

REVIEW

The biochemistry of common aeroallergens

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Introduction

In recent years, our knowledge of the structure of many of the important aeroallergens has increased greatly due to the application of sophisticated molecular biological and protein chemistry techniques. We now know the complete amino acid sequences of more than 50 allergens, and the number of clinically significant allergens remaining to be sequenced is rapidly growing smaller. The data obtained from such studies have been extensively reviewed [1] and they clearly indicate that, from a structural standpoint, allergens are indistinguishable from conventional antigens, and that factors known to contribute to antigenicity *per se* will apply to allergens entering the respiratory tract. However, it has become apparent too that many allergens demonstrate a range of biochemical activities suggesting these properties may also contribute to immunogenicity [2]. In this article, the available information on the physicochemical characteristics and biochemical properties of many of the individual protein allergens from common aeroallergenic sources such as plants, fungi and bacteria (Tables 1–6), dust mites and insects (Table 7, 8), animals (Tables 6, 9) and birds (Table 10) have been collated.

The data indicate that the majority of allergens can be divided into several broad groupings, based either on demonstrable biological activity or significant homology with proteins of known function. They include (a) hydrolytic and non-hydrolytic enzymes, (b) enzyme inhibitors, (c) proteins involved in transport and (d) regulatory proteins. The general properties of the allergens comprising these groups are discussed, as is the potential importance of such activities on immune responses to allergens. Throughout this review, we have used the recently recommended allergen nomenclature [3], trivial biochemical names or, in the case of enzymes, the appropriate Enzyme Commission (EC) nomenclature. In addition, we have used the term 'major' only to describe allergens recognized by the majority of individuals sensitive to a particular allergenic source.

Hydrolytic enzymes

Many of the more important domestic and occupational allergens from plants, bacteria, fungi and dust mites are hydrolytic enzymes (EC group 3). These proteins play important roles in a variety of physiological processes and are usually involved in cleaving polymeric substrates by the addition of water. They include proteases, carbohydrases and ribonucleases [4–8].

Proteases

Proteases are divided into four groups based on either the amino acid or metal ion (e.g. zinc) involved in catalysis. They include the serine, cysteine, aspartate and the metalloproteinases. A number of clinically significant allergens possess proteolytic activity (Tables 5, 6, 8) but the most important are those derived from dust mites, plants, mammals and bacteria. The more common allergenic proteases belong to the serine and cysteine protease groups, although allergens belonging to the other two groups have been described (Table 5, 6, 8).

Serine proteases

Serine protease allergens have been identified in extracts of mammalian pancreas, dust mites and bacteria.

Mammalian serine proteases

The mammalian serine protease allergens include pig and bovine trypsin (EC 3.4.21.4) and chymotrypsin (EC 3.4.21.1). They appear to be important occupational allergens (Table 6) in individuals such as parents and nurses of children with cystic fibrosis, and pharmaceutical workers [9–12].

Mite serine proteases

Mite trypsin and chymotrypsin have also been shown to be allergenic (Table 8) and are designated the group 3 and 6 allergens respectively [13–15]. They are similar to the mammalian enzymes with regard to both general physicochemical characteristics, such as size, amino

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Table 1. Physicochemical and biochemical characteristics of grass pollen allergens

