Food Allergy

Molecular and Clinical Practice

Andreas L. Lopata (*ed.*)

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Editor

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Preface

Allergy-related diseases are today recognized as reaching epidemic proportions, with up to 30% of the general population suffering from clinical symptoms ranging from urticaria, rhinitis and asthma to life-threatening anaphylactic reactions.

The main contributors to the increasing prevalence of allergy seem to be very diverse including increasing immunological predisposition ('atopy'), changing food consumption and well as living conditions. The dramatic increase of allergic diseases is not only seen in the developed world, but increasing evidence indicates that also developing countries are considerably affected. Already over fifty percent of the world population is living in Asia, where not only food consumption, but also food allergies are very different from what is mainly published from Western countries. In the research efforts in the field of food allergy two main questions are often asked: What makes one person allergic to a particular food and not the other? Furthermore, Why are some foods and food proteins more allergenic than others? In addition it is very difficult to predict the severity of clinical reaction and the amount of allergen required to elicit these reactions.

Major food allergens from a small number of sources were identified and purified as early as the 1970s. A boost in the number of newly identified allergens was elicited by the general availability of recombinant DNA technology in the late 1980s. The ever-growing IUIS Allergen Nomenclature Database contains currently over 840 allergens from 252 sources and their isoforms and variants. Currently we know about 290 food allergens from 98 different food sources.

Recent developments into the molecular nature of allergenic proteins enabled us to classify most allergens into few protein families with limited biochemical function. Allergenic proteins can be classified into approximately 130 Pfam protein families, while the most important plant and animal food allergens can be found in 8 protein superfamilies and is discussed in detail in Chapters 1 and 2.

The correct diagnosis of a food allergy can be complex, but includes a convincing clinical history as well as the presence of elevated levels of specific IgE antibody to allergenic proteins in a given food. Therefore, detailed knowledge about the food specific allergenic proteins is central to a specific and sensitive diagnostic approach. The different allergens of peanut, egg, fish, shellfish and food contamination parasites and their diagnostic application are detailed in Chapters 3 to 7.

The food industry is one of the largest employers of workers with about 10% and therefore is the allergic sensitisation to food borne proteins at the workplace not surprising. Workers at increased risk of allergic sensitisation include farmers who grow and harvest crops; factory workers involved in food processing, storage and packing; as well as those involved in food preparation (chefs and waiters) and transport and is detailed in Chapter 8.

Research in food allergies and allergens is much more complex than investigating inhalant allergens since food proteins often undergo extensive modifications during food processing. Furthermore these allergenic proteins are embedded in a complex matrix and may undergo physicochemical changes during digestion and subsequent uptake by the gut mucosal barrier and presentation to the immune system, and have been highlighted in Chapter 9.

Furthermore, food processing results often in water-insoluble proteins, which makes the traditional serological analysis of allergenicity difficult as well as detection and quantification in the food matrix. The approaches and problems of quantifying allergen residues in processed food are detailed in Chapter 10.

To characterize allergens better but also develop better diagnostic and therapeutics, recombinant allergens are increasingly utilized.

Unlike natural allergens or allergen extracts, the production of recombinant proteins is not dependent on biological source material composed of complex mixtures of allergen isoforms. The use of recombinant allergens has revolutionized diagnosis, enabling clinicians to identify disease eliciting allergens as well as cross-reactivity pattern, thereby providing us with the tools necessary for personalized allergy medicine and therapeutics and is detailed in Chapter 11.

Food allergy is a growing problem globally carrying a huge socioeconomic burden for patients, families and the community. Although fatalities are fortunately rare, the fear of death is very real for each patient. Currently, there is no cure for any food allergy available, with management strategies focusing on complete avoidance and utilization of adrenaline as the emergency antidote for anaphylaxis. There is a very strong imperative for safe and effective specific therapeutics for food allergy and one strategy based on T-cell epitopes for peanut allergy is detailed in Chapter 12.

We hope that the joined effort by the authors will not only provide pragmatic information for current food allergy research but also serves as a foundation for significant new research that will advance our current knowledge.

