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ALLERGY

Review article

Allergic cross-reactivity: from gene to the clinic

A large number of allergenic proteins have now their complete cDNA sequences determined and in some cases also the 3D structures. It turned out that most allergens could be grouped into a small number of structural protein families, regardless of their biological source. Structural similarity among proteins from diverse sources is the molecular basis of allergic cross-reactivity. The clinical relevance of immunoglobulin E (IgE) cross-reactivity seems to be influenced by a number of factors including the immune response against the allergen, exposure and the allergen. As individuals are exposed to a variable number of allergenic sources bearing homologous molecules, the exact nature of the antigenic structure inducing the primary IgE immune response cannot be easily defined. In general, the 'cross-reactivity' term should be limited to defined clinical manifestations showing reactivity to a source without previous exposure. 'Co-recognition', including by definition 'cross-reactivity', could be used to describe the large majority of the IgE reactivity where co-exposure to a number of sources bearing homologous molecules do not allow unequivocal identification of the sensitizing molecule. The analysis of reactivity clusters in diagnosis allows the interpretation of the patient's reactivity profile as a result of the sensitization process, which often begins with exposure to a single allergenic molecule.

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Allergenic proteins originate from a great variety of sources (pollen, mites, moulds, animal products, venom, foods and latex) and are able to induce the immune system to produce high-affinity immunoglobulin E (IgE) antibodies and/or to trigger allergic symptoms in a sensitized individual. The phenomenon of allergen cross-reactivity occurs when IgE antibodies originally raised against one allergen binds or recognizes a similar protein from another source (1–3). The interaction with such homologous protein can then trigger allergic reactions or can be completely irrelevant for the patient. Such cross-reacting allergens are usually discovered in epidemiological studies or clinical observations. Cloning and sequencing of allergen genes provides the molecular basis of cross-reactivity. For example, it is well known that some pollen allergic patients often display adverse reactions after ingestion of certain fresh fruits, vegetables and nuts. Several clinical syndromes have been described such as those associated with birch, mugwort, and ragweed pollen. They can be collectively termed pollen-food syndrome (PFS) (4). For instance, molecular cloning led to the identification of the Bet v 1 and Bet v 2 group of proteins as the molecules involved in the birch PFS.

Two (or more) allergens are cross-reactive if IgE antibodies or a T cell receptor reacts with both (2). However, as high affinity IgE antibodies are central for the clinical manifestation of cross-reactivity, here we will only discuss cross-reactions at the antibody level.

Cross-reactivity, co-sensitization and co-recognition

The clinical relevance of cross-reactivity seems to be influenced by a number of factors including the host (the immune response against the allergen), exposure and the allergen (1). In general, repeated exposure to the allergen is required for allergic reactions. In addition, the levels of specific IgE antibodies and their affinity are important aspects for allergic cross-reactions. High affinity antibodies are necessary to trigger IgE-mediated cellular responses by trace amounts of allergens. However, data concerning affinity threshold for triggering reactions by cross-reactive proteins is still insufficient.

The structural characteristics of proteins are major determinants of cross-reactivity. The IgE cross-reactions are because of shared features at the level of primary and tertiary structure of proteins. In general, cross-reactivity seems to require more than 70% sequence identity. Proteins having $< 50\%$ sequence identity are very seldom cross-reactive (1). Another important aspect to be considered is sequence similarities of allergens to human homologues, which could lead to autoreactive

IgE antibodies. For instance, IgE antibodies reacting with human profilin and manganese-superoxide dismutase (MnSOD) have been described in some patients with pollinosis and fungal allergy, respectively (5, 6). Exceptions occur when postsynthetic modifications (e.g. glycosylation) are involved in cross-reactivity between unrelated proteins. These cross-reactive carbohydrate determinants (CCD) will be briefly discussed below.

The exact nature of the antigenic structure inducing the primary IgE immune response cannot be easily defined. Usually, subjects are exposed to a variable number of allergenic sources bearing homologous molecules (e.g. several grass species). Even in the case of a mono-exposure to a single species (e.g. birch pollen without exposure to other Fagales pollen), very likely subjects are exposed to a variable number of different isoforms from a particular allergen (7). In the routine diagnostic approach, patients are tested with allergenic extracts or molecules showing the highest IgE reactivity and it is usually assumed that those are the sensitizers. This assumption has not been unequivocally demonstrated. For instance, it is possible to record stronger skin test reactivity with pollen extracts to which patients are not exposed to, although such findings could be linked to the quality of extracts used for testing (8). More striking are the observations that certain genetically engineered molecules obtained by site-directed mutagenesis or gene shuffling can display higher IgE-binding activity than the original wild type allergens (9). In the latter case, patients have not been exposed to such molecules. Thus it is conceivable that homologous allergens found in sources to which patients are not exposed could show higher IgE binding activity than the primary sensitizer.

In general, the term 'cross-reactivity' should be used to describe clearly defined clinical features showing the reactivity to a source without previous exposure (10). The more comprehensive term 'co-recognition', including by definition 'cross-reactivity', could be usefully adopted to define the large majority of the IgE reactivity where co-exposure to a number of sources bearing homologous molecules does not allow the identification of the sensitizer. There are a few exceptions (i.e. cat Fel d 1, Api m 1 from bee venom and chicken egg proteins) where the sensitizing source can be identified with a high degree of certainty. For example, the reactivity to Fel d 1-homologous molecules from 'big cats' is certainly determined by primary sensitization to Fel d 1 via exposure to cat-derived material (11). In such limited number of cases the term cross-reactivity could be appropriate, as the primary sensitizer is known. In most of the cases the sensitizing source is postulated because of the high level of exposure (e.g. peanut allergen). Thus, assuming that IgE antibodies are the essential part in the reactivity, they should be central to evaluate the relationships among molecules. Recently, an interesting report from Fernandes et al. (10) clearly highlights how IgE co-recognition of allergenic molecules might lead to a potential reactivity without prior exposure. Mite or other arthropods tropomyosin sensitization via the inhalation route is suspected to lead to reactivity to shrimp tropomyosin without any previous exposure via the digestive tract. The IgE reactivity to mite group 10 allergenic epitopes that are present in different allergenic sources, as well as IgE to cockroach Per a 7 might thus lead to cross-reactivity to crustacean homologous molecules (Pen a 1). A similar example is reported in a recent study dealing with the IgE reactivity to Bet v 1 in a birch-free area (12). As in the tropomyosin story with Orthodox Jews, IgE reactivity to the major birch allergen has been detected without prior exposure to the related source. Again, considering IgE toward epitopes shared by molecules of the Fagales group 1 as the central point, the phenomenon of reactivity to an 'absent' source can be regarded as 'normal' or even an 'expected' one. Many other examples reported in the literature may have a similar interpretation (e.g. cockroach sensitization in Norway (13).

Finally, the term 'co-sensitization' underlies the presence of IgE toward epitopes that are not shared between allergenic sources or molecules. The co-sensitization to allergenic sources detected by means of allergenic extracts is a common finding (e.g. grasses and mites) but it does not reflect the pattern of allergenic molecules and of specific IgE antibodies. A subject with isolated grass sensitization might have IgE to several allergenic molecules (e.g. grass group 1, 2, 4, 5) all restricted to the grass family (14). Conversely, a subject sensitized to olive and pellitory pollen and to cat and *Alternaria* might react to Ole e 1, Par j 2, Fel d 1 and Alt a 1 allergens. Co-sensitization to four different molecules is detected in the latter case as well, although they belong to four different sources. Even if the subjects in both cases are co-sensitized to the same number of molecules they will experience different patterns of symptoms, as their triggering allergens can be present at different time points. It is up to the clinical allergologist to differentiate what is relevant in the interventional approach for patients with multiple sensitization and how their immunological reactivity can be interpreted using different criteria.

Cross-reactive molecules

Presently, the allergen databank (http://www.allergen.org) from the Allergen Nomenclature Sub-Committee of the International Union of Immunological Societies (IUIS) contains a list with more than 400 allergens and almost 200 isoallergens. For most of these allergenic proteins originating from various sources, the complete cDNA sequences have been determined and in some cases also the 3D structures (see selected examples in Fig. 1). From these data it is now clear that most allergens can be grouped into a small number of structural protein families, regardless of their biological

source. As mentioned above, structural similarity among proteins from diverse sources is the molecular basis of cross-reactivity.

Tables 1–4 list 28 major groups of cross-reactive proteins from various sources. Allergens in six of these groups belong to some families of pathogenesis-related (PR) proteins from plants (PR-2, PR-3, PR-5, PR-10, PR-12 and PR-14). The PR proteins are divided into 14 families. They are induced in response to infections by pathogens (fungi, bacteria and viruses), by wounding or other stresses including drought, flooding, freezing temperature, ozone and ultraviolet B light (UV-B). Plants expressing higher levels of certain PR proteins are more resistant to environmental stress and disease and their selection for agricultural use could then contribute to an increase in the allergenicity of cultivated plants (15). The aspects of plant PR proteins as allergens and environmental issues have been addressed in several review articles (16–19).

Eleven groups of allergens show sequence homology to a variety of enzymes including proteases (plant and mites cysteine proteases, Grass pollen group 1), glycolytic enzymes (moulds and latex enolase), superoxide dismutase (latex, moulds and human manganese SOD), carbohydrate active enzymes (weed pollen pectate lyase, latex/fruit glucanase and chitinase, chicken egg lysozyme, insect venom hyaluronidase and milk alpha-lactalbumin), and esterases (insect venom phospholipase A1 and A2). Other groups of allergens are transport proteins (food nsLTP, Fagales pollen group 1, milk beta-lactoglobulin and casein), protease inhibitors (egg, ovomucoid and ovoalbumin), regulatory proteins (pollen/food profilins and pollen calcium-binding proteins), structural proteins (arthropods tropomyosins and fish parvalbumins), and storage proteins (plant albumins and globulins). In case of some allergens, it has been suggested that their enzymatic activity might function as pro-allergic adjuvant. For example, the cysteine protease activity of Der p 1 enhances its own permeability in the bronchial epithelium, increases IgE production by cleaving the low-affinity IgE receptor (CD23) on B cells and monocytes, and decreases the proliferation of Th1 cells by cleaving the IL-2 receptor (CD25) (20, 21). However, it is still unclear to what extent enzymatic activities or other biochemical functions of allergens are involved in the process of sensitization and allergic reactions (22).