Allergen	Frequency of reactivity (%)	Mol. weight (kDa)	Number of amino acid residues	pI	Potential glycosylation site(s)	Biochemical identity or homology
Subfamily: Pooideae						
Sweet verna (<i>Anthoxanthum odoratum</i>)						
Anto o 1	?	30	?	?	?	Function unknown; shows homology with groups 1–3 grass allergens
Bent grass (<i>Agrostis alba</i>)						
Agr a 1	?	30	?	?	?	Function unknown; shows homology with groups 1–3 grass allergens
Orchard grass (<i>Dactylis glomerata</i>)						
Dac g 1	>95	30	?	5.9	Yes	Function unknown; shows homology with groups 1–3 grass allergens
Dac g 2	75	11	98	4.8	No	Function unknown; shows homology with groups 1–3 allergens
Dac g 5 (DG3 allergen)	>90	25–28	?	?	?	Ribonuclease (?)
Red fescue (<i>Festuca elatior</i>)						
Fes e 1	?	?	?	?	?	Function unknown; shows homology with groups 1–3 grass allergens
Velvet grass (<i>Holcus lanatus</i>)						
Hol l 1	?	34	?	?	?	Function unknown; shows homology with groups 1–3 grass allergens
Hol l 5	?	30	?	4.8–6	?	Ribonuclease (?)
<i>Hordeum vulgare</i>						
Hor v 5	?	30	?	?	?	Ribonuclease (?)
Rye grass (<i>Lolium perenne</i>)						
Lol p 1	>90	26	240	5.1	Yes	Function unknown; shows homology with groups 1–3 grass allergens
Lol p 2	60	11	97	5.0	No	Function unknown; shows homology with groups 1–3 grass allergens
Lol p 3	70	11	97	8.3	No	Function unknown; shows homology with groups 1–3 grass allergens
Lol p 4	74	57	?	9	Yes	Function unknown

Table 1. Continued

Allergen	Frequency of reactivity (%)	Mol. weight (kDa)	Number of amino acid residues	pI	Potential glycosylation site(s)	Biochemical identity or homology
Lol p 5	80	29–31	283–314	6–10	No	Ribonuclease?
Lol p 10	?	12	?	?	?	Cytochrome c; shows homology with group 10 grass allergens
Lol p 11	65	15	134	5.6	Yes	Function unknown; shows homology with tree allergen Ole e 1 and soybean trypsin inhibitor
Timothy grass (<i>Phleum pratense</i>)						
Phl p 1	80	26	239	8.7	Yes	Function unknown; shows homology with groups 1–3 grass allergens
Phl p 2	62	11	96	4.4	No	Function unknown; shows homology with groups 1–3 grass allergens
Phl p 5	80	29	287	7.0	No	Ribonuclease (?)
Phl p 6	?	12	110	5.5	Yes	Possible cleavage product of Phl p 5
Kentucky blue grass (<i>Poa pratense</i>)						
Poa p 1	?	33–36	?	6.4–9.1	?	Function unknown; shows homology with groups 1–3 grass allergens
Poa p 9(5)	>95	28–35	286–345	8.8–9.3	No	Ribonuclease (?)
Poa p 10	?	12	?	?	?	Cytochrome c; shows homology with group 10 grass allergens
<i>Secale creale</i>						
Sec c5	?	30	?	5–5.5	?	Ribonuclease (?)
Subfamily: Panicoideae						
Bermuda grass (<i>Cynodon dactylis</i>)						
Cyn d 1	100	25–34	?	6.0–6.8	Yes	Function unknown; shows homology with groups 1–3 grass allergens
BG 60a	>40	34–60	?	9.7	Yes	Function unknown; shows homology with groups 1–3 grass allergens
Cyn d 10	?	12	?	?	?	Cytochrome c; shows homology with group 10 grass allergens
Johnson grass (<i>Sorghum halepense</i>)						
Sor h 1	?	30	?	?	?	Function unknown; shows homology with groups 1–3 grass allergens

Table 1. Continued

Allergen	Frequency of reactivity (%)	Mol. weight (kDa)	Number of amino acid residues	pI	Potential glycosylation site(s)	Biochemical identity or homology
Canary grass (<i>Phalaris aquatica</i>)						
Pha a 1	77	34	?	?	?	Function unknown; likely to show homology with group 1 allergens
All grasses						
Profilin	30	14	?	?	?	Protein involved in actin polymerization

