelnut

Sensitization to Cor a 14 is correlated with severe allergic symptoms in pediatric patients with hazelnut allergy (18 out of 20 patients) (Table 1, 2).[48] In the Mediterranean region, severe allergic symptoms associated with sensitization to Cor a 8, and Cor a 1 correlated with oral symptoms associated with birch pollen sensitization in northern and central Europe.[49] The higher the severity of the allergic reactions, the higher is the sensitization to Cor a 9 and Cor a 14[49] (AUC for Cor a 9 and Cor a 14 = 0.87, and 0.80, respectively) (Table 2). An extensive study was conducted in Europe to predict the severity of symptoms in patients with hazelnut allergy. Analyses for Cor a 1, Cor a 2, Cor a 8, Cor a 9, Cor a 11, Cor a 12, and Cor a 14 showed that 21% of patients with severe symptoms were negative for all these components.[50] Sensitization to Cor a 9 and Cor a 14 was positively associated and Cor a 1 was negatively associated with severe symptoms during a double-blind placebo-controlled food challenges (Table 2).[50] An investigation carried out in the United States to assess the pattern of allergic sensitization to hazelnuts revealed that children (<3 years old) were mainly sensitized to Cor a 9 and Cor a 14 (Table 2).[51] Analysis of patients with multiple allergies showed that sIgE antibody titers for Cor a 14 were significantly higher in patients who were positive for double-blind placebo-controlled food challenges than in those who were negative, and that sIgE antibody titers did not substantially differ in response to other allergens.[52] Analysis of Japanese pediatric patients indicated that high sIgE antibody titers for Cor a 9 could improve the diagnostic accuracy for identifying hazelnut allergy (Table 2).[53]

Cashew

There are many reports pertaining to cashew allergy on the diagnostic performance of Ana o 3, which has a high ratio of single sensitization in patients with anaphylactic symptoms (Table 1, 2).[54–56] The sIgE antibody titer of Ana o 3 is useful in distinguishing between positive and negative results in oral food challenge tests in Japanese pediatric patients (allergic, n = 26; tolerant, n = 69) (AUC, 0.791) (Table 2).[55] Both cashew nuts and pistachios belong to the family Anacardiaceae. A retrospective, single-center study conducted in France indicated that sIgE antibody titers for Ana o 3 and the ratio of Ana o 3/total IgE shows better clinical performance in predicting pistachio allergy than sIgE antibody titers to pistachio (n = 25; allergic for pistachio, n = 8; sensitized-only, n = 17) (Table 2).[56]

Vicilin/Legumin

Peanut

In peanut-allergic patients in Iceland, half of the patients sensitized to peanuts were negative for Ara h 2, and the sIgE antibody titer for Ara h 1 contributed to the prediction of peanut allergy for these patients (Table 1, 2).[57] A characteristic motif containing cysteine residues (see section Vicilin) is present at the N-terminal extension region of Ara h 1 (Table 1, Fig. 1G).[12] A recombinant protein against a peptide derived from the N-terminal amino acid residues of Ara h 1 showed an sIgE antibody titer in 25 out of 55 patients with peanut allergy studied in the Netherlands (Table 2).[58]

Walnut and hazelnut

As described above, there are several reports highlighting the importance of vicilin and legumin for CRD. In the study from Switzerland and others, the sIgE antibody titer against Jug r 4, and vicilin fractions were associated with systemic reactions to walnut (Table 1, 2).[43] Sensitization to Cor a 9 is highly correlated with severe allergic symptoms (Table 2).[49–51]

Macadamia nut and almond

Vicilin-derived peptides with antimicrobial properties (Mac i 1), obtained from macadamia nuts (Table 1), reacted intensely with the sera of 29% (24/82) of nut-allergic patients tested, including three patients with macadamia nut allergy who showed systemic

Table 2
Clinical characteristics of plant-derived food allergen components from studies published between 2015 and 2021.