Below we give a short description of cross-reactive molecules grouped as in Tables 1–4, but with no intention of exhausting the published data on allergens. For additional information, the reader is referred to given specialized review articles and to specialized databases listed in Table 5.

Fagales pollen – group 1 (PR-10)

Bet v 1, Cor a 1, Aln g 1, Car b 1 and other major pollen allergens from Fagales trees belong to the PR-10 family of proteins. These pollen allergens exist as multiple isoforms showing a high degree of sequence similarity. Sensitization by these isoallergens frequently leads to cross-reactions with homologous proteins in apple, cherry, apricot, pear, hazelnut, carrot, celery and other vegetables (Table 1). Bet v 1 is the best characterized allergen in this group. The crystal structure of Bet v 1l, a naturally occurring hypoallergenic isoform, was recently determined in complex with deoxycholate (23). Furthermore, ligand binding studies with Bet v 1l (23), Bet v 1.2801 (24), and with Pru av 1 (25) showed interaction with various phytosteroids. Thus, it has been suggested that Bet v 1 and related PR-10 proteins might function as general plant-steroid carriers (23).

The IgE cross-reactivity of Bet v 1 and its homologous proteins is one of the main causes of PFS for patients allergic to pollen from trees of the Fagales order. The IgE binding to Bet v 1 and homologous proteins is conformation dependent and cross-reactivity is assumed to be due to very similar three-dimensional structures. Interestingly, the three-dimensional structure of Bet v 1l, a low IgE binding isoform, does not show significant differences when compared with the structures of high IgE-binding Bet v 1.2801 and Pru av 1 molecules. Although no sequential epitopes have been identified for the Bet v 1 family, single amino acid residues/positions involved in epitope formation were successfully identified by site-directed mutagenesis. Amino acid positions 10, 30, 57, 112, 113 and 125 were shown to be crucial for IgE recognition of pollen Bet v 1 and food homologues Mal d 1 and Api g 1 (26, 27). Position 111/112 was also for Mal d 1 (28) and Pru av 1 (29). In addition, glutamic acid in position 45 was shown to be important for the IgE binding activity of Bet v 1 (30) and Pru av 1 (31).

Profilins

Profilin is involved in the regulation of actin polymerization and signal transduction of phosphatidylinositol pathway. Profilin is now considered as a ubiquitous cross-reactive plant allergen and sensitized patients typically react to a broad range of pollen and food sources (8, 32). Just to cite a few examples, profilin is responsible for cross-reactions between birch/mugwort pollen-celery-spices, grass pollen-celery-carrots and tree pollen-hazelnut (Table 1). It is believed that IgE cross-reactivity is mostly because of the highly conserved three-dimensional structure of profilins and not to similarities at the level of amino acid sequence (33). Three major epitope regions involved in IgE binding, including the amino and carboxy-terminal alpha-helices of birch profilin were identified, which are also highly conserved regions interacting with the physiologic ligands actin and proline-rich peptides (34). Despite extensive cross-reactivity among plant profilin and to the human homologue as well, it seems that a large proportion of IgE reactivities to profilins is clinically irrelevant (35, 36). The lack of

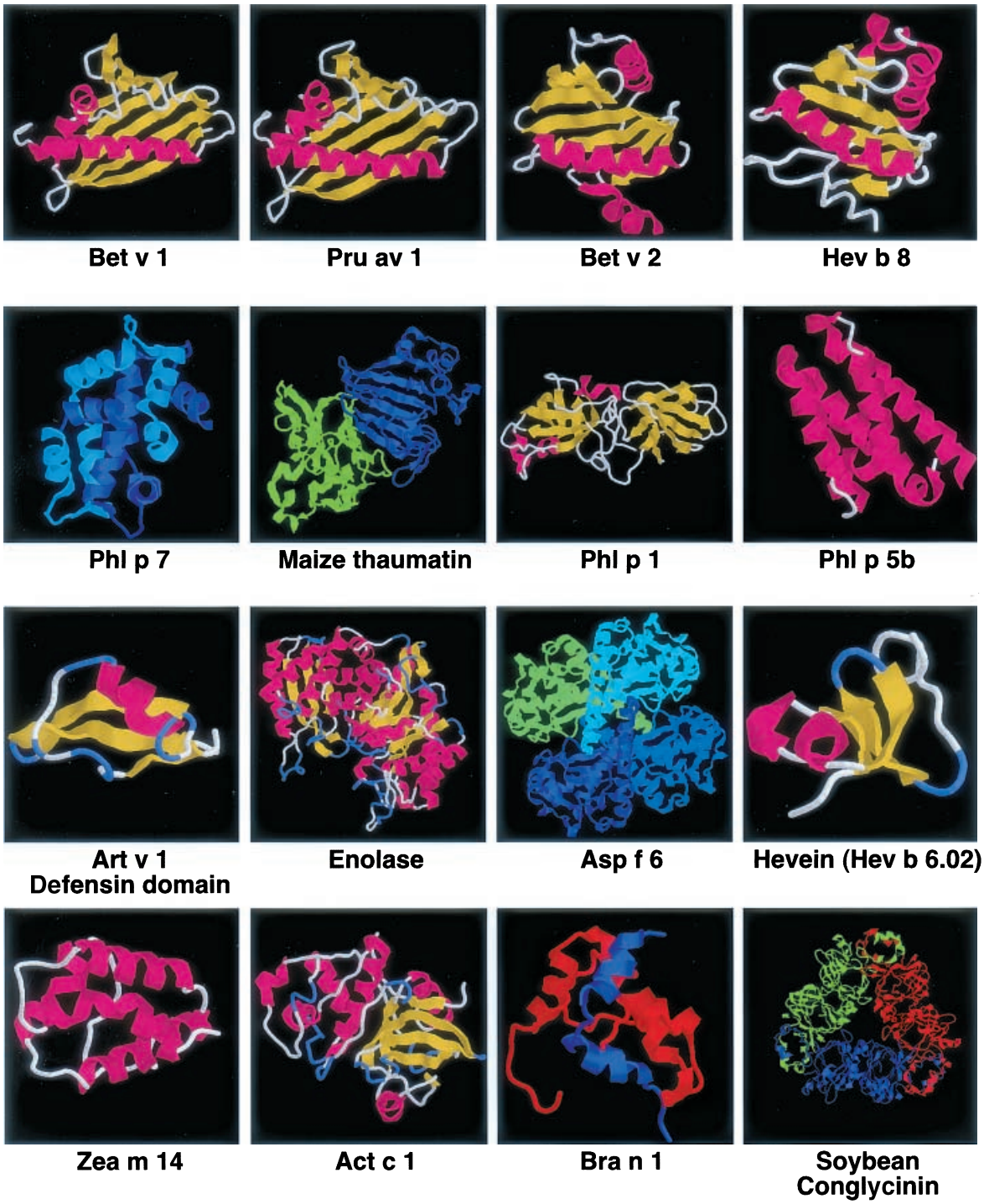

Figure 1. Cartoon representation of the three-dimensional structure of cross-reactive allergens. List of allergens with their PDB codes in parenthesis: birch pollen Bet v 1 (1BV1), cherry Pru av 1 (1EO9), birch pollen Bet v 2 (1CQA), latex Hev b 8 (1G5U), timothy grass pollen Phl p 7 (1K9U), maize thaumatin (1DU5), timothy grass pollen Phl p 1 (1N1O), timothy grass pollen Phl p 5 (1L3P), mugwort pollen Art v 1 (54), *Saccharomyces cerevisae* enolase (3ENL), *Aspergillus fumigatus* Asp f 6 (1KKC), latex Hev b 6.02 (1HEV), maize Zea m 14 (1MZL), kiwi Act c 1 (2ACT), oilseed rape Bra n 1 (1PNB), soyabean conglycinin (1IPK), soyabean glycinin (1OD5), turkey egg ovomucoid (1OMU), chicken egg Gal d 2 (1OVA), chicken egg Gal d 3 (1OVT), chicken egg Gal d 4 (1E8L), bovine milk Bos d 4 (1F6S), bovine milk Bos d 5 (1GXA), house dust mite Der f 2 (1AHK), house dust mite Der p 2 (1KTJ), carp Cyp c 1 (1B8R), bee venom Api m 1 (1POC), bee venom Api m 2 (1FCV), yellow-jacket venom Ves v 5 (1QNX). For single chain allergen molecules, secondary structure elements are displayed in red (helices), yellow (beta sheets), blue/white (loops and turns), with the exception of Der f 2. Each chain of oligomeric allergens is displayed in a different colour.