Molecular weights (all subunit values) and pI in this and subsequent tables are expressed either as calculated (i.e. non-glycosylated) values obtained using sequence analysis software (Mac Vector; IBI, New Haven, USA) and data downloaded from the Genbank, PIR or Swiss Protein sequence databanks, or obtained from original references where complete sequence data were unavailable. Homologies were derived from original references and/or updated using appropriate algorithms. The frequencies of reactivities are expressed as the percentage of an appropriate population responding to the source from which the allergen was derived using data from the literature. The allergen nomenclature used is that recommended by the IUIS Allergen Nomenclature Committee [3].? indicates that information was not available or could not be found.

acid sequence, the capacity to undergo autolysis, and substrate specificities. In addition to trypsin and chymotrypsin, recent data indicate that mite extracts contain another allergenic serine protease with collagenolytic activity [16,17].

Bacterial serine proteases

In contrast to the similarities demonstrated by the mite and mammalian serine proteases described above, the bacterial serine protease allergens, designated subtilisins (EC 3.4.21.14), do not appear to be structurally related. They are extracellular enzymes (Table 6) which play a role in sporulation and are well characterized occupational allergens in the detergent industry. The two most important allergens, marketed as Alcalase and Esperase, are derived from *Bacillus subtilis* and *B. licheniformis* respectively. Their role in allergic disease has been extensively investigated, and subsequent industrial reforms have greatly reduced the risk of sensitization [18,19].

Cysteine proteases

A number of allergenic cysteine proteases have been isolated from plants, dust mites and helminthic parasites but of these, the most clinically important are the group 1 allergens from mites.

Mite cysteine proteases

Mite cysteine proteases, designated the group 1 allergens (Table 7), are recognized by most mite allergic individuals, and sequences of enzymes from *D. pteronyssinus*, *D. farinae* and *Euopglyphus maynei* have been determined [4,20,21]. They are synthesized by cells lining the gastrointestinal tract of the mite [22] as preproteins consisting of a leader peptide (about 18 residues) and a propeptide (about 80 residues), together with the mature protein [20]. The amino acid sequences of the group 1 allergens are very similar (81–84% identity), with most of the differences occurring in the N-terminal region and C-terminal half of the molecule [20]. Homology searches indicate that the group 1 allergens are structurally similar to the mammalian enzymes cathepsin B and H, and the plant enzymes actinidin, papain and bromelain. The overall degree of homology between Der p 1 and papain is about 31% [4,23] but this increases significantly in those regions known to be involved in the catalytic site.

Plant cysteine proteases

The cysteine proteases from plants have also been shown to be important allergens in occupational settings (Table 6). The most important are the structurally and immunologically similar enzymes papain (EC 3.4.22.2) from the latex of the papaya, and bromelain (EC

Table 2. Physicochemical and biochemical characteristics of weed pollen allergens

Allergen	Frequency of reactivity (%)	Mol. weight (kDa)	Number of amino acid residues	pI	Potential glycosylation site(s)	Biochemical identity or homology
Heliantheae						
Short ragweed (<i>Ambrosia artemisiifolia</i>)						
Amb a 1	>90	40	370	5.1	Yes	Function unknown; shows homology with pectate lyase, Amb a 2 and Cry j 1
Amb a 2	>90	41	371	5.7	Yes	Function unknown; shows homology with pectate lyase, Amb a 2 and Cry j 1
Amb a 3	51	11	101	5.5	Yes	Function unknown; shows homology with the stellacyanin electron transport proteins
Amb a 5	17	5	45	9.1	No	Function unknown
Amb a 6	21	11	108	>11	No	Function unknown; shows homology with lipid transferase
Amb a 7	20	12	?	?	?	Function unknown; shows homology with the plastocyanin electron transport proteins
Amb a 10	?	12	?	?	?	Cytochrome c
Cystatin	30	10	91	5.0	No	Cysteine protease inhibitor; shows homology with cystatins
Giant and western ragweed (<i>A. trifida</i> , <i>A. psilostachya</i>)						
Amb t 5	?	4	40	8.4	No	Function unknown
Amb p 5	?	4	40	8.4	No	Function unknown
Anthemideae						
Mugwort (<i>Artemisia vulgaris</i>)						
Art v 1	>70	47	?	4.4	Yes	Function unknown
Art v 2	33	20	?	4.1–4.8	Yes	Function unknown
Urticaceae						
Wall pellitory (<i>Parietaria spp</i>)						
Par o 1	100	14	?	4.6	Yes	Function unknown; possibly similar to Par j 1 (see Amb a 6)
Par j 1.01	100	13	?	5.0	Yes	Shows homology with phospholipid transfer protein (see Amb a 6)
Par j 1.02	100	11	?	5.0	Yes	