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Biomolecular and Clinical Aspects of Food Allergy

Heimo Breiteneder

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1.1 INTRODUCTION

Allergenic proteins are able to elicit Th2-polarized immune responses in predisposed individuals. As compared to the presently known number of protein architectures, allergenic proteins can be classified into a highly limited number of protein families (Radauer et al. 2008a). Version 30.0 of the protein family database Pfam (http://pfam.xfam.org/) describes 16,306 protein families (Finn et al. 2014). The structural database of allergenic proteins (SDAP; http://fermi.utmb.edu/) (Ivanciuc et al. 2003) assigns all allergens to 130 Pfam families. The most important plant and animal food allergens can be found in eight protein superfamilies discussed below. Our understanding why exactly these proteins are able to induce a specific IgE response in certain individuals is still incomplete. Allergenic proteins seem to be able to modulate the communication between innate and adaptive immune cells by interacting with pattern recognition receptors, which results in a Th2 polarization of the adaptive immune response (Karp 2010, Platts-Mills and Woodfolk 2011, Pulendran et al. 2010, Ruiter and Shreffler 2012, Willart and Hammad 2010, Wills-Karp 2010). Recent discoveries have shown that group 2 innate lymphoid cells are able to translate epithelial cell-derived alarmins into downstream adaptive type-2 responses (Scanlon and McKenzie 2015).

The toxin hypothesis of allergy has now gained interest and offers an alternative understanding of why certain proteins are targeted by IgE (Palm et al. 2012, Tsai et al. 2015). This hypothesis offers plausible explanations for allergenic components of insect venoms, proteins that have been altered by environmental toxins or proteins that carry ligands that present a certain danger to a host's cells. Why only few of the individuals who are exposed to the allergen raise an IgE response

is most likely rooted in the way the incoming signals are processed. It has been shown that monocyte-derived dendritic cells from birch pollen allergic and non-allergic subjects displayed distinct signal transduction pathways following the contact with the major birch pollen allergen Bet v 1 (Smole et al. 2015). The situation is less clear for food allergens. Certain lipids directly bound as ligands by the allergen or when present in the allergen source seem to play a role in the allergic sensitization process (Bublin et al. 2014). Moreover, plant seed storage proteins of the cupin and prolamin superfamilies have the capacity to damage cells, which might induce danger signals in exposed innate immune cells resulting in allergic sensitization (Candido Ede et al. 2011).

1.2 PROLAMIN SUPERFAMILY

Plant seeds are a major source of dietary proteins. Seed storage proteins such as the prolamins are a source of amino acids for use during germination and seedling growth. The prolamin superfamily comprises several families of proteins with limited sequence homology. The prolamins which gave the superfamily its name are the major seed storage proteins in most cereal seeds. They possess two or more unrelated structural domains, one of which contains repeated sequences. Parts of the non-repetitive domain of one group of the sulfur-rich prolamins are homologous with sequences present in a large group of low molecular seed proteins including the 2S albumins, the non-specific lipid proteins (nsLTPs) and the cereal inhibitors of α-amylase and trypsin (Kreis et al. 1985). They all share a conserved cysteine skeleton, which contains eight cysteine residues. The prolamin superfamily seems to be of a much more recent origin than the cupin seed storage proteins. The 2.2S spore storage protein matteucin of the ostrich fern is related to the 2S albumins of angiosperms whose common ancestors lived more than 300 million years ago (Rodin and Rask 1990). nsLTPs are abundant in liverworts, mosses and land plants but have not been found in any algae indicating that they have evolved only after plants had conquered land (Edstam et al. 2011).

1.2.1 Prolamins

The prolamins which are characterized by high levels of glutamine and proline residues are restricted to the grasses including major cereals such as wheat, barley and rye (Shewry et al. 1995). The prolamin seed storage proteins of wheat are the major components of gluten, which determines the quality of the flour for bread making. The complex mixture of cereal storage proteins, the gluten, consists of roughly equal amounts of gliadins and glutenins (Tatham and Shewry 2008). Gliadins are monomeric proteins, which interact by noncovalent forces. Based on their electrophoretic mobility they are divided into the fast moving α/β-gliadins, the intermediate γ-gliadins, and the slowly moving ω-gliadins. The glutenins are polymers of individual proteins that are linked by interchain disulfide bridges. Glutenins can be classified into high molecular weight (HMW) and low molecular weight (LMW) groups. The sulfur-rich prolamins are quantitatively the major prolamin group in wheat, barley and rye, and they include polymeric and monomeric proteins (Shewry and Tatham 1990). Wheat-dependent exercise-induced anaphylaxis (WDEIA) is associated with ω_5-gliadins (Tatham and Shewry 2008) while both gliadins and glutenins appear to be implicated in baker's asthma (Quirce and Diaz-Perales 2013).