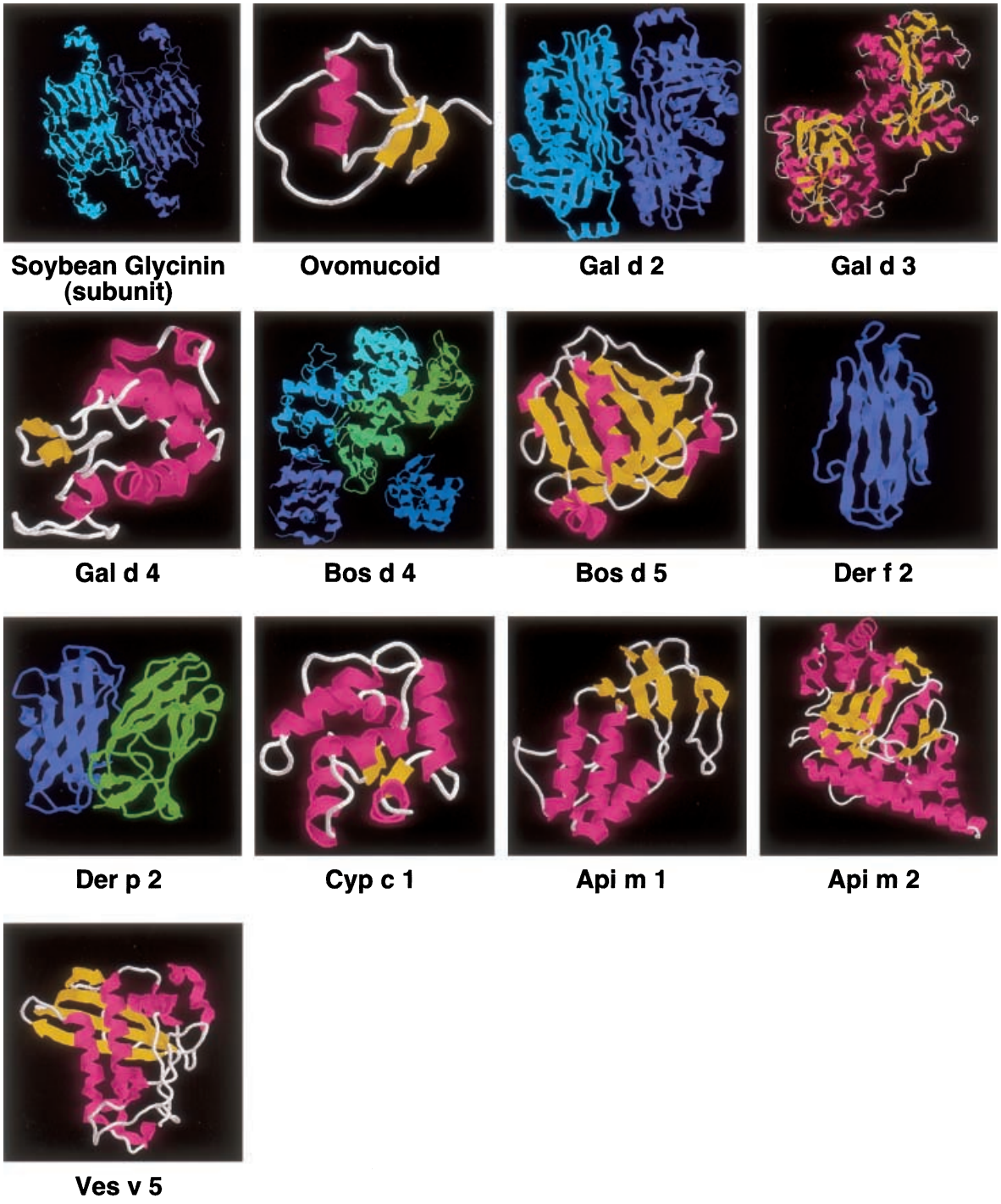

Figure 1. Continued.

correlation between profilin sensitization and clinical manifestation of allergic reactions or even autoimmune diseases might be partially explained by recent findings showing that mast cell exocytosed chymase cleaves human and birch pollen profilin causing a reduction in their IgE binding activity. The destruction of the IgE-binding epitopes of profilin by chymase might thus hinder further mast cell activation and limit the allergic responses to profilin in sensitized individuals (37).

Pollen calcium-binding proteins – polcalcins

Calcium-binding allergens containing 2 EF-hands (Bet v 4, Aln g 4, Ole e 3, Cyn d 7, Phl p 7 and Bra r 1), 3 EF-hands (Bet v 3), and 4 EF-hands (Jun o 4, Ole e 8)

Table 1. Allergenic molecules grouped on the basis of their functions and sources

Allergen	Function	Pollens			Fruits and vegetables	Legumes, nuts and seeds	Latex	Human
		Trees	Grasses	Weeds				
Fagales, group 1	Plant steroid hormone transporter, PR-10	***Aln g 1****			***Api g 1****	***Cor a 1.04****		
		Bet v 1*			***Dau c 1****	***Gly m 4****		
		Car b 1*			***Mal d 1****			
		Cas s 1*			***Pru ar 1****			
		Cor a 1*			***Pru av 1****			
		Fag s 1			***Pyr c 1****			
		Que a 1*						
Profilins	Actin-binding protein	***Bet v 2****	***Cyn d 12****	***Amb a ?***	***Ana c 1****	***Ara h 5****	***Hev b 8****	***Hom s ?***
		Car b 2	***Lol p 12***	***Art v 4****	***Api g 4****	***Bra n ?***		
		Cor a 2*	***Ory s 12***	***Che a ?***	***Aspa o ?***	***Cor a 2****		
		Fra e 2	***Phl p 12****	***Hel a 2****	***Cap a 2****			
		Ole e 2*	***Poa p 12***	***Mer a 1****	***Cit l ?***			
		Pho d 2*	***Zea m 12***	***Par j 3****	***Cuc m ?***			
		Pla a ?		***Zyg f ?***	***Cuc p ?***			
					Cuc s ?			
					Dau c 4*			
					Gly m 3*			
					Lit c 1*			
					Lyc e 1*			
					Mal d 4*			
					Mus xp 1*			
					Pru av 4*			
					Pru p 4*			
					Pyr c 4*			
Polcalcins	Calcium-binding protein	***Aln g 4***	***Cyn d 7***	***Amb a ?***				
		Bet v 3*	***Phl p 7***	***Art v ?***				
		Bet v 4*		***Bra n ?***				
		Car b ?		***Bra r ?***				
		Fra e 3		***Che a ?***				
		Jun o 4*		***Par j ?***				
		Ole e 3*						
		Ole e 8*						
		Syr v 3						
Oleaceae, group 1	Trypsin inhibitor	***Fra e 1****	**Lol p 11***	**Che a 1***				
		Lig v 1*	**Phl p 11***	**Pla l 1***				
		Ole e 1*		**Sal k ?**				
		Syr v 1*						
Thaumatins	PR-5	**Jun a 3***			**Act c 2***			
					Cap a 1*			
					Mal d 2*			
					Pru av 2*			
					Vit v ?			

* Allergenic molecules listed in the Official International Union of Immunological Societies Allergen Nomenclature website (http://www.allergen.org).
In bolditalic are reported crossreactive molecules. In bold molecules with function and sequence homologies but lacking demonstrated crossreactivity.
Act c, *Actinidia chinensis* (Kiwi); Aln g, *Alnus glutinosa* (Alder); Amb a, *Ambrosia artemisiifolia* (Short ragweed); Ana c, *Ananas comosus* (Pineapple); Api g, *Apium graveolens* (Celery); Ara h, *Arachis hypogaea* (Peanuts); Art v, *Artemisia vulgaris* (Mugwort); Aspa o, *Asparagus officinalis*; Bet v, *Betula verrucosa* (Birch); Bra n, *Brassica napus* (Rapeseed); Bra r, *Brassica rapa* (Turnip); Cap a, *Capsicum annuum* (Bell pepper); Car b, *Carpinus betulus* (Hornbeam); Cas s, *Castanea sativa* (Chestnut); Che a, *Chenopodium album* (Goosefoot); Cit l, *Citrullus lanatus* (Watermelon); Cor a, *Corylus avellana* (Hazel); Cuc m, *Cucumis melo* (Muskmelon); Cuc p, *Cucurbita pepo* (Zucchini); Cuc s, *Cucumis sativus* (Cucumber); Cyn d, *Cynodon dactylon* (Bermuda grass); Dau c, *Daucus carota* (Carrot); Fag s, *Fagus sylvatica* (Beech); Fra e, *Fraxinus excelsior* (Ash); Gly m, *Glycine max* (Soy); Hel a, *Helianthus annuus* (Sunflower); Hev b, *Hevea brasiliensis* (Latex); Hom s, *Homo sapiens*; Jun a, *Juniperus ashei* (Mountain cedar); Jun o, *Juniperus oxycedrus* (Prickly juniper); Lig v, *Ligustrum vulgare* (Common privet); Lit c, *Litchi chinensis*; Lol p, *Lolium perenne* (Rye grass); Lyc e, *Lycopersicon esculentum* (Tomato); Mal d, *Malus domestica* (Apple); Mer a, *Mercurialis annua* (Annual mercury); Mus xp, *Musa x paradisiaca* (Banana); Ole e, *Olea europaea* (Olive); Ory s, *Oryza sativa* (Rice); Par j, *Parietaria judaica* (Pellitory); Phl p, *Phleum pratense* (Timothy grass); Pho d, *Phoenix dactylifera* (Date palm); Pla a, *Platanus acerifolia* (London plane tree); Pla l, *Plantago lanceolata* (English plantain); Poa p, *Poa pratensis* (Kentucky blue grass); Pru ar, *Prunus armeniaca* (Apricot); Pru av, *Prunus avium* (Cherry); Pru p, *Prunus persica* (Peach); Pyr c, *Pyrus communis* (Pear); Que a, *Quercus alba* (White oak); Sal k, *Salsola kali* (Russian thistle); Syr v, *Syringa vulgaris* (Common lilac); Vit v, *Vitis vinifera* (Grape); Zea m, *Zea mays* (Corn); Zyg f, *Zygophyllum fabago* (Syrian bean-caper).

Table 2. Allergenic molecules grouped on the basis of functions and sources

Allergen	Function	Pollens			Fruits and vegetables	Legumes, nuts and seeds	Latex	Moulds	Human
		Trees	Grasses	Weeds					
Grasses, group 1	β-Expansin		***Agr a 1***						
			Ant o 1						
			Ave s 1						
			Cyn d 1*						
			Dac g 1*						
			Fes e 1						
			Fes p 1						
			Hol l 1*						
			Hor v 1						
			Lol p 1*						
			Ory s 1*						
			Pha a 1*						
			Phl p 1*						
			Phr a 1						
			Poa p 1*						
			Sec c 1						
			Sor h 1*						
			Tri a 1						
			Zea m 1*						
Grasses, group 5	Unknown		***Ant o 5***						
			Ave s 5						
			Cyn d 5						
			Dac g 5*						
			Fes e 5						
			Fes p 5						
			Fes r 5						
			Hol l 5						
			Hor v 5						
			Imp c 5						
			Lol p 5*						
			Pha a 5						
			Phl p 5*						
			Phr a 5						
			Poa p 5*						
			Sec c 5						
Ragweed, group 1	Pectate lyase	**Cha o 1**		**Amb a 1***					
		Cry j 1*		**Art v ?**					
		Cup a 1*							
		Cup s 1*							
		Jun a 1*							
Compositae, group 1	PR-12, defensin domain			**Art v 1***					
				Hel a ? (SF18)					
				Par h 1					