3.4.22.32) from pineapples [5,24,25]. As mentioned previously, they demonstrate sequence homology with mite cysteine proteases but immunological cross-reactivity between mite and plant cysteine proteases has yet to be reported.

Aspartate proteases

Enzymes belonging to the aspartate proteases such as pepsin have been shown to be allergenic in both occupational settings such as the cheese making and

Table 3. Physicochemical and biochemical characteristics of tree pollen and latex allergens

Allergen	Frequency of reactivity (%)	Mol. weight (kDa)	Number of amino acid residues	pI	Potential glycosylation site(s)	Biochemical identity or homology
Betulaceae						
Birch pollen (<i>Betula verrucosa</i>)						
Bet v 1 (isoforms 1a to 1n)	>95	17	159	5.2	Yes	Function unknown; shows homology with Fagales group 1 allergens, plant pathogenesis-related proteins
Bet v 2	10	14	133	4.9	No	Profilin, plays role in actin polymerization
Bet v 3	<10	20	?	?	?	Calmodulin (?)
Alder (<i>Alnus glutinosa</i>)						
Aln g 1	>90	17	159	5.3	No	Function unknown; shows homology with group 1 Fagales allergens and plant pathogenesis-related proteins
Corylaceae						
Hornbeam pollen (<i>Carpinus betulus</i>)						
Carb b 1	>90	17	159	5.8	Yes	Function unknown; shows homology with group 1 Fagales allergens and plant pathogenesis-related proteins
Hazel (<i>Corylus avellana</i>)						
Cor a 1(1/11)	>90	17	159	5.3	Yes	Function unknown; shows homology with group 1 Fagales allergens and plant pathogenesis-related proteins
Fagaceae						
White Oak (<i>Quercus alba</i>)						
Que a 1	>90	17	?	?	?	Function unknown; shows homology with group 1 Fagales allergens and plant pathogenesis-related proteins
European chestnut (<i>Castanea sativa</i>)						
Cas s 1	?	22	?	?	?	Function unknown; shows homology with group 1 Fagales allergens and plant pathogenesis-related proteins
Apple 18kDa allergen						
	?	18	?	5.5	?	Function unknown; shows homology with group 1 Fagales allergens and plant pathogenesis-related proteins