Biochemical name	Plant/Component	Result	Country	Ref
2S albumin	Peanut/Ara h 1, Ara h 3, Ara h 6	Sensitization to Ara h 1, Ara h 3, and Ara h 6 contributed to the prediction of peanut allergy in patients negative for Ara h 2	Iceland	57
	Sesame/Ses i 1	Ses i 1-sIgE titer was a more effective measure for sesame allergy diagnosis than skin prick test	Japan	35,36
	Soybean/Gly m 8	sIgE titer for Gly m 8 showed a high area under the curve (AUC) for discriminating between allergic and tolerant subjects	USA	39
		sIgE titer for Gly m 8 showed the highest AUC among 14 allergen components for discriminating between allergic and tolerant subjects	Japan	37,40
	Walnut/Jug r 1	Jug r 1 showed high specificity and AUC for the prediction of walnut allergy	UK	44
		Sensitization to Jug r 1 was associated with walnut allergy	Japan	45
		Measurement of sIgEs for Jug r 1 was benefical in diagnosing walnut allergy	Korea Republic	46
		Sensitization to Jug r 1 was found in most patients allergic to walnut	Israel	47
	Walnut/Jug r 1, Jug r 4, Vicilin fraction	Titers of sIgEs for Jug r 1, Jug r 4, and vicilin fractions were associated with systemic reactions to walnut	Switzerland and others	43
	Hazelnut/Cor a 14	Measurement of sIgEs for Cor a 14 was benefical in diagnosing hazelnut allergy in pediatric patients	Belgium	48
		Double-blind placebo-controlled food challenge-positive patients had significantly higher titers of IgE for Cor a 14 than challenge-negative patients	USA	51
	Hazelnut/Cor a 9, Cor a 14	Sensitization to Cor a 9 and Cor a 14 was highly correlated with severe allergic symptoms	Netherlands	49
		Sensitization to Cor a 9 and Cor a 14 was positively associated with severe symptoms	Netherlands and others	50
		Children aged <3 years were mainly sensitized to Cor a 14 together with Cor a 9	USA	51
	Cashew/Ana o 3	Single sensitization of patients with Ana o 3 was highly associated with anaphylactic symptoms	Poland	54
		Measurement of IgE against Ana o 3 was benefical in distinguishing between positive and negative results in oral food-challenge tests in children	Japan	55
		Measurement of sIgE against Ana o 3, and its ratio to total IgE levels, showed better clinical performance than that of sIgE to pistachio	France	56
	Buckwheat/Fag e 1, Fag e 2, Fag e 5	Concomitant sensitization to Fag e 1, Fag e 2, and Fag e 5 appeared to correlate with buckwheat allergy	Austria and others	61
Vicilin/Legumin	Peanut/Ara h 1, Ara h 3, Ara h 6	Sensitization to Ara h 1, Ara h 3, and Ara h 6 contributed to peanut allergy prediction in patients negative for Ara h 2	Iceland	57
	Peanut/Ara h 1 propeptide	The N-terminal peptide of Ara h 1 reacted intensely with sera of about half of patients with peanut allergy	Netherlands	58
	Walnut/Jug r 1, Jug r 4, Vicilin fraction	Levels of sIgEs against Jug r 1, Jug r 4, and vicilin fractions were associated with systemic reactions to walnut	Switzerland and others	43
	Hazelnut/Cor a 9, Cor a 14	Sensitization to Cor a 9 and Cor a 14 was highly correlated with severe allergic symptoms	Netherlands	49
		Sensitization to Cor a 9 with Cor a 14 was positively associated with severe allergic symptoms	Netherlands and others	50
		Children aged < 3 years were mainly sensitized to Cor a 14 together with Cor a 9	USA	51
	Hazelnut/Cor a 9	High sIgE levels for Cor a 9 improve diagnostic accuracy in distinguishing hazelnut allergy	Japan	53
	Macadamia/Mac i 1	Vicilin-like N-terminal peptides react intensely with sera of patients with nut allergy, including three macadamia-allergic patients that have systemic symptoms	Netherlands	59
	Almond/Pru du 8	Pru du 8 reacted with one-third of sera from patients with almond allergy	USA	60
	Buckwheat/Fag e 1, Fag e 2, Fag e 5	Concomitant sensitization to Fag e 1, Fag e 2, and Fag e 5 appeared to correlate with buckwheat allergy	Austria and others	61
	Buckwheat/Fag e 3	Levels of sIgE against Fag e 3 aided in predicting results from oral food-challenge testing	Japan	62
	Soybean/Gly m 5	A fusion protein composed of Gly m 8 and the N-terminal region of Gly m 5.0201 improved the diagnostic accuracy of soybean allergy in pediatric patients	Japan	40
ω-5 gliadin	Wheat/Tri a 19	Among wheat allergy components, sIgE levels to Tri a 19 correlated best to food challenge outcomes	Sweden	64
β-Amylase	Wheat/Tri a 17	Sensitization to Tri a 17 was associated with severe allergic reactions in sensitized patients	Austria	65
ns-LTP	Peach/Pru p 3	Sensitization to Pru p 3 was associated with systemic symptoms in central Europe	Austria	66
		Sensitization to several lipid transfer proteins was a risk factor for anaphylaxis	Spain	68

(*continued on next page*)

Table 2 (*continued*)

Biochemical name	Plant/Component	Result	Country	Ref
GRP	Peach/Pru p 7	Pru p 7 sensitization was associated with clinical severity	France	29
		Sensitization to Pru p 7 was a predominant cause of severe peach allergy and associated with sensitization to cypress pollen	Italy	70
		Sensitization to Pru p 7 was correlated with systemic symptoms and anaphylaxis	Japan	71
Bet v 1/PR-10/Profilin	Hazelnut/Cor a 1	Sensitization to Cor a 1 was negatively associated with severe symptoms during a double-blind placebo-controlled food challenge	Netherlands and others	50
	Hazelnut/Cor a 1	Measurement of sIgEs for Cor a 1 improved diagnostic accuracy in hazelnut allergy	Japan	53
	Peach/Pru p 1, Pru p 4	Sensitization to Pru p 1 and Pru p 4 was correlated with local symptoms	Japan	71

AUC, area under the curve.

symptoms (Table 2).[59] In an IgE immunoblotting-based assessment, a peptide derived from almond vicilin (Pru du 8) also reacted with the sera of 6 out of 18 patients with almond allergy, diagnosed by double-blind placebo-controlled food challenges (Table 2).[60]

Buckwheat

Allergen components were analyzed for subjects sensitized with buckwheat (n = 52; allergic, n = 11; sensitized, n = 41).[61] In this study, vicilin was reported as a new allergen, Fag e 5 (Table 1), and co-sensitization with vicilin, legumin, and 2S albumin has been reported to be useful in predicting buckwheat allergy (Table 2). Fag e 3, the putative N-terminal domain of vicilin, is also useful for predicting the diagnostic results of oral food challenge tests (n = 60; symptomatic, n = 20; asymptomatic, n = 40) (AUC = 0.893) (Table 2).[62]

Soybean

Gly m 5 is composed of three subunits, viz. Gly m 5.0101, Gly m 5.0201, and Gly m 5.0301 (Table 1). Gly m 5.0101 and Gly m 5.0201 are composed of an extension region and a core region (see the section Vicilin). The extension region is less likely to cause serological cross-reactivity due to its low sequence similarity among different plant species. Furthermore, I and collaborators showed that this region contains epitopes for many patients with soybean allergy.[40] Supposedly, developing an allergen component that fused the extension region of Gly m 5.0201 (high clinical sensitivity) to Gly m 8 (high clinical specificity) improved clinical performance (n = 91; symptomatic, n = 40; asymptomatic, n = 51; AUC = 0.801) (Table 2).