* Allergenic molecules listed in the Official International Union of Immunological Societies Allergen Nomenclature website (http://www.allergen.org).
In bolditalic are reported crossreactive molecules. In bold molecules with function and sequence homologies but lacking demonstrated crossreactivity.
Act c, *Actinidia chinensis* (Kiwi); Agr a, *Agrostis alba* (Redtop grass); Alt a, *Alternaria alternata*; Amb a, *Ambrosia artemisiifolia* (Short ragweed); Ant o, *Anthoxanthum odoratum* (Sweet vernal grass); Art v, *Artemisia vulgaris* (Mugwort); Asp f, *Aspergillus fumigatus*; Ave s, *Avena sativa* (Cultivated oat); Bra r, *Brassica rapa* (Turnip); Cand a, *Candida albicans*; Cas s, *Castanea sativa* (Chestnut); Cha o, *Chamaecyparis obtusa* (Japanese cypress); Cla h, *Cladosporium herbarum*; Cry j, *Cryptomeria japonica* (Japanese cedar); Cup a, *Cupressus arizonica* (Arizona cypress); Cup s, *Cupressus sempervirens* (Mediterranean cypress); Cyn d, *Cynodon dactylon* (Bermuda grass); Dac g, *Dactylis glomerata* (Orchard grass); Fes e, *Festuca elatior* (Reed fescue); Fes p, *Festuca pratensis* (Meadow fescue); Fes r, *Festuca rubra*; Hel a, *Helianthus annuus* (Sunflower); Hev b, *Hevea brasiliensis* (Latex); Hol l, *Holcus lanatus* (Velvet grass); Hom s, *Homo sapiens*; Hor v, *Horduem vulgare* (Barley); Imp c, *Imperata cylindrica* (Cogon grass); Jun a, *Juniperus ashei* (Mountain cedar); Lol p, *Lolium perenne* (Rye grass); Mus xp, *Musa x paradisiaca* (Banana); Ory s, *Oryza sativa* (Rice); Par h, *Parthenium hysterophorus* (Feverfew); Pen c, *Penicillium citrinum*; Pers a, *Persea americana* (Avocado); Pha a, *Phalaris aquatica* (Canary grass); Phl p, *Phleum pratense* (Timothy grass); Phr a, *Phragmites australis* (Common reed); Poa p, *Poa pratensis* (Kentucky blue grass); Rho m, *Rhodotorula mucilaginosa*; Sac c, *Saccharomyces cerevisiae* (Baker's yeast); Sec c, *Secale cereale* (Rye); Sor h, *Sorghum halepense* (Johnson grass); Tri a, *Triticum aestivum* (Wheat); Vit v, *Vitis vinifera* (Grape); Zea m, *Zea mays* (Corn).

Table 2. (Continued)

Allergen	Function	Pollens			Fruits and vegetables	Legumes, nuts and seeds	Latex	Moulds	Human
		Trees	Grasses	Weeds					
Enolases	Glycolytic enzyme						***Hev b 9****	***Alt a 11**** ***Asp f 22**** ***Cand a ?*** ***Cla h 6**** ***Pen c 22**** ***Rho m 1**** ***Sac c ?***	
SOD	Manganese super oxide dismutase						***Hev b 10****	***Asp f 6**** ***Sac c ?***	***Hom s ?***
Glucanase	PR-2				***Mus xp ?***		***Hev b 2****		
Chitinase	PR-3				***Act c ?*** ***Bra r 2*** ***Cas s 5**** ***Mus xp ?*** ***Pers a 1**** ***Vit v ?***		***Hev b 6.02**** **Hev b 11***		

domains were identified as pollen-specific cross-reactive proteins (Table 1). This group of allergens may thus be used as markers for multiple pollen sensitization (8). The IgE recognition of calcium-binding allergens seems to be conformation dependent and influenced by bound calcium. A comparison between allergens with 2-, 3- and 4-EF hand domains showed that Phl p 7 is the most cross-reactive allergen among polcalcins (38).

Oleaceae pollen – group 1

Ole e 1, the major allergen of olive pollen, is a polymorphic glycoprotein with three disulphide bonds. Several homologues have been identified in other Oleaceae pollen including Lig v 1, Fra e 1 and Syr v 1, which are responsible for a high degree of IgE cross-reactivity among Oleaceae plants (39) (Table 1). The IgE recognition of Ole e 1 depends on the integrity of the disulphide bonds (40). Moreover, the glycan moiety is able to bind IgE and seems to induce the release of histamine from basophils (41). Although Ole e 1-homologous proteins have been characterized in pollen from non-Oleaceae plants (e.g. grasses, English plantain and Chenopodium), IgE cross-reactivity seems to be very low. This is probably because of the low sequence similarity (30–44%) between Ole e 1 and non-Oleaceae homologues.

Thaumatin-like proteins (PR-5)

The PR-5 homologous allergens have been characterized in fruits (cherry Pru av 2, apple Mal d 2, kiwi Act c 2, grape Vit v ?), bell pepper (Cap a 1), and in pollen from mountain cedar (Jun a 3) (Table 1). Because of sequence similarities to thaumatin, the sweet-tasting protein from the African shrub *Thaumatococcus daniellii*, PR-5 proteins are also referred to as thaumatin-like proteins. Recombinant Mal d 2 was produced in tobacco plants and shown to exhibit antifungal activity against *Fusarium oxysporum* and *Penicillium expansum*, indicating a function in plant defense against fungal pathogens (42). Sequence similarities among thaumatin-like allergens indicate a potential for IgE cross-reactivity, although this has not yet been demonstrated.

Grass pollen – group 1 (β-expansin)

Group 1 allergens are the most prominent allergens in pollen from grasses and have been characterized in at least 19 grass species [reviewed by Andersson and Lidholm (43) (Table 2)]. They are polymorphic, N-glycosylated, and contain seven conserved cysteine residues in the N-terminal part of the protein. Group 1 allergens are homologous to expansins, a family of plant proteins involved in cell wall loosening and extension. The exact biochemical mechanism of action of expansins and the identity of their target site of action is still uncertain. Using purified natural and recombinant group 1 allergens (Phl p 1 and Lol p 1) extensive IgE cross-reactivity was demonstrated among different grass species (44, 45).

Five continuous IgE epitopes were identified for Phl p 1. These IgE epitopes seem to be conserved among group 1 allergens from other grass species. However, a considerable proportion of Phl p 1-specific IgE is also directed to conformational Phl p 1 epitopes (46).

Grass pollen – group 5

Group 5 allergens seem to be phylogenetically confined as they have been identified only in members of the Poodieae grass subfamily [reviewed by Andersson and Lidholm (43)]. Nevertheless, they are important cross-reactive allergens for the species where they are expressed (Table 2). Multiple sequence variants have

Table 3. Allergenic molecules grouped on the basis of functions and sources

Allergen	Function	Pollens			Fruits and vegetables	Legumes, nuts and seeds	Latex	Egg	Milk
		Trees	Grasses	Weeds					
nsLTP	Non-specific Lipid Transfer Protein, PR-14	**Ole e 7**		***Art v 3****	***Aspa o 1****	***Ara h ?***			
				Par j 1*	***Dau c ?***	***Bra o ?***	***Hev b 12****		
				Par j 2*	***Hor v ?***	***Bra r ?***			
				Par o 1*	***Lac s 1****	***Cas s 8****			
					Mal d 3*	***Cor a 8****			
					Pru ar 3*	***Jug r 3****			
					Pru av 3*				
					Pru d 3*				
					Pru du ?				
					Pru p 3*				
					Pyr c 3				
					Tri a ?				
					Tri s ?				
					Vit v 1*				
					Zea m 14*				
Plant proteases	Cystein protease				***Act c 1****	**Gly m 1**			
					Ana c ? (Bromelain)				
					Car p ? (Papain)				
					Fic c ? (Ficin)				
Plant albumins	2S albumin					***Ana o ?***			
						Ara h 2*			
						Ara h 6*			
						Ara h 7*			
						Ber e 1*			
						Bra j 1*			
						Bra n 1*			
						Jug n 1*			
						Jug r 1*			
						Pru du ?			
						Ric c 1*			
						Ric c 3			
						Ses i 1*			
						Ses i 2*			
						Sin a 1*			
Plant globulins (1)	7S globulin					***Ana o 1****			
						Ara h 1*			
						Cor a 11*			
						Gly m ?			
						Jug n 2*			
						Jug r 2*			
						Len c 1*			
						Pis s 1			
						Ses i 3*			
Plant globulins (2)	11S globulin					***Ana o 2****			
						Ara h 3*			
						Ara h 4*			

* Allergenic molecules listed in the Official International Union of Immunological Societies Allergen Nomenclature website (http://www.allergen.org).

† Reported cross-reactivity with homologous proteins in other avian eggs.

‡ Reported cross-reactivity with homologous proteins in other mammal milk.

In bolditalic are reported crossreactive molecules. In bold molecules with function and sequence homologies but lacking demonstrated crossreactivity.

Act c, *Actinidia chinensis* (Kiwi); Ana c, *Ananas comosus* (Pineapple); Ana o, *Anacardium occidentale* (Cashew); Ara h, *Arachis hypogaea* (Peanuts); Art v, *Artemisia vulgaris* (Mugwort); Aspa o, *Asparagus officinalis*; Ber e, *Bertholletia excelsa* (Brazil Nut); Bos d, *Bos domesticus* (Cow); Bra j, *Brassica juncea* (Oriental Mustard); Bra n, *Brassica napus* (Rapeseed); Bra o, *Brassica oleracea* (Broccoli); Bra r, *Brassica rapa* (Turnip); Car p, *Carica papaya* (Papaya); Cas s, *Castanea sativa* (Chestnut); Cor a, *Corylus avellana* (Hazel); Dau c, *Daucus carota* (Carrot); Fag e, *Fagopyrum esculentum* (Buckwheat); Fic c, *Ficus carica* (Fig); Gal d, *Gallus domesticus* (Hen); Gly m, *Glycine max* (Soy); Hev b, *Hevea brasiliensis* (Latex); Hor v, *Horduem vulgare* (Barley); Jug n, *Juglans nigra* (Black walnut); Jug r, *Juglans regia* (Walnut); Lac s, *Lactuca sativa* (Garden lettuce); Len c, *Lens culinaris* (Lentils); Mal d, *Malus domestica* (Apple); Ole e, *Olea europaea* (Olive); Par j, *Parietaria judaica* (Pellitory); Par o, *Parietaria officinalis* (Pellitory); Pis s, *Pisum sativum* (Garden pea); Pru ar, *Prunus armeniaca* (Apricot); Pru av, *Prunus avium* (Cherry); Pru d, *Prunus domestica* (European plum); Pru du, *Prunus dulcis* (Almond); Pru p, *Prunus persica* (Peach); Pyr c, *Pyrus communis* (Pear); Ric c, *Ricinus communis* (Castor bean); Ses i, *Sesamum indicum* (Sesame); Sin a, *Sinapis alba* (White mustard); Tri a, *Triticum aestivum* (Wheat); Tri s, *Triticum spelta* (Spelt); Vit v, *Vitis vinifera* (Grape); Zea m, *Zea mays* (Corn).