1.2.2 Bifunctional Inhibitors

Plants have evolved a certain degree of resistance to insect pests that feed on plant tissues. Six types of proteinaceous α-amylase inhibitors are found in higher plants (Svensson et al. 2004). The bifunctional inhibitors impede digestion by acting on insect gut α-amylases and proteinases such as trypsin (Franco et al. 2002). A large family of these inhibitors, also referred to as CM proteins for their presence in chloroform/methanol extracts, is found in cereals seeds (Svensson et al. 2004). Several of these proteins are α-amylase/trypsin inhibitors while others inhibit only α-amylase or trypsin. These inhibitors consist of 120 to 160 amino acids, have a high α-helical content, and possess ten cysteine residues which form five disulfide bonds

(Oda et al. 1997). Tri a 28 (syn. 0.19 α-amylase inhibitor form wheat) acts as a homodimer (Oda et al. 1997) whereas the wheat inhibitor 0.28 and the corresponding barley inhibitor BMAI-1 (Hor v 15) are monomers (Sanchez-Monge et al. 1992). Current immunological and clinical data point to the α-amylase/trypsin inhibitor family as the main culprit of Baker's asthma (Salcedo et al. 2011).

1.2.3 2S Albumins

2S albumins are a water-soluble storage protein group widely present in mono- and dicotyledonous seeds (Candido Ede et al. 2011). They are encoded by a multigene family, which results in the presence of several isoforms in individual plants. They are synthesized as a single large precursor, which is then processed to give rise to two subunits that are held together by disulfide bonds. Typically, the 2S albumins comprise four α-helices and four to five disulfide bonds (Moreno and Clemente 2008). Although the major function of 2S albumins is the storage of amino acids, antifungal and antibacterial properties of several 2S albumins and thus their role in plant defense against pathogens were described (Candido Ede et al. 2011). A novel antimicrobial protein, SiAMP2, of the 2S albumin family was identified in sesame seeds and its inhibition of the growth of the human pathogenic bacterium *Klebsiella* was described (Maria-Neto et al. 2011). The 2S albumins of *Brassica napus* were able to significantly damage the fungal plasma lemma and to cause its permeabilization (Barciszewski et al. 2000). The number of 2S albumins that are described as food allergens is still increasing (Moreno and Clemente 2008). Many of the highly important seed, tree nut and legume allergens belong to the 2S albumins. Among them are Ara h 2, Ara h 6, and Ara h 7 from peanut (Burks et al. 1992, Kleber-Janke et al. 1999), Jug r 1 from walnut (Teuber et al. 1998), Ses i 1 and Ses i 2 from sesame seeds (Beyer et al. 2002a, Pastorello et al. 2001), Ber e 1 from Brazil nut (Pastorello et al. 1998), and Ana o 1 from cashew (Robotham et al. 2005). Ber e 1 serves as a model protein for studies of intrinsic allergenicity of food proteins (Alcocer et al. 2012).

1.2.4 Nonspecific Lipid Transfer Proteins (nsLTPs)

The nsLTPs are a family of allergens of high importance. They are divided into the 9 kDa nsLTP1 and the 7 kDa nsLTP2 subfamilies (Kader 1996). NsLTP1 are primarily found in aerial organs while nsLTP2 are expressed in roots. Both nsLTP1 and nsLTP2 are found in seeds. Members of both subfamilies are compact cysteine-rich proteins, which are made up of four or five α-helices that are held together by four conserved disulfide bridges. The α-helices enclose a hydrophobic cavity that enables them to transfer various lipid ligands between lipid bilayers *in vitro* (Lascombe et al. 2008). NsLTPs are involved in key cellular processes such as stabilization of membranes, cell wall organization and signal transduction but they also play important roles in resistance to biotic and abiotic stress, plant growth and development (Liu et al. 2015). Besides their various biologic roles in plants, nsLTPs are a large group of heat- and proteolysis-resistant allergens (Egger et al. 2010). The type 1 nsLTPs are able to elicit severe type 1 reactions to fresh fruits such as peach in predisposed individuals in Southern Europe and the Mediterranean region. NsLTPs are regarded as panallergens due to their presence in a variety of plant tissues including seeds, fruits and vegetative tissues (Salcedo et al. 2007). In addition, nsLTPs1 were described as inhalant allergens in pollen of many flowering plants including *Parietaria judaica* (Duro et al. 1996), olive tree (Tejera et al. 1999), and mugwort (Gadermaier et al. 2009).