Wheat ω-5 gliadin and β-amylase

sIgE antibody titers to Tri a 19 have been frequently used in the diagnosis of wheat food-dependent exercise-induced anaphylaxis and FA (Table 1).[63,64] β-amylase (Tri a 17) was positive for 6 out of 8 patients who had a history of wheat-induced anaphylaxis (Table 1, 2).[65]

Peach ns-LTP

In the Mediterranean region, ns-LTPs are the major causes of plant-food allergies and are often associated with severe allergic reactions (Table 1).[22] In the analysis of IgE reactivity in an Austrian cohort, 10 out of 13 patients were sensitive to Pru p 3, suggesting that Pru p 3 sensitization is a risk factor for severe allergic symptoms in central Europe.[66] To know the sensitization pattern of ns-LTPs in the northern area, subjects (n = 35) born and residing in the United Kingdom, with prior diagnosis of ns-LTP allergy and sensitization to Pru p 3 were compared with those (n = 15) having PFAS by microarray immunoassay.[67] The tendency of sensitization to ns-LTPs and the other components in the United Kingdom cohort is similar to those of the Mediterranean a region. In a study conducted in patients positive for Pru p 3 (n = 421) in Spain, patients sensitized to five or more ns-LTPs (including Pru p 3, Jug r 3, Ara h 9, Cor a 8, and Art v 3) showed high frequency of systemic reactions (Table 2).[68]

Peach GRP

The distribution, clinical features, and molecular relevance of Pru p 7 sensitization among subjects suspected of peach allergy in different regions of France have been investigated (Table 1).[29] Sensitization to Pru p 7 was found in 171 (54%) of all subjects and 123 (62%) of 198 subjects diagnosed with peach allergy, more than half of whom were not sensitive to any other peach allergen (Table 2).[29] The cypress pollen allergen homologous to Pru p 7 may be the underlying cause of severe peach allergy.[69] However, among Italian patients allergic to cypress pollen, investigation for Pru p 7 sensitization revealed that only 2.8% of the patients were monosensitive to Pru p 7 (Table 2).[70] This suggests that the prevalence of allergy to Pru p 7 is low in Italy with regional differences in Pru p 7 sensitization. In Japan, in patients with systemic reactions, including patients who experienced anaphylaxis (n = 15), and oral or throat mucosal symptoms (n = 12), systemic symptoms and anaphylaxis correlated with sensitization to Pru p 7 (Table 2).[71]

Bet v 1/PR-10 and profilin

In Europe, sensitization to Cor a 1 was negatively associated with more severe symptoms during double-blind placebo-controlled food challenges (Table 1, 2).[50] Analysis of Japanese pediatric patients indicated that low sIgE antibody titers against Cor a 1 improve diagnostic accuracy in hazelnut allergy (Table 2).[53] A comparison between local and systemic reaction groups of patients with peach allergy in Japan revealed that sensitization to Pru p 1 and Pru p 4 was correlated with local symptoms in these patients (Table 2).[71]

Allergen component IgE epitopes

In general, epitopes of allergens that bind to IgE antibodies can be divided into linear epitopes formed by the primary structure (continuous amino acid residues) and conformational epitopes formed by a three-dimensional structure.[1] The search for epitopes correlated with clinical symptoms has been conducted for precise diagnosis.[2] There are many reports regarding the linear epitopes of plant food allergen components.[15,72] However, there are only a few reports on the identification of conformational epitopes, owing to limitations in the analytical methods used.[73] Herein, I introduce

details about conformational epitopes of both seed storage and PFAS-related proteins of plant-derived food allergen components.

Peanut 2S albumin

Because disulfide bonds are important for maintaining the conformation of 2S albumin (Fig. 1A, 2A), the disruption of the disulfide bonds by chemical reduction and alkylation change the conformation. The IgE-binding ability of alkylated Ara h 2 (Table 1) was greatly reduced compared with that of the native molecule.[74] Interestingly, when the sIgE antibody titer to epitopes in the peanut-allergic patient group was standardized, the frequency of identification of the linear epitope for Ara h 2 and Ara h 6 was inversely correlated with the severity of clinical symptoms.[75] Furthermore, the conformational epitopes of peanut 2S albumin were identified by screening by a phage display library containing 12 amino-acid random peptides using the EpiSearch tool (http://curie.utmb.edu/episearch.html) (Fig. 3A).[76] These findings suggest that the conformational epitope of 2S albumin may be useful in clinical diagnosis in the future.

Cashew and almond legumin

Legumin of seed storage proteins have conformational epitopes (Table 1, Fig. 1B–D).[77–79] Immunoblotting using a murine monoclonal antibody, chimeric molecule analysis, molecular modeling, and electron microscopy revealed that conformational epitopes of Ana o 2 reside on the acidic chain of legumins (Fig. 2B), depend on the association of basic chain, and are sensitive to denaturation.[77,78] Conformational epitope of Pru du 6 determined by using murine monoclonal antibody, and hydrogen deuterium exchange mass spectrometry comprises discontinuous strands with close proximity (Fig. 3B).[79]

Peach ns-LTP

The binding of ns-LTP to lipid partially affected its conformation (Fig. 1M, 2F) and the lipid binding changes the interaction of IgE antibodies with ns-LTP.[80,81] Art v 3, an ns-LTP of mugwort pollen, has been suggested to cross-react with Pru p 3. Art v 3 have conformational epitopes located in three different regions on the protein surface.[82,83] A comparison with a previously reported epitope of Pru p 3 revealed that the three-dimensional structural epitope of Art v 3 might be involved in cross-reactivity.

Bet v 1/PR-10

Conformational epitopes are important for allergen components belonging to the Bet v 1/PR-10 family related to PFAS (Table 1). The epitope for Bet v 1, one of the primary allergens causing PFAS, analyzed by a Fab fragment of a monoclonal murine IgG antibody was identified around the loop connected by two β strands.[84] Analysis of an artificial protein whose Bet v 1 molecular surface structure was partially replaced by that of the homologous protein celery Api g 1 (Table 1) revealed the presence of several epitopes all over the molecular surface.[85] Epitopes have also been reported for Bet v 1/PR-10 molecules from the seeds. Analysis using engineered recombinant variants of a non-allergenic Bet v 1-type model protein indicated that six IgE-binding regions accounted for more than 80% of the total IgE-binding capacity of Gly m 4 (Fig. 1K, 3C).[86]

Immunotherapy using recombinant allergen components

Allergen immunotherapy (AIT) is an important therapeutic modality for allergic diseases.[87] Although immunotherapy has been performed using foods and extracts, this method has the risk of inducing allergic symptoms, and may not be effective when the content of the allergen to be treated is low. Vaccines and therapeutic agents with reduced risk are being developed by modifying B-cell epitopes and preserving T-cell epitopes using recombinant proteins of allergen components.[88] Disrupting disulfide bonds, modifying conformational epitopes, and designing chimeric proteins can help optimizing the immunogenicity of allergen components for effective AIT.[89] Herein, I describe plant-derived food allergen components designed for AIT.