Table 3. (Continued)

Allergen	Function	Pollens			Fruits and vegetables	Legumes, nuts and seeds	Latex	Egg	Milk
		Trees	Grasses	Weeds					
				Ber e 2*					
				Cor a 9*					
				Fag e ?					
				Gly m ?					
Avian Proteins†	Ovomucoid							***Gal d 1****	
	Ovalbumin							***Gal d 2****	
	Transferrin							***Gal d 3****	
	Lysozyme							***Gal d 4****	
	α-Livetin							***Gal d 5****	
Mammal Proteins‡	Lactalbumin								***Bos d 4****
	Lactoglobulin								***Bos d 5****
	Casein								***Bos d 8****

been characterized in several species but no sequence similarities were found suggesting a possible functional and biologic role for group 5 allergens.

A major IgE-binding epitope was identified comprising the first alanine-rich motif (aa 56–165) of Phl p 5 (47, 48). In addition, synthetic peptides (49), site-directed mutagenesis and short deletions located critical amino acid residues or regions involved in IgE recognition of Lol p 5 (50) and Phl p 5 (51).

Ragweed pollen – group 1 (pectate lyase)

Group 1 allergens from ragweed pollen comprises a family of closely related proteins (52). Ragweed Amb a 1 and homologous allergens in cypress and cedar pollen display sequence similarities to pectate lyases. Japanese cedar Cry j 1 was shown to have pectate lyase enzyme activity (53). Because pectate lyase is a pectin-degrading enzyme involved in the formation of pollen tubes during germination and fruit ripening, it is widely distributed in the environment and could be considered a potential cross-reactive allergen (Table 2). However, studies demonstrating the role of Amb a 1 in IgE cross-reactions are lacking.

Compositae pollen – group 1 (PR-12)

The major allergen of mugwort pollen, Art v 1, is a secreted protein with an N-terminal cysteine-rich domain homologous to plant defensins and a C-terminal proline/hydroxyproline-rich region (54). Homologous allergens have been characterized in other *Compositae* pollen (55, 56), but no data is available concerning IgE cross-reactivity of this group of allergens (Table 2).

Enolases

The glycolytic enzyme enolase was first identified as an allergen in *Saccharomyces cerevisiae* (57). Subsequently, enolase was also shown to be an important allergen of the mould *Cladosporium herbarum* (58). Extensive IgE cross-reactivity was detected between enolases from several moulds including *C. herbarum, Alternaria alternata, Candida albicans, Aspergillus fumigatus, Penicillium citrinum, Rhodotorula mucilaginosa, Fusarium solani* (59–62). Enolase was also described in natural rubber latex and shown to cross-react with *A. alternata* enolase (63) (Table 2). A major IgE-binding epitope (aa 120–189) was identified, which cross-reacts with other mould enolases (59). As for profilins and polcalcins causing multiple pollen sensitization (8), IgE co-recognition of enolases from unrelated sources could be the molecular basis for multiple sensitization to fungi (64).

Manganese-superoxide dismutase

The MnSOD is a ubiquitous enzyme in prokaryotes and eukaryotes involved in physiologic responses to oxygen toxicity. It has been detected as a major allergen in *A. fumigatus* and shown to cross-react with human and *S. cerevisae* MnSOD (65). The natural rubber latex homologue, Hev b 10, also cross-reacts with the human and *A. fumigatus* MnSOD (66). Thus, MnSOD could be considered as a potential autoallergen of the mould-latex group (Table 2).

β-1,3-Glucanases (PR-2)

The majority of plant β-1,3-glucanases are endoglucanases hydrolyzing polymers of the β-1,3-glucans, essential components of most fungi. Hev b 2, a latex basic β-1,3-glucanase (67, 68), was shown to be involved in the latex-fruit syndrome (reviewed in (16, 18, 69, 70) and to cross react with homologous allergens from banana, potato and tomato (71) (Table 2).

Class I (basic) chitinases (PR-3)

In most cases plant chitinases are endochitinases that hydrolyze chitin polymers, which are major components

Table 4. Allergenic molecules grouped on the basis of functions and sources

Allergen	Function	Mites	Cockroaches and other Arthropods	Crustacean and Mollusks	Fishes and Amphibians	Nematodes	Insect venom
Mite, group 1	Cystein protease	**Blo t 1***					
		Der f 1*					
		Der m 1*					
		Der p 1*					
		Der s 1					
		Eur m 1					
Mite, group 2	Unknown	***Aca s 2***					
		Der f 2*					
		Der p 2*					
		Der s 2					
		Eur m 2*					
		Gly d 2*					
		Lep d 2*					
		Pso o 2					
		Tyr p 2*					
Tropomyosins	Muscle contraction control	***Blo t 10****	***Chi k 10****	***Cha f 1***		***Ani s 3****	
		Der f 10*	***Per a 7****	***Cra g 1***			
		Der p 10*		***Hal d 1***			
		Lep d 10*		***Hal m 1***			
				Hel as 1*			
				Hom a 1*			
				Met e 1*			
				Pan s 1			
				Par f 1			
				Pen a 1*			
				Pen i 1*			
				Pen m 1*			
				Pena o 1			
				Per v 1			
				Tod p 1*			
				Tur c 1*			
Parvalbumins	Calcium-binding protein				***Cyp c 1***		
					Gad c 1*		
					Gad m 1*		
					Ran e ?		
					Sal s 1*		
					Sco a 1		
					Sco j 1		
					Sco s 1		
					Sti l ?		
					The c 1		
PLA1	Phospholipase A1						***Dol m 1****
							Pol a 1*
							Pol e 1*
							Sol i 1
							Ves g 1
							Ves m 1*
							Ves s 1
							Ves v 1*
							Vesp c 1*
							Vesp m 1*
PLA2	Phospholipase A2						***Api m 1****
							Bom t 1
							Bom p 1*
Hyaluronidases	Hyaluronidase						***Api m 2****
							Bom p ?
							Dol m 2*
							Pol a 2*

Table 4. (Continued)

Allergen	Function	Mites	Cockroaches and other Arthropods	Crustacean and Mollusks	Fishes and Amphibians	Nematodes	Insect venom
							Pol e 2
							Ves g 2
							Ves m 2*
							Ves s 2
							Ves v 2*
Antigen 5	Unknown						***Dol a 5****
							Dol m 5*
							Pol a 5*
							Pol d 5
							Pol e 5*
							Pol f 5*
							Pol g 5*
							Pol m 5*
							Pol s 5
							Sol i 3*
							Ves f 5*
							Ves g 5*
							Ves m 5*
							Ves p 5*
							Ves s 5*
							Ves v 5*
							Ves vi 5*
							Vesp c 5*
							Vesp m 5*

* Allergenic molecules listed in the Official International Union of Immunological Societies Allergen Nomenclature website (http://www.allergen.org).
In bolditalic are reported crossreactive molecules. In bold molecules with function and sequence homologies but lacking demonstrated crossreactivity.
Aca s, *Acarus siro*; Ani s, *Anisakis simplex*; Api m, *Apis mellifera* (Honey bee); Blo t, *Blomia tropicalis*; Bom p, *Bombus pennsylvanicus* (American bumble bee); Bom t, *Bombus terrestris* (Bumble bee); Cha f, *Charybdis feriatus* (Crab); Chi k, *Chironomus kiiensis* (Midge); Cra g, *Crassostrea gigas* (Oyster); Cyp c, *Cyprinus carpio* (Carp); Der f, *Dermatophagoides farinae*; Der m, *Dermatophagoides microceras*; Der p, *Dermatophagoides pteronyssinus*; Der s, *Dermatophagoides siboney*; Dol a, *Dolichovespula arenaria* (Yellow hornet); Dol m, *Dolichovespula maculata* (White-faced hornet); Eur m, *Euroglyphus maynei*; Gad c, *Gadus callaris* (Codfish); Gad m, *Gadus morhua* (Atlantic cod); Gly d, *Glycyphagus domesticus*; Hal d, *Haliotis diversicolor* (Abalone); Hal m, *Haliotis midae* (Abalone); Hel as, *Helix aspersa* (Snail); Hom a, *Homarus americanus* (American lobster); Lep d, *Lepidoglyphus destructor*; Met e, *Metapenaeus ensis* (Greasyback shrimp); Pan s, *Panulirus stimpsoni* (Spiny Lobster); Par f, *Parapenaeus fissurus* (Shrimp); Pen a, *Penaeus aztecus* (Brown shrimp); Pen i, *Penaeus indicus* (Shrimp); Pen m, *Penaeus monodon* (Black tiger shrimp); Pena o, *Penaeus orientalis* (Shrimp); Per a, *Periplaneta americana* (American cockroach); Per v, *Perna viridis* (Mussel); Pol a, *Polistes annularis* (Paper wasp); Pol d, *Polistes dominulus* (Mediterranean paper wasp); Pol e, *Polistes exclamans* (Paper wasp); Pol f, *Polistes fuscatus* (Wasp); Pol g, *Polistes gallicus* (Wasp); Pol m, *Polistes metricus* (Wasp); Pol s, *Polybia scutellaris* (Wasp); Pso o, *Psoroptes ovis* (Sheep scab mites); Ran e, *Rana esculenta* (Frog); Sal s, *Salmo salar* (Atlantic salmon); Sco a, *Scomber australasicus* (Pacific mackerel); Sco j, *Scomber japonicus* (Spotted mackerel); Sco s, Scomber scombrus (Atlantic mackerel); Sol i, *Solenopsis invicta* (Fire ant); Sti l, *Stizostedion lucioperca* (Perch); The c, *Theragra chalcogramma* (Alaska pollack); Tod p, *Todarodes pacificus* (Japanese flying squid); Tur c, *Turbo cornutus*; Tyr p, *Tyrophagus putrescentiae*; Ves f, *Vespula flavopilosa* (Yellow jacket); Ves g, *Vespula germanica* (Yellow jacket); Ves m, *Vespula maculifrons* (Eastern yellow jacket); Ves p, *Vespula pensylvanica* (Yellow jacket); Ves s, *Vespula squamosa* (Southern yellow jacket); Ves v, *Vespula vulgaris* (Yellow jacket); Ves vi, *Vespula vidua* (Wasp); Vesp c, *Vespa crabro* (European hornet); Vesp m, *Vespa mandarinia* (Giant Asian hornet).

of the exoskeleton of insects and cell walls of most fungi. Hev b 6.01, a basic class I endochitinase has been identified as the major cross-reactive allergen in the latex-fruit syndrome [reviewed in (16, 18, 69, 70)]. Hev b 6.01 (prohevein) is post-translationally modified to yield an N-terminal fragment designated hevein (Hev b 6.02) with homology to PR-3 proteins and a C-terminal domain (Hev b 6.03) similar to PR-4 proteins. Hev b 6.02-homologous proteins were identified in banana, avocado, chestnut, kiwi, peaches, strawberries and citrus (Table 2). Recombinant endochitinase from avocado (rPers a 1) showed comparable enzymatic activity to the natural counterpart and inhibited fungal growth (72). Karisola et al. (73) showed that the IgE binding ability of hevein is essentially determined by its N-terminal and C-terminal regions and that major IgE-binding epitopes of hevein are conformational.