Table 3. Continued

Allergen	Frequency of reactivity (%)	Mol. weight (kDa)	Number of amino acid residues	pI	Potential glycosylation site(s)	Biochemical identity or homology
Oleaceae						
Olive pollen (<i>Olea europaea</i>)						
Ole e 1	>90	16	145	6.1	Yes	Function unknown; shows limited homology with soybean trypsin inhibitor, Lol p 11, LAT52 (tomato) and Zmc13 (corn)
Ole e 2	>90	8	?	?	?	Function unknown
Lilac (<i>Syringa vulgaris</i>)						
Syr v 1	16	145	?	Yes		Shows homology with Ole e 1
<i>Fraxinus excelsior</i>						
Fra e 1	?	16	?	?	?	Shows homology with Ole e 1
<i>Ligustrum vulgare</i>						
Lig v 1	?	?	?	?	?	Shows homology with Ole e 1
Taxodiaceae						
Japanese cedar pollen (<i>Cryptomeria japonica</i>)						
Cry j 1	> 85	41–46	353	8.9–9.2	Yes	Pectate lyase; shows homology with bacterial pectate lyase and Amb a 1 and 2
Cry j 2	76	37	460	?	Yes	Polymethylgalacturonase
Cupressaceae						
Japanese juniper (<i>Juniperus rigida</i>)						
70 kDa allergen	100	70	?	?	Yes	Function unknown
Mountain cedar (<i>Juniperus ashei</i>)						
GP 40	?	40	?	?	Yes	Function unknown
Cypress						
<i>Cupressus sempervirens</i>						
42 kDa Allergen	81	42	?	?	?	Function unknown
<i>Cupressus arizonica</i>						
43 kDa Allergen	?	43	?	?	Yes	Function unknown
Euphorbiaceae						
Rubber tree (latex) (<i>Hevea brasiliensis</i>)						
Hev b 1	23–>80	15	137	4.8	No	Rubber elongation factor, exists as homotetramer with mol. wt. of 58 kDa and pI of 8.5
Prohevein	83	20	186	?	No	Lectin, causes latex agglutination

Table 3. Continued

Allergen	Frequency of reactivity (%)	Mol. weight (kDa)	Number of amino acid residues	pI	Potential glycosylation site(s)	Biochemical identity or homology
23 kDa	76	23	?	?	?	Shows some homology with rubber elongation factor
25–27 kDa allergen	?	25	?	?	?	Function unknown
Hevamine	3	30	273			Chitinase/lysozyme
36 kDa allergen	21	30	?	?	?	Endo-1,3- β -glucosidase
46/110 kDa allergen	?	46/110	?	?	?	Function unknown
Profilin	11	14		3.5		Plays role in actin polymerization
Leguminosae						
Mesquite (<i>Prosopis juliflora</i>)						
10 kDa allergen	?	10	?	?	Yes	Function unknown
20 kDa allergen	?	20	?	?	Yes	Function unknown

pharmaceutical industries, and in domestic settings. These proteases have an aspartic acid residue in their catalytic site and are generally most active in an acid environment. The major aspartate protease allergens include pepsin (EC 3.4.23.1) [26], renin (EC 3.4.23.6) [27], *Candida albicans* acid protease (EC 3.4.23.24) [28] and a recently described cockroach protease, Bla g 2 [29], all of which are structurally similar. The occupationally important aspartate proteases are derived from porcine and bovine stomach tissue, and the fungus *Cryphonectria* (previously *Endothia*) *parasitica*.

Metalloproteinases

In addition to the above proteases, it has been reported that the metalloproteinase collagenase (EC 3.4.24.3) from *Clostridium histolyticum* may also be a potent allergen in occupational settings [30].

Carbohydrases

A variety of carbohydrases have been found to be allergenic. They include amylase, cellulase, xylanase, lysozyme and polygalacturonase, and all cleave appropriate bonds (e.g. 1,4- β -D- or 1,4- α -D) within oligo- and polysaccharides. The allergenic carbohydrases

have been found in dust mites, fungi, seed flours and mammalian pancreatic extracts (Tables 4,5,7).

Amylase

Amylases are enzymes which cleave glycosidic links in polysaccharides such as starch, and several have been shown to be important occupational and domestic allergens. For example, the chemically and immunologically distinct α - (EC 3.2.1.1) and/or β -amylases (EC 3.2.1.2) from wheat and barley [31] (Table 4), and α -amylase from *Aspergillus oryzae* (Table 5) have been shown to be important allergens in the baking industry [5,32,33]. Mammalian amylases have also been shown to be allergenic in process or pharmaceutical workers [34], and an enzymatically equivalent protein from dust mites has been shown to be allergenic [35]. Limited N-terminal sequence data indicate little, if any, homology between the mite enzyme and fungal or mammalian amylase, although it shows homology with amylases from insects such as *Bombix mori*.