Peanut 2S albumin

For peanut allergy, molecules with reduced IgE antibody binding ability, such as 2S albumin (Table 1), have been designed, and their potential as immunotherapeutic agents has been reported. To maintain the desensitizing potential, allergen-modified mutants with conserved T-cell stimulation capacity and reduced IgE binding were generated for AIT.[90] Most of the known linear epitopes are located in the flexible loop of Ara h 2, whereas T-cell epitopes have been identified mainly in the α helix (Fig. 1A). An important point for designing the molecules is that the loop was modified, but the disulfide bonds, which affect the higher-order structure, were not

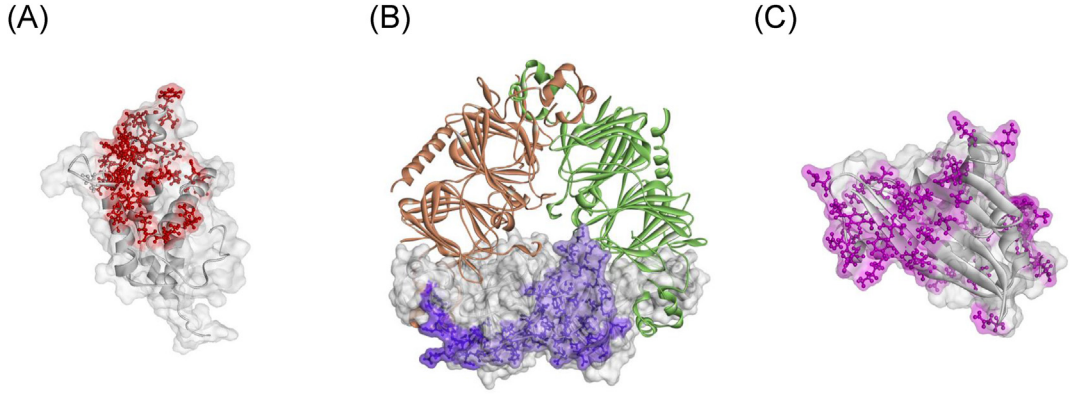

Fig. 3. Conformational epitopes of plant-derived allergen components. **(A)** Peanut 2S albumin (Ara h 2) as reported by Chen *et al.*[76] Residues related to a conformational epitope are shown as ball and stick models. **(B)** Almond legumin (Pru du 6) as reported by Willison *et al.*[79] **(C)** Soybean PR-10 (Gly m 4) as reported by Husslik *et al.*[86] Residues related to conformational epitopes are shown using ball and stick models. Molecular surfaces composed by conformational epitopes are depicted in red (A), blue (B) and pink (C). The models of the other two monomers which form a trimer are included in (B).

altered. In another report, a hypoallergenic Ara h 2 mutant was designed by reduction and alkylation to remove conformational epitopes, and by modification to remove linear epitopes.[91] This molecule abolished the binding of IgE, and, as expected, showed no anaphylaxis in mice, but induced T-cell proliferation. The administered amount of 16.3 μg for a mouse almost corresponds to 56 mg in a human weighing 65 kg. This amount is lower than the maximum doses used for peanut oral immunotherapy (estimated amount of Ara h 2 is 400 mg). Additionally, the effects of peanut allergens (Ara h 1 and Ara h 2) (Table 1) conjugated with plant viruses as potential vaccines for immunotherapy were examined in mice; they were highly safe and induced specific IgG antibodies and reduced local reactions after skin-prick tests.[92]

Peach ns-LTP

Various attempts have been made to design Pru p 3 for AIT (Table 1). In patients with systemic allergic reactions to peaches and/or peanuts, peach and peanut desensitization and immunological changes were evaluated after one year of Pru p 3 sublingual immunotherapy.[93] Both desensitization and immunological changes were induced for peach as well as for other food allergens, such as peanut, which are associated with the induction of severe reactions.[93] The effects of subcutaneous immunotherapy with reduced-alkylated and wild-type Pru p 3 were tested in mice.[94] Wild-type Pru p 3 was highly effective compared with reduced-alkylated Pru p 3; low allergenicity and structural instability of the reduced-alkylated Pru p 3 may be contributing factors. In addition, using oligonucleotides adjuvanted with the T-cell epitope peptide of Pru p 3 resulted in Treg induction in mice, without an increase in Pru p 3-specific IgE levels.[95]

Bet v 1/PR-10

In the studies on the AIT against Bet v 1/PR-10 molecules (Table 1), 16-week sublingual immunotherapy using recombinant Mal d 1 (25 μg/day, n = 20), but not recombinant Bet v 1 (25 μg/day, n = 20), remarkably improved birch pollen-related apple allergy in Austrian patients.[96,97] Allergen-specific IgE-blocking IgG antibodies were associated with clinical efficacy.[96,97] In a one-year AIT using a folding variant of recombinant Bet v 1 against birch-related soybean allergy, a hypoallergenic variant of Bet v 1 was effective, although the data were not significant.[98] Furthermore, IgG antibodies induced by a sublingual immunotherapy of Bet v 1 showed cross-blocking activity on many related allergens of Bet v 1/PR-10 family.[99]

Concluding remarks

Knowledge of the correlation between allergen components and clinical symptoms has become increasingly important in the routine diagnosis and treatment of FAs. Research on designed and engineered allergen components using information related to three-dimensional structures and epitopes in plant-related FAs will be useful for diagnosis and immunotherapy. The application of novel technologies in FAs is expected to lead to the development of more precise diagnostic and therapeutic methods.

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Conflict of interest

The author has no conflict of interest to declare.