Nonspecific lipid transfer protein, nsLTP (PR-14)

Plant nsLTPs are involved in the transport of lipids and phospholipids across membranes. They also have potent antifungal and antibacterial activities. nsLTPs usually cause fruit allergy, particularly those of the Rosaceae family (apples, peach and apricot), without concomitant pollen hypersensitivity (Table 3). In addition, patients allergic to nsLTPs tend to have a higher rate of more severe symptoms, which can also reach multiple organs. This is in contrast to the food homologues of the Fagales group 1 allergens where pollen Bet v 1 seems to be the

Table 5. Major allergen databases and their most relevant features (updated July 2003)

Name	URL	Data source	Allergenic molecules	Allergen sequences	Biochemistry molecular biology	Clinical and epidemiological data	Internal search engine	Internal data cross-linking	Computational tools	User alert (newsletter)	References	Last update
Official Allergen Nomenclature (IUIS Subcommittee)	http://www.allergen.org	Submission	All	Yes	Yes	No	No	No	No	No	++	July 3, 2003
All-Allergy	http://allallergy.net	Literature	All	No	No	Yes	Yes	No	No	Yes	++	Not supplied
Allergome	http://www.allergome.org	Literature	All	Links	Yes	Yes	Yes	Yes	No	Yes	++++	25 July 2003
FARRP	http://www.allergenonline.com	Protein databases and Medline	All	Links	Yes	No	Yes	No	Yes	Yes	None	8 April 2003
Food Allergy Information Page (BIFSD)	http://www.iit.edu/s˜gendel/fa.htm	Literature	All	Links	No	No	No	No	No	No	+	Not supplied
Protall	http://www.ifrn.bbsrc.ac.uk/protall	Literature	Food	Links	Yes	Yes	Yes	No	No	No	+++	Not supplied
SDAP	http://fermi.utmb.edu/SDAP/sdap_src.html	Protein databases	All	Links	Yes	No	Yes	No	Yes	No	None	Not supplied

IUIS, International Union of Immunological Societies; FARRP, Food Allergy and Resource Program; BIFSD, Biotechnology Information for Food Safety; SDAP, Structural Database of Allergenic Proteins.

sensitizing molecule and leads to more mild and localized cross-allergic reactions. These differences were attributed to the structural features of nsLTP, which has four disulphide bridges keeping four alpha helices packed together and results in extreme resistance to proteolysis, heat denaturation and pH changes. This enables them to survive the digestive tract environment, to cause IgE sensitization and to elicit severe symptoms. Therefore, they behave as complete food allergens (17, 19, 74). Interestingly, allergens showing limited sequence homology to fruit LTPs have also been described in *Parietaria* pollen (Par j 1) and soyabean (Gly m 1). The major IgE-binding epitope of Par j 1 was mapped to the first 30 amino terminal residues, with Cys14 and Cys29 being essential for maintaining the structure of the epitope (75).

Plant cysteine proteases

This class of proteases includes enzymes from fruits such as papain from papaya, ficin from fig, bromelain from pineapple and actinidin from kiwi (Table 3). The IgE cross-reactions were shown among these plant cysteine proteases (76–79), but not with group 1 cysteine proteases from house dust mites.

Plant albumins and globulins: seed storage proteins

Seed storage proteins have been traditionally characterized according to their solubility as albumins (soluble in low-salt buffers) and globulins (soluble in high-salt buffers), and by their sedimentation constants (e.g. 7S, 11S globulins and 2S albumins). Albumins and globulins are the major seed storage proteins of angiosperms and important cross-reactive food allergens (4, 17).

The 2S albumins are heterodymeric proteins with the two subunits linked by disulphide bonds. Allergenic 2S albumins were identified in mustard seeds (Sin a 1, Bra j 1), oilseed rape (Bra n 1), Brazil nut (Ber e 1), walnut (Jug r 1), peanut (Ara h 2, 6, 7) and others (see Table 3). Although one immunodominant linear epitope was identified in Jug r 1, strong evidence for the existence of conformational epitopes was also obtained (80).

The 7S globulins or vicilins are trimeric proteins and were identified as allergens in peanut (Ara h 1), cashew nut (Ana o 1), walnut (Jug r 2), soyabean (conglycinin) and several other nuts and seeds (Table 3). Overlapping peptides were used to identify the IgE-binding epitopes of Ara h 1. At least 23 different linear IgE-binding epitopes, located throughout the length of Ara h 1, were identified. Four of the peptides were immunodominant IgE-binding epitopes being recognized by more than 80% of the patients tested (81).

Legumins or 11S globulins are hexameric proteins. Each subunit is made up of an acidic and a basic chain linked by disulphide bonds. Among others, allergenic 11S globulins were characterized in peanut (Ara h 3, 4),

hazelnut (Cor a 9), cashew nut (Ana o 2), and soyabean (glycinin G1 and G2). The IgE-binding epitopes of glycinin G1 acidic chain were mapped to residues G217–V235 and G253–I265 and are similar to those identified for Ara h 3 (82).

Avian proteins

Allergens have been described in both egg white and yolk [reviewed by Poulsen et al. (83)]. The egg white proteins, ovomucoid, ovalbumin, ovotransferrin and lysozyme, were named in the allergen nomenclature as Gal d 1–d 4, respectively (Table 3). The egg yolk allergen, alpha-livetin (chicken serum albumin), was designated Gal d 5. Extensive cross-reactivity has been described among various avian eggs (84), but the clinical significance has not been extensively evaluated. Gal d 5 was identified as a cross-reactive allergen in the bird feather-egg-syndrome (85) and associated with reactions to chicken meat (86–88).

Mammalian proteins

Several milk proteins have been identified as allergens including alpha-lactalbumin (Bos d 4), beta-lactoglobulin (Bos d 5) and casein (Bos d 8) [reviewed by Wal (89); Table 3]. Both linear and conformational IgE-binding epitopes were identified in milk allergens. However, clear relationships between structure and allergenicity are not yet established. For casein, a highly conserved region corresponding to the major phosphorylation site was found to be an immunodominant IgE-binding epitope. Cross-reactivity among a variety of mammalian milk has been shown in *in vitro* and oral challenge studies (90–92). Interestingly, camel and mare's milk showed a low level of cross-reactivity with milk from cow, sheep, goat and others.

Mites – group 1 (cysteine protease)

Group 1 allergens from house dust mites are highly polymorphic cysteine proteases (93) that induce both species-specific and cross-reactive IgE antibodies. This causes a diversity of results for cross-reactivity tests (94). A recent study showed a lack of IgE cross-reactivity between Der p 1 and Blo t 1, very likely because the two allergens share only 35% sequence homology (95).

Mites – group 2

Mite group 2 allergens Der p 2, Der f 2 and Eur m 2 share 83–85% sequence identity (93) and are highly cross-reactive. However, Lep d 2 and Tyr p 2 did not react with sera from patients with IgE to *Dermatophagoides* species. The allergenic cross-reactivity between Der p 2, Der f 2, and Eur m 2 seems to be due to a conserved antigenic surface, whereas the lack of cross-reactivity with Lep d 2 and Tyr p 2 correlates with multiple substitutions across this surface (96).

Tropomyosin

The muscle protein tropomyosin is an important allergen in many invertebrates including shrimp (Pen a 1), cockroach (Per a 7), and house dust mites (Der p 10 and Der f 10). Because of a high degree of conservation and sequence identity, strong IgE cross-reactivity was reported among tropomyosins from various invertebrate species (94). Using synthetic peptides, Ayuso et al. (97) showed that Pen a 1-specific IgE antibodies recognize homologous amino acid sequences in Der p 10, Der f 10, Per a 7 and Hom a 1allergens, thus providing the molecular basis of arthropod cross-reactivity. In this context, studies have shown that patients allergic to house dust mite and/or cockroach show IgE reactivity to shrimp Pen a 1 without previous exposure to shrimp (10) or that mite immunotherapy can induce allergic reactions to shrimp and snail (98).

Parvalbumin

Parvalbumin is the dominating fish allergen (83). Fish parvalbumin is a very stable calcium-binding protein: exposure to extremes in pH and temperature do not alter its IgE reactivity. Similarly to calcium-binding proteins from pollen, depletion of calcium drastically reduces IgE binding to fish parvalbumins (99). Recombinant carp parvalbumin was used to demonstrate IgE cross-reactivity with cod, tuna and salmon parvalbumins (100).

Phospholipase A1 and A2, hyaluronidase

Several major venom allergens from different insects of the Hymenoptera order have been characterized (101). Phospholipases A1 isolated from venom of three species of yellow jackets, white-faced hornets, European hornets and paper wasps showed variable IgE cross-reactivity, suggesting that there are multiple antigenic determinants and that individuals respond to different determinants. No general patterns of cross-reactivity could be observed (102–104). Vespid phospholipase A1 has no sequence similarity and no cross-reactivity with phospholipase A2 from bee venom. Cross-reactivity between hyaluronidases from *Vespula vulgaris* and *Dolichovespula maculata* has been demonstrated (103, 104).

Insect venom antigen 5

The antigen 5 (Ag5) proteins from various *Vespula* species share about 95% sequence identity and are highly cross-reactive. *Dolichovespula* and *Polistes* Ag5 allergens show 58–67% sequence homology and display only partial cross-reactivity within the Vespidae family (101). Conserved surface patches of the yellow-jacket Ves v 5

and homologous allergens in various yellow-jackets, hornets and paper wasps were suggested to be involved in their antigenic cross-reactivity (105).