Lysozyme

Lysozymes (N-acetylmuramoylhydrolase; EC 3.2.1.17) are ubiquitous enzymes which cleave 1,4- β -D-glycosidic bonds between N-acetyl-D-glucosamine and N-acetyl-

Table 4. Physicochemical and biochemical characteristics of seed allergens

Allergen	Frequency of reactivity (%)	Mol. weight (kDa)	Number of amino acid residues	pI	Potential glycosylation site(s)	Biochemical identity or homology
Soybean (<i>Glycine max</i>)						
Trypsin-inhibitor (B)	?	20	181	4.5	No	Kunitz protease inhibitor
Lipoxygenase	?	94	839	5.9	Yes	Lipoxygenase
Castor bean (<i>Ricinus communis</i>)						
Ric c 1	?					2S storage albumin; shows homology with 2S storage proteins from wheat, mustard, barley and rice
Short chain		4	34	11.1	No	
Long chain		7	64	7.0	No	
Ric c2	?	47	?	?	?	Crystallloid protein
Yellow mustard seed (<i>Sinapis alba</i> L.)						
Sin a 1	?					2S storage albumin; shows homology with 2S storage proteins from wheat, barley and rice
Short chain		4	39	12.2	No	
Long chain		10	88	10.0	No	
Oriental mustard seed (<i>Brassica juncea</i>)						
Bra j 1	?					2S storage albumin; shows homology with 2S storage proteins from yellow mustard, wheat, barley and rice
Short chain		4	37	12.5	No	
Long chain		10	92	10.4	No	
Barley (<i>Hordeum vulgare</i>)						
BMAI-1	?	14	132	6.6	Yes	Barley α -amylase/trypsin inhibitor; shows homology with wheat allergens and 2S seed storage allergens
BDAI-1	?	13	122	4.9	Yes	Barley α -amylase/trypsin inhibitor; shows homology with wheat allergens and 2S seed storage allergens
α -amylase	>96	64	?	?	?	1,4- α -D-glucan glucanohydrolase
β -amylase	>96	60	?	?	?	1,4- α -D-glucan maltohydrolase
Rice (<i>Oryza sativa</i>)						
Ory s 1	?	15	135	6.6	No	α -Amylase inhibitor; shows homology with wheat and barley α -amylase/trypsin inhibitor allergens
Wheat (<i>Triticum turgidum</i>)						
CM16	?	13	119	4.8	Yes	Wheat α -amylase/trypsin inhibitor; shows homology with barley allergens and 2S seed storage allergens
WMAI-1	?	13	123	6.6	Yes	Wheat α -amylase/trypsin inhibitor; shows homology with barley allergens and 2S seed storage allergens
Agglutinin	?	17	171	8.8	No	Lectin
27 kDa allergen	?	27	?	?	?	Shows homology with acyl-CoA oxidase from barley and rice