Plant food nsLTPs1 have been identified in fruits such as peach (Pastorello et al. 1999), apple (Zuidmeer et al. 2005), and grapes (Pastorello et al. 2003), in vegetables such as asparagus (Diaz-Perales et al. 2002), corn (Pastorello et al. 2000), and celery (Gadermaier et al. 2011), and in various nuts including hazelnut (Offermann et al. 2015). Cross-reactivities between nsLTPs1 from closely related plants are frequently observed but decreases with evolutionary distance. The kiwi fruit nsLTP1 does not cross-react with the peach nsLTP1 (Bernardi et al. 2011). Similarly, the nsLTP1s from olive pollen and *Parietaria judaica* pollen neither cross-react with each other nor with other plant food nsLTP1s such as the one from peach (Tordesillas et al. 2011). In contrast, sensitization to the nsLTP1 from peach is

Table 1.1 Selected allergens of the prolamin superfamily.

Protein family	Allergen source	Allergen designation
Prolamin	Wheat (*Triticum aestivum*)	Tri a 19: ω-5-glaidin
		Tri a 20: γ-gliadin
		Tri a 21: α/β-gliadin
		Tri a 26: high molecular weight glutenin
		Tri a 36: low molecular weight glutenin
Bifunctional inhibitor	Wheat (*Triticum aestivum*)	Tri a 15: monomeric α-amylase inhibitor
		Tri a 28: dimeric α-amylase inhibitor 0.19
		Tri a 29: tetrameric α-amylase inhibitor CM1/CM2
		Tri a 30: tetrameric α-amylase inhibitor CM3
	Rye (*Secale cereale*)	Sec c 38: dimeric α-amylase/ trypsin inhibitor
2S albumin	Brazil nut (*Bertholletia excelsa*)	Ber e 1
	Cashew nut (*Anacardium occidentale*)	Ana o 3
	Hazelnut (*Corylus avellana*)	Cor a 14
	Peanut (*Arachis hypogaea*)	Ara h 2, Ara h 6, Ara h 7
	Sesame (*Sesamum indicum*)	Ses i 1, Ses i 2
	Walnut (*Juglans regia*)	Jug r 1
Non-specific lipid transfer protein type 1	Apple (*Malus domestica*)	Mal d 3
	Celeriac (*Apium graveolens*)	Api g 2
	Cherry (*Prunus avium*)	Pru av 3
	Corn (*Zea mays*)	Zea m 14
	Grape (*Vitis vinifera*)	Vit v 1
	Hazelnut (*Corylus avellana*)	Cor a 8
	Peach (*Prunus persica*)	Pru p 3
Non-specific lipid transfer protein type 2	Celeriac (*Apium graveolens*)	Api g 6
	Tomato (*Solanum lyopersicum*)	Sola l 6

frequently present with a sensitization to the mugwort nsLTP1 in the Mediterranean region. A primary sensitization to the peach nsLTP1 can lead to a respiratory allergy based on the cross-reactivity of peach and mugwort nsLTPs (Sanchez-Lopez et al. 2014). The first allergenic type 2 nsLTP, detected as a heat-resistant protein in celeriac, showed only a very limited cross-reactivity to the tape 1 nsLTP from celeriac (Vejvar et al. 2013). Recently, a type 2 nsLTP was identified as an allergen present in tomato seeds (Giangrieco et al. 2015).

1.3 CUPIN SUPERFAMILY

At present, the cupin superfamily contains 57 families. The members of this superfamily possess one or more conserved cupin domain, a characteristic β-barrel (Latin *cupa* = barrel) that evolved in a prokaryotic organism and was then passed on into the plant kingdom (Khuri et al. 2001). The cupin domain is used for a large number of biological functions and is found in fungal spherulins that are produced upon spore formation, in proteins that bind saccharose, or in germins whose function depends on the binding of manganese ions by the cupin domain (Dunwell et al. 2000). Cupins are highly thermostable, a trait that has most likely evolved in thermophilic archaea and that can still be found in today's plant food allergens. The cupin domain was duplicated in flowering plants giving rise to the so-called bicupin seed storage proteins (Dunwell and Gane 1998), the 7S and 11S globulins which are described as major allergens of peanut, tree nuts and various seeds (Mills et al. 2002, Radauer and Breiteneder 2007, Willison et al. 2014). The cupin seed storage proteins are primarily an energy source and provide amino acids during seed germination. In addition, they are also involved in the defense of many plant species against fungi and insects (Candido Ede et al. 2011).