Cross-reactive carbohydrate determinants

Carbohydrate side chains of allergenic glycoproteins contribute to IgE cross-reactivity. Therefore, characterization of postsynthetic modifications is highly relevant for the definition of the allergenic structure. The biochemical features and their diagnostic relevance have been recently reviewed by van Ree (106). Using allergenic extracts, IgE antibodies directed toward glycans seem to show the widest pattern of cross-reactivity among those presently known (107, 108). It mainly involves plant- and insect-derived glycoproteins. Fucose and xylose glycan residues are relevant for IgE cross-recognition (109). Among allergenic plants and insects, several native molecules have been reported carrying one or more IgE-binding glycan side-chains (Grass groups 1,4,11,13, Oleaceae group 1, Parietaria group 1, Api g 5, Api m 1, Ara h 1, Art v 1, Cry j 1, Cup a 1, Hev b 2, Lyc e 2, Par h 1, Pla l 1).

The IgE-binding carbohydrates were also described in other nonallergenic plant-derived molecules (e.g. pineapple bromelain, horseradish peroxidase, maize polyamine oxidase, *Cucurbita pepo* ascorbate oxidase, kidney bean phytohaemagglutinin) (108–111). The presence of nonallergenic IgE-reactive glycoproteins in unrelated sources may thus influence and extend the pattern of CCD reactivity when allergenic extracts are tested (108, 112). Moreover, the CCD-IgE co-recognition of similar carbohydrate structures on unrelated sources may lead to *in vitro* false positive results in diagnostic tests (14).

The clinical relevance of CCD is still questioned. Several reports demonstrating poor or absent biological activity of CCD (112, 113) were recently followed by studies showing *in vivo* (HRP) (108) or *in vitro* (Api g 5, HRP, Lyc e 2, Ole e 1) (41, 114, 115) histamine release activity of certain glycoproteins. Such biologic activity seems not to be shared by all CCD-bearing molecules and not present in all CCD-IgE reactive subjects (108). Differences in the glycan number or affinity of IgE antibodies could account for such differences (106).

Bioinformatics applied to allergens: can cross-reactivity be predicted?

An interesting approach to study relationships of allergenic molecule is to compare protein structures using computer algorithms (116). This approach is becoming increasingly relevant as genetically modified foods must undergo evaluation for their allergenicity (117–120). The sequence comparison approach is commonly used when the biological nature of a new allergenic protein must be identified. Dedicated algorithms (BLAST, FASTA, ClustalW) available within protein and nucleotide sequence platforms (EMBL, SwissProt, Expasy, PIR) allow the comparison of a new given sequence with all the protein sequences submitted to the databases. This search verifies if any protein with a variable degree of identity, similarity or homology has ever been found before in other organisms. In the case the identified sequence belongs to an already known group of allergenic molecules the possible immunological relationship might be suspected (121).

As the number of identified allergenic molecules increase efforts are taken to create dedicated resources for allergenicity search (122). Search tools available within such databases allow the screening of linear amino acid sequences within known allergenic proteins (121, 122). This approach has been recently compared and implemented with the motif-based analysis resulting in a greater chance to identify allergenic proteins (123). A further improvement in the protein evaluation is the comparison considering their 3D-structure (124). This should overcome the difficulties of linear sequence comparisons, which does not disclose conformational epitopes of allergenic molecules.

A current limitation of computer-based approaches to predict allergenicity is the lack of general rules that can be applied to any molecule. The presence of 'unique allergens' (123), i.e. allergenic molecules without similar counterparts, suggest the existence of potential new allergens. This can be expected as the list of allergenic sources and allergenic molecules is still expanding and some of the available allergen sequences are only partial sequences (123).

The structural relationship between plant-derived proteases (papain, bromelain and chymopapain) was used to model the 3D structure of the major mite allergen, Der p 1 (125), although there is no *in vitro* IgE data or clinical evidences corroborating this relationship. The functional and structural similarities among proteins can be restricted to a portion of the molecule (e.g. enzymatic active site) and have no implications for allergic reactions. Similarly, the sequence similarity between some allergenic proteins (e.g. Ole 1 and Phl p 11, CBP from fish and plants) has never found an immunochemical, epidemiological, or clinical support. A future strategy should consider defining criteria to be applied in the identification of new allergenic molecules. This should lead to the most complete nonredundant catalogue of allergenic molecules. At the moment, an integrated approach using molecular- and clinical-based evidences is highly recommended.

Clinical considerations of cross-reactivity in food allergies

The following comments will focus on practical aspects of symptomatology, diagnosis and therapy of allergic cross-reactivity to foods.

Pollen-associated food intolerance

Up to 80% of birch-pollen allergic patients suffer from immediate itching in the mouth and throat as well as local edema after eating a variety of fruit, nuts and vegetables, the so-called oral allergy syndrome (OAS). This condition is characterized by the symptoms mentioned because of cross-reactivity between aeroallergens, the initial source of sensitization and ingested allergens. Aeroallergens may be birch- (e.g. tree nuts, pomaceous fruit and stone-fruit), grass- (e.g. legumes, tomatoes), ragweed- (e.g. melon) or mugwort-pollen (e.g. celery, spices), the list of related foods is ever expanding. If one considers the continuous increase of pollen-allergic patients, this represents a growing problem. Most patients suffering from inhalant pollinosis react to two or more cross-allergic foods.

A significant proportion, namely 8.7% of patients with the OAS also reacts with systemic symptoms (126). Definition of allergens has led to clinically significant conclusions: the symptoms related to Bet v 1-related proteins tend to be mild (patients with correlated pollinosis, mainly in the northern parts of Europe), whereas the correlation with lipid-transfer-proteins (observed mainly in the southern parts of Europe) seems to be associated with more severe clinical symptoms.

Many patients tend to continue consumption of fruit or vegetables they react to ignoring mild oral symptoms, but their attention should be drawn to the principal possibility of anaphylactic reactions. This risk of systemic reactions increases with clinical allergy without sensitization to pollen, positive skin tests, systemic symptoms to another related food and history of reactions to cooked foods, the latter usually being tolerated. Regarding skin tests, patients reacting to commercial extracts are more likely to experience severe reactions than patients reacting to fresh food only (127).

A birch pollen specific T-cell response has been hold responsible for worsening atopic eczema in birch pollen-allergic patients after oral challenge with birch pollen-related foods (128).

Latex-associated food allergy

Between 30 and 80% of patients with latex allergy report symptoms (anaphylaxis, asthma, eczema and OAS) to associated foods. An increasing number of mainly 'exotic' fruit and vegetables (e.g. banana, avocado, kiwi, fig, pineapple, papaya, passion fruit, peach, pear, walnut, hazelnut, almond, grapefruit, melon, strawberry, potato, tomato, spinach, lettuce, celery and many spices) has been associated with latex allergy since the first report in 1989 (129). These foods belong to different botanical families. Evaluation often gets complex because of multiple possible cross-reactions with different pollen and foods. Patients with concomitant pollinosis tended to react with celery and Rosaceae fruit. The sequence of sensitization is still not clear.

Peanut, soyabean and other legumes

Peanut-allergic patients do not seem to react to other legumes like beans, peas, lupines, lentils, even if *in vitro* and skin tests show sensitization (130, 131). Thus elimination of all legumes in patients who react to only one legume seems to be unwarranted. Only soyabeans have been shown to cross-react and cause severe symptoms, but at this time there are not enough data to recommend soyabean avoidance in soya-tolerant, peanut-allergic patients (132). Cross-sensitization between peanut and tree nuts has been reported in up to 50% of atopic patients without significant clinical relevance.

Peanut is the most common food causing life-threatening reactions mainly in the American region, partly perhaps because of the cooking practice after one study (133): roasting seems to enhance the allergenicity of peanuts. Refined peanut oil does not contain considerable allergens (134) because of the use of very high temperatures, whereas other modes of processing seem to have little influence on allergenicity. A recent study reported loss of reactivity in about 20% of children, a good news relativized by the possibility of recurrence (135). Mothers should avoid peanuts during pregnancy and lactation, and peanut proteins should not be introduced to infants for the first 3 years (136). As peanut allergy is responsible for about 50% of deaths caused by food allergy and of an ever increasing incidence and prevalence not only in North America (137), intensified research has led to new therapeutic and prophylactic measures, namely anti-IgE therapy increasing the threshold of sensitivity (138) and long-term protection through vaccination with recombinant proteins in a mouse model (139).

Tree nuts

As with peanuts, clinical reactions to tree nuts tend to be severe and potentially fatal. Cross-reactions with peanuts have been described, and tree nuts are important allergens in the birch-pollen-food association. Several studies have demonstrated multiple sensitivity to tree nuts, even if there is a lack of studies with oral food challenges (OFC) because of the frequency of severe anaphylaxis. The latter also justifies recommendations to avoid the whole family of nuts, even if this seems unnecessary in many cases. Contrary to the situation with peanuts, roasting of hazelnuts seems to reduce allergenicity (140).

Milk and mammalian meat

Cow's milk allergy is a common disease mainly during childhood. Changing to milk of other animal species to avoid allergic reactions is seldom successful as extensive cross-allergies to goat's milk (92% cross-reactivity!) (141) and sheep's milk have been described. Only camel's milk in an *in vitro* – study (91) and mare's milk in a clinical study (92) seem to be useful in this respect. Cross-reactions of

milk-allergic children to beef have been observed in nearly 10% (142), cooking reducing the allergenicity of beef (143). The development of allergy to mare's milk after inhalation allergy to horse dander has been reported (144), as well as respiratory allergy to cow dander and food allergy to cow's milk, the so-called milk-dander syndrome (145). The porc (pork)–chat (cat) syndrome (146) has been described as cross-allergy between cat dander and pork meat, that is cross-reactivity between dander and meat of two different mammalian species.

Eggs and avian meat

A number of cross-reacting proteins between different avian eggs have been described (84), but there are still not enough data for clinical complications. A case of clinical reactivity to eggs from duck and goose without sensitization to hen egg proteins has been described (147).

Even if avian meat allergy is relatively rare (148), cross-reactions to different kinds of avian meat occur especially if the patient does not react to eggs (87, 88). We saw a patient who developed OAS and asthma to chicken-, duck-, goose-, turkey hen-, quail-, guinea fowl- and even fish eagle- and/or owl-meat (offered in the jungle of Sarawak) without reacting to eggs (T. Hawranek, unpublished data). Sensitization to α-livetin found in feathers, avian meat and egg (Gal d 5) seems to be responsible for the so-called bird-egg syndrome (149), a syndrome combining allergy to egg yolk with pre-existing inhalation allergy to bird feathers (150). There also exists a so-called egg-bird syndrome (151), where allergy to birds follows that to eggs.