Table 5. Physicochemical and biochemical characteristics of fungal allergens

Allergen	Frequency of reactivity (%)	Mol. weight (kDa)	Number of amino acid residues	pI	Potential glycosylation site(s)	Biochemical identity or homology
<i>Alternaria alternata</i>						
Alt a 1 (Alt a ₁ ¹⁵⁶³ , Alt a Bd)	<80	14	?	4–4.5	Yes	Function unknown
Alt a 2	?	6	?	9.5–9.8	?	Function unknown
70 kDa protein	?	70	?	3.5	?	Function unknown
Alt a 6	8	11	113	?	?	Ribosomal P ₂ protein
Alt a 7	7	22	205	?	?	Shows homology with yeast YCP4 of unknown function
Alt a 10	51	53	496	?	?	Shows homology with aldehyde dehydrogenase
<i>Aspergillus fumigatus</i>						
Asp f 1	85	17	149	9.3	No	Ribonuclease; shows homology with mitocillin
Asp f 2	?	36	?	4.8	Yes	Function unknown
70 kDa allergen	?	70	?	?	?	Function unknown
65 kDa allergen	?	65	?	?	?	Function unknown; shows homology with heat shock protein 90
<i>Aspergillus niger</i>						
¹ Pectinase I	–	35	337	3.9	Yes	Poly(1,4- α -D-galacturonide) glycanohydrolase
² Cellulase	8	–	–	–	–	1,4-(1,3;1,4)- β -D-glucan glucanhydrolase
² Flaviastase	–	–	–	–	–	Protease
Glucoamylase (G1)	5	66	615	3.9	Yes	1,4- α -D-glucan glucanhydrolase
<i>Aspergillus oryzae</i>						
Asp o 2	56	52	478	4.3	Yes	α -Amylase (1,4- α -D-glucan glucanohydrolase)
Lactase	–	?	?	?	?	1,4- β -D-galactoside galactohydrolase
<i>Candida albicans</i>						
37 kDa allergen	?	37	?	?	?	Aldolase
40 kDa allergen	?	40	?	?	Yes	Alcohol dehydrogenase
43 kDa allergen	?	43	?	?	?	Phosphoglycerate kinase
48 kDa allergen	37	47	440	5.4	Yes	Enolase
Acid protease	75	35	?	?	?	Aspartate protease
<i>Cladosporium herbarium</i>						
Cla h 1	>60	13	?	3.7–4.4	?	Function unknown
Cla h 2	43	19	?	5.0	Yes	Function unknown
Cla h 3	36	53	497	?	?	Shows homology with aldehyde dehydrogenase
Cla h 4	22	11	111	3.9	Yes	Ribosomal P ₂ protein
Cla h 5	22	22	205	?	?	Shows homology with yeast YCP4 of unknown function
Cla h 6	20	48	441	?	?	Shows homology with enolase
<i>Cryphonectria parasitica</i>						
Renin	?	34	330	3.9	No	Aspartate protease; shows homology with mammalian and cockroach pepsins

Table 5. Continued

Allergen	Frequency of reactivity (%)	Mol. weight (kDa)	Number of amino acid residues	pI	Potential glycosylation site(s)	Biochemical identity or homology
<i>Penicillium notatum</i>						
64 kDa allergen	46	64	?	?	?	Function unknown
68 kDa allergen	56	62	?	5.4–5.5	Yes	Function unknown; shows homology with β -N-acetylglucosaminidase from <i>Candida albicans</i>
<i>Psilocybe cubensis</i>						
16 kDa allergen	?	16	?	?	?	Cyclophilin
<i>Rhizopus nigricans</i>						
Rhiz IIIb	?	12	?	4.8	Yes	Function unknown
Rhiz VIb	?	14	?	3.6–3.8	Yes	Function unknown
<i>Saccharomyces cerevisiae</i>						
40 kDa allergen	?	37	347	6.2	Yes	Alcohol dehydrogenase
48 kDa allergen	39–68	47	436	6.2	Yes	Enolase
<i>Trichophyton tonsurans</i>						
Tri t 1	73	30	?	?	?	Function unknown; shows limited homology with exo β -1,3-glucanase
<i>Trichoderma viride</i>						
Cellulase	?	–	–	–	–	1,4-(1,3;1,4)- β -D-glucan glucanhydrolase
Xylanase	?	?	?	?	?	1,4- β -D-Xylan xylanhydrolase

muramic acid. They are usually associated with defence against gram positive bacteria but some have been shown to be involved in digestion. The enzyme from hen egg white has long been known to be an important food allergen (Table 10) but it has recently been recognized that it, together with other egg white allergens, are relatively important aeroallergens in egg processing plants [36]. In addition to the egg white lysozyme allergen, a latex allergen, designated heva-mine, has been shown to be a bifunctional carbohydrase with both chitinase (N-acetylglucosamine polymer cleaving enzyme; EC 3.2.1.14) and lysozyme activity [37].