1.3.1 Vicilins (7S globulins)

The 7S globulin seed storage proteins are trimeric proteins that are also referred to as vicilins, as they are primarily found in the Viciae group of legumes. The monomers of these proteins are products of

a multigene family that are proteolytically processed during their maturation and glycosylated by varying degrees dependent on the plant species (Marcus et al. 1999). Many major plant food allergens are vicilins, including Ara h 1 from peanut (Burks et al. 1991), Gly m 5 from soybean (Ogawa et al. 1995), Ana o 1 from cashew (Wang et al. 2002), Jug r 2 from walnut (Teuber et al. 1999), Len c 1 from lentil (Lopez-Torrejon et al. 2003), Ses i 3 from sesame (Beyer et al. 2002a), and Cor a 11 from hazelnut (Lauer et al. 2004).

1.3.2 Legumins (11S globulins)

The 11S globulins are the seed storage proteins of many mono- and dicotyledonous plants. They are also referred to as legumins as they were primarily studied in legume seeds. Legumins are hexameric proteins that consist of two associated viclin-like trimers (Dunwell et al. 2000). The monomers, like in their vicilin counterparts, are the products of multigene families. In contrast to the vicilin monomers, the legumin monomer is proteolytically cleaved into an acidic and a basic chain that are held together by a disulfide bond. Legumins are only rarely glycosylated. Various allergens of

Table 1.2 Selected allergens of the cupin superfamily.

Protein family	Allergen source	Allergen designation
Vicilin (7S globulins)	Cashew nut (*Anacardium occidentale*)	Ana o 1
	Hazelnut (*Corylus avellana*)	Cor a 11
	Peanut (*Arachis hypogaea*)	Ara h 1
	Sesame (*Sesamum indicum*)	Ses i 3
	Soybean (*Glycine max*)	Gly m 5
	Walnut (*Juglans regia*)	Jug r 2
Legumin (11S globulins)	Brazil nut (*Bertholletia excelsa*)	Ber e 2
	Cashew nut (*Anacardium occidentale*)	Ana o 2
	Hazelnut (*Corylus avellana*)	Cor a 9
	Peanut (*Arachis hypogaea*)	Ara h 3
	Sesame (*Sesamum indicum*)	Ses i 6, Ses i 7
	Soybean (*Glycine max*)	Gly m 6
	Walnut (*Juglans regia*)	Jug r 4

legume seeds, tree nuts, and seeds belong to the legumin protein family. They include Ara h 3 from peanut (Rabjohn et al. 1999), Gly m 6 from soybean (Beardslee et al. 2000), Ana o 2 from cashew nut (Wang et al. 2003), Cor a 9 from hazelnut (Beyer et al. 2002b), and Ses i 6 and Ses i 7 from sesame seeds (Beyer et al. 2007).

1.4 EF-HAND SUPERFAMILY

The EF-hand motif is the most common calcium-binding motif found in proteins where two α-helices connected by a loop form a calcium-binding structure (Lewit-Bentley and Rety 2000). Proteins that contain EF-hand motifs have functions as diverse as calcium buffering in the cytosol, signal transduction between cellular compartments or muscle contraction. EF-hand motifs are found in certain pollen allergens, the polcalcins, as well as in the major fish allergens, the parvalbumins. Plant EF-hand and animal EF-hand proteins do not cross-react with each other.

1.4.1 Parvalbumins

Parvalbumins are present in high concentration in the white muscle of many fish species and are highly cross-reactive major allergens (Lee et al. 2011). Parvalbumins possess three characteristic EF-hand motifs (Ikura 1996) of which only two are able to bind calcium ions (Declercq et al. 1991). Parvalbumins play an important role in relaxing muscle fibers by binding free intracellular calcium ions (Pauls et al. 1996). Binding of the calcium ligand is necessary for the correct conformation of parvalbumin. Loss of the ligand leads to a change in conformation, which results in the loss of the ability to bind IgE (Bugajska-Schretter et al. 1998, Bugajska-Schretter et al. 2000). Calcium-bound parvalbumin displays a high stability to denaturation by heat or degradation by proteolysis (Elsayed and Aas 1971, Filimonov et al. 1978, Griesmeier et al. 2010, Somkuti et al. 2012). Parvalbumins can be classified into two evolutionary lineages, the α- and the β-parvalbumins, which share similar architectures. In general, only β-parvalbumins are allergenic. However, an allergenic α-parvalbumin from frog was

Table 1.3 Selected allergenic parvalbumins.