References

1. Ebisawa M, Ito K, Fujisawa T. Japanese guidelines for food allergy 2020. *Allergol Int* 2020;**69**:370–86.
2. Foong R-X, Santos AF. Biomarkers of diagnosis and resolution of food allergy. *Pediatr Allergy Immunol* 2021;**32**:223–33.
3. McWilliam VL, Perrett KP, Dang T, Peters RL. Prevalence and natural history of tree nut allergy. *Ann Allergy Asthma Immunol* 2020;**124**:466–72.
4. Radauer C, Breiteneder H. Evolutionary biology of plant food allergens. *J Allergy Clin Immunol* 2007;**120**:518–25.
5. Schwager C, Kull S, Behrends J, Röckendorf N, Schocker F, Frey A, et al. Peanut oleosins associated with severe peanut allergy-importance of lipophilic allergens for comprehensive allergy diagnostics. *J Allergy Clin Immunol* 2017;**140**:1331–8. e8.
6. Bublin M, Breiteneder H. Cross-reactivity of peanut allergens. *Curr Allergy Asthma Rep* 2014;**14**:426.
7. Breiteneder H. Thaumatin-like proteins-a new family of pollen and fruit allergens. *Allergy* 2004;**59**:479–81.
8. Inomata N. Gibberellin-regulated protein allergy: clinical features and cross-reactivity. *Allergol Int* 2020;**69**:11–8.
9. Moreno FJ, Clemente A. 2S albumin storage proteins: what makes them food allergens? *Open Biochem J* 2008;**2**:16–28.
10. Smeekens JM, Bagley K, Kulis M. Tree nut allergies: allergen homology, cross-reactivity, and implications for therapy. *Clin Exp Allergy* 2018;**48**:762–72.
11. Rasheed F, Markgren J, Hedenqvist M, Johansson E. Modeling to understand plant protein structure-function relationships-implications for seed storage proteins. *Molecules* 2020;**25**:873.
12. Zhang J, Payne CD, Pouvreau B, Schaefer H, Fisher MF, Taylor NL, et al. An ancient peptide family buried within vicilin precursors. *ACS Chem Biol* 2019;**14**:979–93.
13. Huang AHC. Plant lipid droplets and their associated proteins: potential for rapid advances. *Plant Physiol* 2018;**176**:1894–918.
14. Tatham AS, Shewry PR. Allergens to wheat and related cereals. *Clin Exp Allergy* 2008;**38**:1712–26.
15. Matsuo H, Yokooji T, Taogoshi T. Common food allergens and their IgE-binding epitopes. *Allergol Int* 2015;**64**:332–43.
16. Sander I, Rihs H-P, Brüning T, Raulf M. A further wheat allergen for baker's asthma: Tri a 40. *J Allergy Clin Immunol* 2016;**137**:1286.
17. Carlson G, Coop C. Pollen food allergy syndrome (PFAS): a review of current available literature. *Ann Allergy Asthma Immunol* 2019;**123**:359–65.
18. Fernandes H, Michalska K, Sikorski M, Jaskolski M. Structural and functional aspects of PR-10 proteins. *FEBS J* 2013;**280**:1169–99.
19. Fukutomi Y, Sjölander S, Nakazawa T, Borres MP, Ishii T, Nakayama S, et al. Clinical relevance of IgE to recombinant Gly m 4 in the diagnosis of adult soybean allergy. *J Allergy Clin Immunol* 2012;**129**:860–3.
20. Rodríguez Del Río P, Díaz-Perales A, Sánchez-García S, Escudero C, Ibáñez MD, Méndez-Brea P, et al. Profilin, a change in the paradigm. *J Investig Allergol Clin Immunol* 2018;**28**:1–12.
21. Kapingidza AB, Pye SE, Hyduke N, Dolamore C, Pote S, Schlachter CR, et al. Comparative structural and thermal stability studies of Cuc m 2.0101, Art v 4.0101 and other allergenic profilins. *Mol Immunol* 2019;**114**:19–29.
22. Asero R, Piantanida M, Pinter E, Pravettoni V. The clinical relevance of lipid transfer protein. *Clin Exp Allergy* 2018;**48**:6–12.
23. Deng S, Yin J. Mugwort pollen-related food allergy: lipid transfer protein sensitization and correlation with the severity of allergic reactions in a Chinese population. *Allergy Asthma Immunol Res* 2019;**11**:116–28.
24. Pasquato N, Berni R, Folli C, Folloni S, Cianci M, Pantano S, et al. Crystal structure of peach Pru p 3, the prototypic member of the family of plant non-specific lipid transfer protein pan-allergens. *J Mol Biol* 2006;**356**:684–94.
25. Leone P, Menu-Bouaouiche L, Peumans WJ, Payan F, Barre A, Roussel A, et al. Resolution of the structure of the allergenic and antifungal banana fruit thaumatin-like protein at 1.7 **Å**. *Biochimie* 2006;**88**:45–52.
26. Oliveira-Lima M, Benko-Iseppon AM, Ribamar Costa Ferreira Neto J, Rodriguez-Decuadro S, Kido EA, Crovella S, et al. Snakin: structure, roles and applications of a plant antimicrobial peptide. *Curr Protein Pept Sci* 2017;**18**:368–74.
27. Tuppo L, Alessandri C, Pomponi D, Picone D, Tamburrini M, Ferrara R, et al. Peamaclein-a new peach allergenic protein: similarities, differences and misleading features compared to Pru p 3. *Clin Exp Allergy* 2013;**43**:128–40.
28. Sénéchal H, Šantrůček J, Melčová M, Svoboda P, Zídková J, Charpin D. A new allergen family involved in pollen food-associated syndrome: snakin/gibberellin-regulated proteins. *J Allergy Clin Immunol* 2018;**141**:411–4.
29. Sénéchal H, Keykhosravi S, Couderc R, Selva MA, Shahali Y, Aizawa T, et al. Pollen/Fruit syndrome: clinical relevance of the cypress pollen allergenic gibberellin-regulated. *Allergy Asthma Immunol Res* 2019;**11**:143–51.
30. Ehrenberg AE, Klingebiel C, Östling J, Larsson H, Mattsson L, Vitte J, et al. Characterization of a 7 kDa pollen allergen belonging to the gibberellin-