Cereal grains

Cross-reaction to multiple grains (wheat, oat, barley, rye, millet, sorghum, maize and rice) is possible, but uncommon. Although cross-sensitization in skin prick tests (SPT) is a frequent finding, only 21% of 145 children with positive SPT reacted clinically (152). The early introduction of cereals into children's diet was reported to be a risk factor for grass pollen asthma (153). In the diagnostic workup of anaphylactic reactions after consumption of flour one should also consider the possibility of anaphylaxis because of ingestion of mites in mite-contaminated food in mite-sensitized patients (154, 155).

Fish

Allergic reactions have to be distinguished from nonallergic (i.e. toxic or histamine-caused) symptoms. Cross-reaction between different fish is very common and may demand avoidance of the whole group, even if allergy to isolated species has been described. As fish represents an important food, oral challenge tests often are unavoidable. Processing such as cooking and canning leads to a reduction of allergenicity (156).

Crustaceans and other shellfish

Invertebrate tropomyosin is the panallergen linking crustaceans (crabs, lobster and shrimps) with mollusks (squids, scallops, clams and oysters) as well as parasites (e.g. anisakis) and insects (e.g. house dust mites). Cross-reaction between different crustaceans is common and often severe and may demand avoidance of the whole group, those between crustaceans and mollusks is reported, but not well defined. Oral challenge tests often are desired by the patients. The rate of cross-reactions with mollusks seems to be lower. Both in crustacean- and mollusk-allergic patients, specific immunotherapy (SIT) with house dust mites may represent a risk (see below).

House dust mites

There are multiple reports on cross-reactivity between house dust mites and snails, the symptomatology ranging from urticaria to asthma and anaphylaxis. Clinically less well documented are cross-reactive allergens of crustaceans and bivalves (94). As in most other cross-relations between inhalant allergens and food-allergens, the latter seems to be the consequence of the former.

Liliaceae

Bronchial asthma, rhinoconjunctivitis, contact dermatitis and anaphylactic reactions have been described for the subfamily of aliolideas (garlic, onion, chives and leek) (157–164), but despite their wide use their occurrence seems to be rare. Cross-reactivity has been demonstrated between aliolideas and asparagoidea (asparagus). Though there are common epitopes, there seems to be a much smaller amount of allergen in onion than in asparagus (165).

Moulds and mushrooms

Similar to cross-reactions between respiratory allergy to animal dander and to food derived from the same animals, the coincidence of respiratory allergy to moulds and food-allergy to mushrooms has been reported several times (166–168). In one such patient cross-allergy to spinach was described (169).

Diagnosis issues

Diagnostic tests for food allergy pose problems not yet solved, especially with regard to cross-reactive allergens. These tend to elicit positive *in vitro* and *in vivo* tests without clinical relevance, whereas commercially available food extracts often lead to false-negative results, partly because of a lack of standardization as well as enzymatic degrading processes. Prick-to-prick tests with fresh food show a high sensitivity and are used widely,

remaining the reference method of skin tests to diagnose food allergy, but are not standardized at all, bear the risk of systemic reactions and often give false-positive results. Determination of specific IgE by RIA (radio immuno assay) or by FEIA (fluorescence enzyme immuno assay) proves sensitization, but says little about clinical relevance even if the risk of a clinical reaction rises with increasing concentration of specific IgE (170). A negative test result makes clinical relevance unlikely. In infants under the age of 1 year a positive RAST-result suggests clinical relevance because tolerance has hardly developed yet. An essential test for research in cross-allergy is the so-called RAST (enzyme-linked immunosorbent assay)-inhibition test, performed to rule out coincidental cross-reactivity and to prove that there exist common epitopes between two foods for example. Patch tests with food may be a helpful tool mainly in the diagnosis of delayed-type reactions (171).

The definition and use of recombinant allergens promises to lead to an improvement of this situation, i.e. to be able to differentiate between clinically irrelevant and relevant sensitization and by this hopefully allow to predict which food may bear danger when eaten for the first time. The expected benefit of cooking may be predicted as well. After first successes with rApi g 1 from celery (172) a recent study (173) demonstrated that recombinant allergens may ameliorate the clinical meaningfulness of diagnostic tests by associating severe clinical symptoms of Spanish patients allergic to cherry with sensitization to recombinant Pru av 3, whereas Swiss patients with less severe symptoms mainly bound to Pru av 1 and Pru av 4, which are related to birch pollen Bet v 1 and Bet v 2, respectively.

As the clinician is left with positive and/or negative results of *in vitro* – and skin tests of uncertain clinical relevance, OFC, particularly the double-blind placebo-controlled food challenge (DBPCFC), after more than 50 years (174) still represent the current mainstay in the diagnosis of a food reaction to avoid extensive unnecessary diets. Clinical history may only be validated in $< 50\%$ by OFC (175). Numerous studies (170, 176–180) have demonstrated that exact evaluation of SPT and specific IgE may reduce the need to perform OFC by approximately 40%, but this is only of little help while clarifying an individual case. A minute clinical history of tolerance has to be executed before challenge tests. Most studies prove that the majority of cross-reactive foods are tolerated, even if positive *in vitro* or skin tests precede. Because of the lability of many cross-reactive proteins, special attention has to be paid to prepare the foods for oral provocation tests. Processing methods like heating (156), fermentation (181), treatment with acidic oxidative potential water (182), freezing or peeling (183) may reduce the allergenicity, but not always for every food (184–186). Because of the possibility of false-negative results because of processing, an open challenge with the 'natural' food has to prove nonreactivity even after a negative blinded challenge test. The decision to perform oral challenge tests has to be discussed with the patient, depending on food preferences, dietary needs or other circumstances. Epidemiological evidence for a possible reaction may help. A table of the approximate risk of clinical reactivity to related foods has been drawn up recently (4). Many patients are content to abstain from foods they have reacted to in the past or foods that got suspicious because of positive *in vitro* or skin tests or known cross-reactivity.

Therapeutic options

Avoidance of foods which have been proven to cause clinical symptoms is the logic most important step to escape new anaphylactic reactions. Patients have to check all food labels and to avoid high-risk situations, mainly eating out. Because of 'hidden allergens', i.e. the contamination of a safe food by utensils or equipment, misleading labels and ingredient switching (187) this often proofs to be an impossible task.

As disodium cromoglycate has to be taken before exposition of the allergenic food, it always comes too late. If consumption has taken place, an interesting starting-point is the prevention of ongoing resorption of food allergens (e.g. peanuts) from the gastrointestinal tract by administering activated charcoal which forms insoluble complexes with peanut proteins no longer able to bind IgE (188). The same mechanism may work for other foods, too.

For birch pollen-associated food allergy SIT with the according pollen has shown to be successful in most studies and case reports (189–193) with symptom reduction rates of up to 84%. The positive effect in a subset of patients both negative clinically and in SPT by the end of SIT was still demonstrable after 30 months in 78% (194). Relapses of the OAS was preceded or associated with a relapse of skin reactivity in all cases. No effect by subcutaneous and oral SIT was observed in an early study (195). One paper reported success with mugwort/ragweed SIT in a patient with associated OAS to fennel, cucumber and melon (196). The SIT with house dust mite has been associated with the induction (98, 197, 198) or deterioration (199) of snail allergy – suggesting to avoid SIT to house dust mites in patients with snail allergy – as well as protection against possible sensitization to snails (200).

The idea of using sublingual/swallow immunotherapy (SLIT) at the location of the OAS seems to be promising, but no data are available until now to the best of our knowledge. The procedure seems to be safe (201).

Sublingual desensitization with the respective native food the patient is allergic to (202–206) represents an interesting alternative starting-point for the treatment of food-allergic patients and has met partial success, even if

the long-term effect of this treatment has yet to be assessed and some studies are poorly controlled. A well-controlled trial with subcutaneous desensitization in peanut-allergic patients (207) showed limited response rates and a high rate of adverse reactions.

One may speculate about future therapies (208) using SIT with panallergens, mutated allergen protein immunotherapy, antigen-immunostimulatory sequence-modulated immunotherapy, peptide immunotherapy, plasmid-DNA immunotherapy, cytokine-modulated immunotherapy and anti-IgE monoclonal antibody therapy. Recently, successful prophylaxis of peanut allergy with a monoclonal anti-IgE-antibody (TNX-901) has been described (138).

For patients with anaphylactic symptoms after food intake, an emergency kit comprising epinephrine, an antihistamine and a corticosteroid should be prescribed. Whether all patients should be provided with injectable epinephrine is widely discussed (209–213), mainly among paediatricians, even if it is regarded as the only medication that is active against collapse. Kemp (214) proposes to take into account several risk factors: age over 5 years, history of respiratory tract involvement in previous reactions, history of asthma requiring preventer medication, peanut or tree nut sensitivity, reactions induced by small amounts of the allergen (215, 216), and perhaps a strongly positive SPT. This issue has yet to be answered, and each case has to be handled individually. As asthma is the main cause of death because of food anaphylaxis and epinephrine is not always sufficient in severe asthma (217), the addition of a rapid-action β-agonist spray to the emergency kit has been suggested (218). Often, the application of epinephrine spray is recommended in case of laryngeal oedema. The use of this emergency kit should be trained with patients, parents of food allergic children, as well as teaching and caring staff of children, as the kits are used appropriately in only 29% of subsequent anaphylactic reactions (219), a number confirmed by most other reports.

Conclusions and future perspectives

The impact of the huge number of studies on allergenic molecules and extracts and their relationships is highly relevant for the clinical allergologist. Of particular interest are those studies showing how the clustering of the allergenic source reactivity can be explained by structural relationships between allergens (8, 38). Before the knowledge about the molecular composition of allergenic extracts and their relationships became available, the general assumption was that patients might show reactivity from one to hundreds of allergenic sources. This view has quickly changed with the discovery of family-restricted homologous molecules [e.g. group 1 grasses (43)] or broad-reactive allergens (e.g. panallergens as plant profilins (36), plant calcium-binding proteins (8, 38), and arthropod/crustacean tropomyosin (97). The analysis of reactivity clusters in diagnosis allows the interpretation of patient's reactivity as the main outcome of the sensitizing process, which often begins with exposure to a single source. A number of different clustering options can be defined within these two extremes. For instance, group 1 Fagales allergen sensitization might include reactivity to pollens belonging to different families as well as plant-derived food taxonomically distant from the Fagales order (36). By the extensive use of the molecule-based approach it is possible to reach the finest resolution of the reactivity spectrum. As for the bioinformatics approach to allergenicity of a molecule, a novel framework should be developed for a joined work of molecular biologists and clinical allergologists.

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