Protein family	Allergen source	Allergen designation
Parvalbumin	Atlantic cod (*Gadus morhua*)	Gad m 1
	Atlantic salmon (*Salmo salar*)	Sal s 1
	Carp (*Cyprinus carpio*)	Cyp c 1
	Rainbow trout (*Oncorhynchus mykiss*)	Onc m 1
	Whiff (*Lepidorhombus whiffagonis*)	Lep w 1

described (Hilger et al. 2002). Gad c 1 was isolated from cod and was the first described allergenic β-parvalbumin (Aas and Jebsen 1967, Elsayed and Bennich 1975). Today, a large number of allergenic β-parvalbumins from a variety of fish species is known (Kuehn et al. 2014, Sharp and Lopata 2014). In addition, two allergenic parvalbumins from red stingray were described (Cai et al. 2010).

1.5 TROPOMYOSIN-LIKE SUPERFAMILY

Tropomyosins are one of three families of the tropomyosin-like superfamily. Tropomyosins are closely related proteins that—together with actin and myosin—are involved in the contraction of muscle fibers. Tropomyosins consist of 40 heptapeptide units and are double stranded, so called coiled-coil, molecules (Li et al. 2002). Tropomyosins are the major allergens of crustaceans and mollusks. Most allergies to shrimps, crabs, lobsters, squids, and shellfish are mediated by tropomyosins. Tropomyosins were originally described as allergenic in shrimps (Daul et al. 1994, Leung et al. 1994, Shanti et al. 1993). Today, tropomyosins are regarded as panallergens of many invertebrate animals (Reese et al. 1999). Tropomyosins of crustaceans and mollusks are highly heat-stable and cross-reactive (Motoyama et al. 2006). Extracts of cooked *Penaeus indicus* shrimps still contained the major allergen Pen i 1 with unchanged IgE-binding capacity (Naqpal et al. 1989). Water-soluble shrimp allergens were also detected in the cooking stock (Lehrer et al. 1990). In seafood processing plants, allergenic tropomyosins are present in aerosols and thus elicit occupational allergies in the work force (Lopata and Jeebhay 2013). Tropomyosins are also inhalant allergens from mites and cockroaches. Although they seem to possess only a

Table 1.4 Selected allergenic tropomyosins.

Protein family	Allergen source	Allergen designation
Tropomyosin: Crustaceans	American lobster (*Homerus americanus*)	Hom a 1
	Crucifix crab (*Charybdis feriatus*)	Cha f 1
	Indian white prawn (*Penaeus indicus*)	Pen i 1
	North Sea shrimp (*Crangon crangon*)	Cra c 1
Tropomyosin: Mollusks	Pacific flying squid (*Todarodes pacificus*)	Tod p 1

limited allergenic potential (Thomas et al. 2010) they are regarded as important for cross-sensitization to tropomyosins of crustaceans and shellfish (Lopata et al. 2010).

1.6 PROFILIN-LIKE SUPERFAMILY

The profilin-like superfamily comprises four member families. One of them, the profilin family, are proteins that are highly conserved in higher plants with sequence identities of at least 75% (Radauer et al. 2006). Profilins are cytoplasmic proteins of 12–15 kDa and are present in all eukaryotic cells. They bind monomeric actin (Schutt et al. 1993) and are involved in the dynamic turnover and restructuring of the actin cytoskeleton (Witke 2004). Profilin from birch pollen was the first profilin that was described as allergenic (Valenta et al. 1991). Subsequently, a large number of cross-reactive profilin allergens were described in pollen of trees, grasses and weeds (Gadermaier et al. 2014, Hauser et al. 2010). As profilin-specific IgE cross-reacts with practically all plant profilins, a profilin sensitization is regarded as a risk factor for allergic reactions to various plant pollen (Mari 2001) and plant foods (Asero et al. 2003, Fernandez-Rivas 2015). However, the clinical relevance of a profilin sensitization is still under discussion (Santos and Van Ree 2011). The clinical relevance of a profilin sensitization was shown for profilins from cantaloupe, watermelon, tomato, banana, pineapple, orange and kaki (Anliker et al. 2001, Asero et al. 2008, Lopez-Torrejon et al. 2005). Recently, profilins were shown to be

Table 1.5 Selected allergenic plant food profilins.

Protein family	Allergen source	Allergen designation
Profilin	Banana (*Musa acuminata*)	Mus a 1
	Cantaloupe (*Cucumis melo*)	Cuc m 2
	Orange (*Citrus sinensis*)	Cit s 2
	Pineapple (*Ananas comosus*)	Ana c 1
	Tomato (*Solanum lycopersicum*)	Sola l 1

complete food allergens capable of eliciting severe reactions in plant food allergic patients that had been exposed to high levels of grass pollen (Alvarado et al. 2014).