regulated protein family from three Cupressaceae species. *Clin Exp Allergy* 2020;**50**:964–72.
31. Iizuka T, Takei M, Saito Y, Rumi F, Zheng J, Lu X, et al. Gibberellin-regulated protein sensitization in Japanese cedar (Cryptomeria japonica) pollen allergic Japanese cohorts. *Allergy* 2021. https://doi.org/10.1111/all.14816.
32. Matricardi PM, Kleine-Tebbe J, Hoffmann HJ, Valenta R, Hilger C, Hofmaier S, et al. EAACI molecular allergology user's guide. *Pediatr Allergy Immunol* 2016;**27**:1–250.
33. Borres MP, Maruyama N, Sato S, Ebisawa M. Recent advances in component resolved diagnosis in food allergy. *Allergol Int* 2016;**65**:378–87.
34. Ebisawa M, Movérare R, Sato S, Borres MP, Ito K. The predictive relationship between peanut- and Ara h 2-specific serum IgE concentrations and peanut allergy. *J Allergy Clin Immunol Pract* 2015;**3**:131–2.e1.
35. Maruyama N, Nakagawa T, Ito K, Cabanos C, Borres MP, Movérare R, et al. Measurement of specific IgE antibodies to Ses i 1 improves the diagnosis of sesame allergy. *Clin Exp Allergy* 2016;**46**:163–71.
36. Yanagida N, Ejiri Y, Takeishi D, Sato S, Maruyama N, Takahashi K, et al. Ses i 1-specific IgE and sesame oral food challenge results. *J Allergy Clin Immunol Pract* 2019;**7**:2084–6.
37. Ebisawa M, Brostedt P, Sjölander S, Sato S, Borres MP, Ito K. Gly m 2S albumin is a major allergen with a high diagnostic value in soybean-allergic children. *J Allergy Clin Immunol* 2013;**132**:976–8.
38. Klemans RJ, Knol EF, Michelsen-Huisman A, Pasmans SG, de Kruijf-Broekman W, Bruijnzeel-Koomen CA, et al. Components in soy allergy diagnostics: Gly m 2S albumin has the best diagnostic value in adults. *Allergy* 2013;**68**:1396–402.
39. Kattan JD, Sampson HA. Clinical reactivity to soy is best identified by component testing to Gly m 8. *J Allergy Clin Immunol Pract* 2015;**3**:970–2.
40. Maruyama N, Sato S, Cabanos C, Tanaka A, Ito K, Ebisawa M. Gly m 5/Gly m 8 fusion component as a potential novel candidate molecule for diagnosing soya bean allergy in Japanese children. *Clin Exp Allergy* 2018;**48**:1726–34.
41. Lyons SA, Clausen M, Knulst AC, Ballmer-Weber BK, Fernandez-Rivas M, Barreales L, et al. Prevalence of food sensitization and food allergy in children across Europe. *J Allergy Clin Immunol Pract* 2020;**8**:2736–46.
42. Lyons SA, Datema MR, Le T, Asero R, Barreales L, Belohlavkova S, et al. Walnut allergy across Europe: distribution of allergen sensitization patterns and prediction of severity. *J Allergy Clin Immunol Pract* 2021;**9**:225–35.
43. Ballmer-Weber BK, Lidholm J, Lange L, Pascal M, Lang C, Gernert S, et al. Allergen recognition patterns in walnut allergy are age dependent and correlate with the severity of allergic reactions. *J Allergy Clin Immunol Pract* 2019;**7**:1560–7.
44. Mew R, Borres M, Sjölander S, du Toit G. A retrospect study into the utility of allergen components in walnut allergy. *Pediatr Allergy Immunol* 2016;**27**:750–2.
45. Sato S, Yamamoto M, Yanagida N, Ito K, Ohya Y, Imai T, et al. Jug r 1 sensitization is important in walnut-allergic children and youth. *J Allergy Clin Immunol Pract* 2017;**5**:1784–6.
46. Lee J, Jeong K, Jeon S, Lee S. Component resolved diagnosis of walnut allergy in young children: Jug r 1 as a major walnut allergen. *Asian Pac J Allergy Immunol* 2019. https://doi.org/10.12932/AP-161118-0443.
47. Elizur A, Appel MY, Nachshon L, Levy MB, Epstein-Rigbi N, Pontoppidan B, et al. Clinical and molecular characterization of walnut and pecan allergy (NUT CRACKER Study). *J Allergy Clin Immunol Pract* 2020;**8**:157–65.
48. Faber M, Graag MD, Heijden CVD, Sabato V, Hagendorens MM, Bridts CH, et al. Cor a 14: missing link in the molecular diagnosis of hazelnut allergy? *Int Arch Allergy Immunol* 2014;**164**:200–6.
49. Masthoff LJN, Mattsson L, Zuidmeer-Jongejan L, Lidholm J, Andersson K, Akkerdaas JH, et al. Sensitization to Cor a 9 and Cor a 14 is highly specific for a hazelnut allergy with objective symptoms in Dutch children and adults. *J Allergy Clin Immunol* 2013;**132**:393–9.
50. Datema MR, van Ree R, Asero R, Barreales L, Belohlavkova S, de Blay F, et al. Component-resolved diagnosis and beyond: multivariable regression models to predict severity of hazelnut allergy. *Allergy* 2018;**73**:549–59.
51. Valcour A, Lidholm J, Borres MP, Hamilton RG. Sensitization profiles to hazelnut allergens across the United States. *Ann Allergy Asthma Immunol* 2019;**122**:111–6.
52. Andorf S, Borres MP, Block W, Tupa D, Bollyky JB, Sampath V, et al. Association of clinical reactivity with sensitization to allergen components in multi-food allergic children. *J Allergy Clin Immunol Pract* 2017;**5**:1325–34.
53. Inoue Y, Sato S, Takahashi K, Yanagida N, Yamamoto H, Shimizu N, et al. Component-resolved diagnostics can be useful for identifying hazelnut allergy in Japanese children. *Allergol Int* 2020;**69**:239–45.
54. Blazowski L, Majak P, Kurzawa R, Kuna P, Jerzynska J. Food allergy endotype with high risk of severe anaphylaxis in children-monosensitization to cashew 2S albumin Ana o 3. *Allergy* 2019;**74**:1945–55.
55. Sato S, Movérare R, Ohya Y, Ito K, Nagao M, Borres MP. Ana o 3-specific IgE is a predictive marker for cashew oral food challenge failure. *J Allergy Clin Immunol Pract* 2019;**7**:2909–11.
56. Amat F, Benharoun-Stern R, Benissa M, Just J. Usefulness of r Ana o 3 assessment before oral food challenge to pistachio. *Pediatr Allergy Immunol* 2021;**32**:615–8.
57. Magnusdottir H, Vidarsdóttir AG, Ludviksson BR, Clausen M, Lund SH, Jensen AB, et al. Ara h 1 and Ara h 6 sensitization causes clinical peanut allergy in Ara h 2-negative individuals. *Int Arch Allergy Immunol* 2019;**178**:66–75.