1.7 BET V 1-LIKE SUPERFAMILY

Bet v 1, the major birch pollen allergen, is the one member that gave this superfamily its name (Breiteneder et al. 1989). The Bet v 1-like superfamily contains at present 103,375 members from 17,750 species (http://pfam.xfam.org/clan/CL0209, accessed November 2015). Proteins with the typical Bet v 1 architecture can be found in all kingdoms of life and hence belong to the earliest proteins that evolved at the beginning of life (Radauer et al. 2008b). The superfamily consists of 14 families including the Bet v family, which comprises 11 subfamilies. Most of the Bet v 1-homologous allergens known today belong to the PR-10 subfamily (Hoffmann-Sommergruber 2002). The cDNA coding for Bet v 1 was discovered on July 3, 1989 and published as a sequence for the first plant allergen (Breiteneder et al. 1989). Birch belongs to the botanical order Fagales which comprises 8 families, some of which produce allergenic pollen such as hazel (Breiteneder et al. 1993), alder (Breiteneder et al. 1992), oak (Wallner et al. 2009), and beech (Hauser et al. 2011).

The association of a birch pollen allergy with an allergy to diverse plant foods is a frequently observed syndrome, which is due to the presence of homologous allergens in these allergen sources (Katelaris 2010, Vieths et al. 2002). The observed clinical symptoms to the various plant foods are generally elicited by IgE that was induced by exposure to Bet v 1. The known structures of Bet v 1 (Gajhede

Table 1.6 Selected allergens of the Bet v 1 family.

Subfamily of the Bet v 1 family	Allergen source	Allergen designation
PR-10	Apple (*Malus domestica*)	Mal d 1
	Celeriac (*Apium graveolens*)	Api g 1
	Cherry (*Prunus avium*)	Pru av 1
	Mung bean (*Vigna radiata*)	Vig r 1
	Peach (*Prunus persica*)	Pru p 1
	Peanut (*Arachis hypogaea*)	Ara h 8
	Soybean (*Glycine max*)	Gly m 4
RRP	Kiwifruit (*Actinidia deliciosa*)	Act d 11
CSBP	Mung bean (*Vigna radiata*)	Vig r 6

et al. 1996), and its homologs form cherry (Neudecker et al. 2001), celeriac (Markovic-Housley et al. 2009), carrot (Markovic-Housley et al. 2009), soybean (Berkner et al. 2009) and peanut (Hurlburt et al. 2013) clearly illustrate the similarities of these molecules' surfaces that explain the clinically observed cross-reactivities. IgE antibodies bind to Bet v 1-related plant food allergens such as Mal d 1 from apple (Vanek-Krebitz et al. 1995), Api g 1 from celeriac (Breiteneder et al. 1995), Ara h 8 from peanut (Mittag et al. 2004), Vig r 1 from mung bean (Mittag et al. 2005), and Bet v 1 homologs from Sharon fruit (Bolhaar et al. 2005) and jackfruit (Bolhaar et al. 2004). Act d 11 is an allergen of the kiwifruit that belongs to the ripening related protein (RRP) subfamily (D'Avino et al. 2011). Vig r 6 from mung beans is another Bet v 1 homolog that belongs to the cytokinin-specific binding protein (CSBP) family.

1.8 THE CASEIN AND THE CASEIN KAPPA FAMILY

All mammalian milks contain multiple casein proteins characterized as α-, β- and κ-caseins (Oftedal 2012). Caseins are members of the unfolded secretory calcium-binding phosphoproteins called SSCP (Kawasaki and Weiss 2003). The α- and β-caseins evolved from tooth and bone-proteins well before the evolution of lactation

Table 1.7 Allergenic caseins of cow's milk.

Protein family	Allergen source	Allergen designation
Casein	Cow's milk (*Bos domesticus*)	Bos d 9: αS1-casein
		Bos d 10: αS2-casein
		Bos d 11: β-casein
Casein kappa	Cow's milk (*Bos domesticus*)	Bos d 12: κ-casein

(Lenton et al. 2015). In mammalian milks, sequestered nanoclusters of calcium phosphate are substructures in casein micelles which allow the calcium and phosphate concentrations to be far in excess of their solubility. The αS1-, αS2- and β-caseins form a shell around amorphous calcium phosphate to form the nanoclusters. These nanoclustes are then assembled into the casein micelles that are stabilized by κ-casein (ten Grotenhuis et al. 2003). α- and β-caseins are members of the casein family (Kawasaki et al. 2011), while κ-caseins are members of the casein kappa family (Ward et al. 1997). Caseins are major food allergens involved in cow's milk allergy, which affects predominantly young children. In European children, the incidence of challenge-proven cow's milk allergy was 0.54% with national incidences ranging from <0.3% to 1% (Schoemaker et al. 2015). Recently, the official nomenclature of allergenic caseins has been changed (Radauer et al. 2014). The name Bos d 8, as it is widely established, was kept to designate the whole casein fraction. However, based on low sequence similarities, Bos d 8 was demerged into four separate allergens: Bos d 9 (aS1-casein), Bos d 10 (αS2-casein), Bos d 11.0101 (β-casein), and Bos d 12.0101 (κ-casein).

1.9 CALYCIN-LIKE SUPERFAMILY

The calycin structural superfamily includes 20 families. Calycins are an example for a superfamily of proteins, which—although they share structural similarities—have unusually low levels of overall sequence conservation. The calycin architecture is based on an eight-stranded β-barrel which forms an internal ligand binding site for small hydrophobic molecules (Flower et al. 1993).

1.9.1 Lipocalins

Lipocalins form a subset of the calycin superfamily. Lipocalins are small extracellular proteins with a large variety of functions which typically revolves around the binding of small hydrophobic ligands such as retinol (Flower et al. 2000). Most of the allergenic lipocalins are not food allergens but important inhalant allergens from mammals and insects (Hilger et al. 2012, Virtanen et al. 2012). The only lipocalin animal food allergen is β-lactoglobulin (Bos d 5) which is a major allergen in cow's milk (Hochwallner et al. 2014) and is absent from human and camel milk (Restani et al. 2009). Bos d 5 is highly stable to proteolytic degradation and acid hydrolysis (Wal 2004).

1.10 CONCLUSIONS

In 1991, the evolutionary biologist Margie Profet published the toxin hypothesis of allergy (Profet 1991). She proposed that the allergic immune response evolved as a defense mechanism to protect the individual from toxic environmental substances such as venoms and toxic plant compounds. Recently, this hypothesis has found experimental proof for bee and snake venoms (Marichal et al. 2013, Palm et al. 2013, Starkl et al. 2015). It is highly plausible that this hypothesis will be confirmed for allergenic components of other insect venoms. Future experiments will have to be performed for plant food allergens and plant food matrices to explore whether they are as innocuous as they were made out to be. In fact, seed storage proteins which are commonly regarded as inert also have functions in plant defense mechanisms (Candido Ede et al. 2011). 2S albumins from passion fruit seeds have been shown to induce plasma membrane permeabilization (Agizzio et al. 2006) and vicilins from cowpea were discovered to interact with the microvilli of the larval midgut epithelium of the bean-feeding cowpea beetle (Oliveira et al. 2014).

The allergens of the various superfamilies have distinct distributions. Allergenic prolamins and cupins are only present

in plants. While the cupin allergens are so far only known as seed storage proteins, allergens of the prolamin superfamily can either be storage proteins or have inhibitory or signal transduction functions. Bet v 1 homologs and profilins are also only known as plant allergens. Allergenic food proteins of the EF-hand superfamily are only known from fish. Likewise, allergenic tropomyosins as food allergens seem to be limited to crustaceans and mollusks. Although lipocalins are also present in plants (Charron et al. 2005), most of them are inhalant animal allergens and only one is an animal food allergen, the β-lactoglobulin from cow's milk. All of these proteins perform a specific biologic function. They become allergenic only when they interact with the immune system of a predisposed individual. It is worth to note, that in general, allergens are restricted to a highly limited number of protein families. That indicates that only a very small number of protein structures are able to induce allergic sensitization or to become involved in such a process. Why this is the case is still unclear. The innate immune system (Herre et al. 2013, Junker et al. 2012, Trompette et al. 2009), binding of ligands to the allergens (Jyonouchi et al. 2011, Mirotti et al. 2013), and adjuvants present in the allergen source seem to play a role (Gilles et al. 2009, Mittag et al. 2013).

When the allergens designated by the WHO/IUIS Allergen Nomenclature Subcommittee (http://www.allergen.org/) are classified by protein families, as was done in this chapter, they become much more manageable. A detailed analysis of the biochemical, structural and immunologic properties of each family of allergens will contribute to the understanding of factors that contribute to the allergenic potential of a protein.

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