58. Aalberse RC, Mueller GA, Derksen NIL, Aalberse JA, Edwards LL, Pomés A, et al. Identification of the amino-terminal fragment of Ara h 1 as a major target of the IgE-binding activity in the basic peanut protein fraction. *Clin Exp Allergy* 2020;**50**:401–5.
59. Ehlers AM, Rohwer S, Otten HG, Brix B, Le T, Suer W, et al. IgE-binding to vicilin-like antimicrobial peptides is associated with systemic reactions to macadamia nut. *Clin Transl Allergy* 2020;**10**:55.
60. Che H, Zhang Y, Jiang S, Jin T, Lyu S, Nadeau KC, et al. Almond (*Prunus dulcis*) allergen Pru du 8, the first member of a new family of food allergens. *J Agric Food Chem* 2019;**67**:8626–31.
61. Geiselhart S, Nagl C, Dubiela P, Pedersen AC, Bublin M, Radauer C, et al. Concomitant sensitization to legumin, Fag e 2 and Fag e 5 predicts buckwheat allergy. *Clin Exp Allergy* 2018;**48**:217–24.
62. Yanagida N, Sato S, Maruyama N, Takahashi K, Nagakura K, Ogura K, et al. Specific IgE for Fag e 3 predicts oral buckwheat food challenge test results and anaphylaxis: a pilot study. *Int Arch Allergy Immunol* 2018;**176**:8–14.
63. Graham F, Caubet JC, Ramadan S, Spoerl D, Eigenmann PA. Specific IgE decision point cutoffs in children with IgE-mediated wheat allergy and a review of the literature. *Int Arch Allergy Immunol* 2020;**181**:296–300.
64. Nilsson N, Sjölander S, Baar A, Berthold M, Pahr S, Vrtala S, et al. Wheat allergy in children evaluated with challenge and IgE antibodies to wheat components. *Pediatr Allergy Immunol* 2015;**26**:119–25.
65. Hofer G, Wieser S, Bogdos MK, Gattinger P, Nakamura R, Ebisawa M, et al. Three-dimensional structure of the wheat β-amylase Tri a 17, a clinically relevant food allergen. *Allergy* 2019;**74**:1009–13.
66. Mothes-Luksch N, Raith M, Stingl G, Focke-Tejkl M, Razzazi-Fazeli E, Zieglmayer R, et al. Pru p 3, a marker allergen for lipid transfer protein sensitization also in central Europe. *Allergy* 2017;**72**:1415–8.
67. Skypala IJ, Cecchi L, Shamji MH, Scala E, Till S. Lipid transfer protein allergy in the United Kingdom: characterization and comparison with a matched Italian cohort. *Allergy* 2019;**74**:1340–51.
68. Ruano-Zaragoza M, Somoza ML, Jiménez-Rodriguez TW, Soriano-Gomis V, Delgado PG, Esteban-Rodriguez A, et al. Lipid transfer protein sensitization: risk of anaphylaxis and molecular sensitization profile in Pru p 3-sensitized patients. *Int Arch Allergy Immunol* 2021;**182**:425–32.
69. Klingebiel C, Chantran Y, Arif-Lusson R, Ehrenberg AE, Östling J, Poisson A, et al. Pru p 7 sensitization is a predominant cause of severe, cypress pollen-associated peach allergy. *Clin Exp Allergy* 2019;**49**:526–36.
70. Asero R, Abbadessa S, Aruanno A, Barilaro G, Barzaghi C, Bignardi D, et al. Detection of gibberellin-regulated protein (Peamaclein) sensitization among Italian cypress pollen-sensitized patients. *J Investig Allergol Clin Immunol* 2020. https://doi.org/10.18176/jiaci.0542.
71. Ando Y, Miyamoto M, Kato M, Nakayama M, Fukuda H, Yoshihara S. Pru p 7 predicts severe reactions after ingestion of peach in Japanese children and adolescents. *Int Arch Allergy Immunol* 2020;**181**:183–90.
72. Liu C, Sathe SK. Food allergen epitope mapping. *J Agric Food Chem* 2018;**66**:7238–48.
73. Breiteneder H. Mapping of conformational IgE epitopes of food allergens. *Allergy* 2018;**73**:2107–9.
74. Apostolovic D, Luykx D, Warmenhoven H, Verbart D, Stanic-Vucinic D, de Jong GAH, et al. Reduction and alkylation of peanut allergen isoforms Ara h 2 and Ara h 6; characterization of intermediate- and end products. *Biochim Biophys Acta* 2013;**1834**:2832–42.
75. Otsu K, Guo R, Dreskin SC. Epitope analysis of Ara h 2 and Ara h 6: characteristic patterns of IgE-binding fingerprints among individuals with similar clinical histories. *Clin Exp Allergy* 2015;**45**:471–84.
76. Chen X, Negi SS, Liao S, Gao V, Braun W, Dreskin SC. Conformational IgE epitopes of peanut allergens Ara h 2 and Ara h 6. *Clin Exp Allergy* 2016;**46**:1120–8.
77. Xia L, Willison LN, Porter L, Robotham JM, Teuber SS, Sathe SK, et al. Mapping of a conformational epitope on the cashew allergen Ana o 2: a discontinuous large subunit epitope dependent upon homologous or heterologous small subunit association. *Mol Immunol* 2010;**47**:1808–16.
78. Robotham JM, Xia L, Willison LN, Teuber SS, Sathe SK, Roux KH. Characterization of a cashew allergen, 11S globulin (Ana o 2), conformational epitope. *Mol Immunol* 2010;**47**:1830–8.
79. Willison LN, Zhang Q, Su M, Teuber SS, Sathe SK, Roux KH. Conformational epitope mapping of Pru du 6, a major allergen from almond nut. *Mol Immunol* 2013;**55**:253–63.
80. Dubiela P, Aina R, Polak D, Geiselhart S, Humeniuk P, Bohle B, et al. Enhanced Pru p 3 IgE-binding activity by selective free fatty acid-interaction. *J Allergy Clin Immunol* 2017;**140**:1728–31.
81. Aina R, Dubiela P, Geiselhart S, Bublin M, Bruschi M, Radauer C, et al. Distinct lipid transfer proteins display different IgE-binding activities that are affected by fatty acid binding. *Allergy* 2019;**74**:827–31.
82. Wildner S, Griessner I, Stemeseder T, Regl C, Soh WT, Stock LG, et al. Boiling down the cysteine-stabilized LTP fold-loss of structural and immunological integrity of allergenic Art v 3 and Pru p 3 as a consequence of irreversible lanthionine formation. *Mol Immunol* 2019;**116**:140–50.
83. Muzio MD, Wildner S, Huber S, Hauser M, Vejvar E, Auzinger W, et al. Hydrogen/deuterium exchange memory NMR reveals structural epitopes involved in IgE cross-reactivity of allergenic lipid transfer proteins. *J Biol Chem* 2020;**295**:17398–410.
84. Mirza O, Henriksen A, Ipsen H, Larsen JN, Wissenbach M, Spangfort MD, et al. Dominant epitopes and allergic cross-reactivity: complex formation between a Fab fragment of a monoclonal murine IgG antibody and the major allergen from birch pollen Bet v 1. *J Immunol* 2000;**165**:331–8.

85. Gepp B, Lengger N, Bublin M, Hemmer W, Breiteneder H, Radauer C, et al. Chimeras of Bet v 1 and Api g 1 reveal heterogeneous IgE responses in patients with birch pollen allergy. *J Allergy Clin Immunol* 2014;**134**:188–94.
86. Husslik F, Nürnberg J, von Loetzen CS, Mews T, Ballmer-Weber BK, Kleine-Tebbe J, et al. The conformational IgE epitope profile of soya bean allergen Gly m 4. *Clin Exp Allergy* 2016;**46**:1484–97.
87. Pajno GB, Passanisi S, Valenzise M, Messina MF, Lombardo F. The evolution of allergen-specific immunotherapy: the near and far future. *Pediatr Allergy Immunol* 2020;**31**:11–3.
88. Tulaeva I, Kratzer B, Campana1 R, Curin1 M, van Hage M, Karsonova A, et al. Preventive allergen-specific vaccination against allergy: mission possible? *Front Immunol* 2020;**11**:1368.
89. Tscheppe A, Breiteneder H. Recombinant allergens in structural biology, diagnosis, and immunotherapy. *Int Arch Allergy Immunol* 2017;**172**:187–202.
90. Bublin M, Kostadinova M, Radauer C, Varga E, Hafner C, Schmidthaler K, et al. Engineering of structural variants of the major peanut allergens Ara h 2 and Ara h 6 for allergen-specific immunotherapy. *J Allergy Clin Immunol* 2019;**143**:1226–9.
91. Tscheppe A, Palmberger D, van Rijt L, Kalic T, Mayr V, Palladino C, et al. Development of a novel Ara h 2 hypoallergen with no IgE binding or anaphylactogenic activity. *J Allergy Clin Immunol* 2020;**145**:229–38.
92. Storni F, Zeltins A, Balke I, Heath MD, Kramer MF, Skinner MA, et al. Vaccine against peanut allergy based on engineered virus-like particles displaying single major peanut allergens. *J Allergy Clin Immunol* 2020;**145**:1240–53.
93. Gomez F, Bogas G, Gonzalez M, Campo P, Salas M, Diaz-Perales A, et al. The clinical and immunological effects of Pru p 3 sublingual immunotherapy on peach and peanut allergy in patients with systemic reactions. *Clin Exp Allergy* 2017;**47**:339–50.
94. Rodriguez MJ, Wangorsch A, Gomez F, Schülke S, Torres MJ, Vieths S, et al. Immunotherapy with native molecule rather than hypoallergenic variant of Pru p 3, the major peach allergen, shows beneficial effects in mice. *J Immunol Res* 2018;**2018**:3479185.
95. Rodriguez MJ, Mascaraque A, Ramos-Soriano J, Torres MJ, Perkins JR, Gomez F, et al. Pru p 3-epitope-based sublingual immunotherapy in a murine model for the treatment of peach allergy. *Mol Nutr Food Res* 2017;**61**. https://doi.org/10.1002/mnfr.201700110.
96. Kinaciyan T, Nagl B, Faustmann S, Frommlet F, Kopp S, Wolkersdorfer M, et al. Efficacy and safety of 4 months of sublingual immunotherapy with recombinant Mal d 1 and Bet v 1 in patients with birch pollen-related apple allergy. *J Allergy Clin Immunol* 2018;**141**:1002–8.
97. Acosta GS, Kinaciyan T, Kitzmüller C, Möbs C, Pfützner W, Bohle B. IgE-blocking antibodies following SLIT with recombinant Mal d 1 accord with improved apple allergy. *J Allergy Clin Immunol* 2020;**146**:894–900.
98. Aglas L, Bethanis A, Chrusciel P, Stolz F, Gruen M, Jaakkola U-M, et al. In vivo induction of functional Inhibitory IgG antibodies by a hypoallergenic Bet v 1 variant. *Front Immunol* 2020;**11**:2118.
99. Grilo JR, Kitzmüller C, Aglas L, Acosta GS, Vollmann U, Ebner C, et al. IgE-cross-blocking antibodies to Fagales following sublingual immunotherapy with recombinant Bet v 1. *Allergy* 2021. https://doi.org/10.1111/all.14817.