Pectin degrading enzymes

Pectin (a polymer of α -(1,4) linked galacturonic acid which may be partially methyl-esterified) is a major plant cell wall constituent and a variety of enzymes are necessary for its degradation. They include the hydrolytic enzymes polygalacturonase (EC 3.2.1.15), polymethylgalacturonase (EC 3.2.1) and pectin methylesterase (EC 3.1.1.11), and the non-hydrolytic transeliminases, pectate lyase (EC 4.2.2.2), pectin lyase (EC 4.2.2.10) and exopolygalacturonate lyase (EC 4.2.2.9). Several of

these enzymes have been shown to be allergenic and include fungal allergens [38] and the group 1 pectate lyase allergen [39] and group 2 polymethylgalacturonase allergen [40] from Japanese cedar pollen (Table 3). Sequence homology searches indicate that both proteins are similar to enzymes from tomato, avocado fruit and maize pollen [40]. The group 1 pectate lyase cedar allergen shows homology with an equivalent allergen from mountain cedar [41] and the ragweed group 1 and 2 allergens (Tables 2,3) [42] suggesting that these allergens may also be enzymatically active. The precise role played by such enzymes in pollen is not known but it has been proposed that they are intimately involved in the utilization of cytoplasmic pectin-containing particles within the pollen grain or, alternatively, in facilitating the development and penetration of the pollen tube into the stigma after pollination [43].

Other carbohydrases

Other carbohydrases have also been shown to be allergenic and include cellulase (EC 3.2.1.4) [44], xylanase (EC 3.2.1.8) [44], lactase (EC 3.2.1.4) [45] and glucoamylase (EC 3.2.1.4) [46,47]. These enzymes are

Table 6. Physicochemical and biochemical characteristics of plant, bacterial and mammalian protease allergens

Allergen	Frequency of reactivity (%)	Mol. weight (kDa)	Number of amino acid residues	pI	Potential glycosylation site(s)	Biochemical identity or homology
Plant proteases						
Pawpaw (<i>Carica papaya</i>) Papain	?	23	212	9.3	No	Cysteine protease; shows homology with bromelain and the group I mite allergens
Pineapple (<i>Ananas comosus</i>) Bromelain	?	23	212	9.0	Yes	Cysteine protease; shows homology with papain and the group I mite allergens
Bacterial proteases						
<i>Bacillus subtilis</i> Alcalase	?	28	275	6.3	Yes	Subtilisin serine protease
<i>Bacillus licheniformis</i> Esperase	?	28	274	6.6	Yes	Subtilisin serine protease
<i>Clostridium histolyticum</i> ¹ Collagenase	?	68–125	–	–	–	Metalloprotease
Mammalian proteases						
Trypsin (porcine)	?	24	223	?	?	Serine protease; shows homology with mite groups 3, 6 and 9 allergens
Chymotrypsin (bovine)	?	25	245	?	?	Serine protease; shows homology with mite groups 3, 6 and 9 allergens
Pepsin (porcine)	?	35	327	?	?	Aspartate protease; shows homology with Bla g 2 and renin

¹ Represents a mixture of proteases.

important industrial enzymes which are used to endohydrolyse appropriate linkages in the breakdown of carbohydrates such as starch, cellulose, hemicellulase and pectin in the food industry (Table 4). They are usually derived from fungi such as *Aspergillus niger* and *Trichoderma viride*, and limited sensitization has been reported in both process workers and laboratory personnel. As yet, precise details of the enzymes involved in the reported sensitivities are lacking since the enzyme activities described are often the result of several distinct enzymes acting in concert (e.g. cellulase or pectinase), or the data were obtained using crude protein mixtures. In addition to these enzymes, sequence homology studies indicate the possible presence of allergens with β -N-acetylglucosaminidase (EC 3.2.1.30) activity in

Penicillium notatum [48] and endo-1,3- β -glucosidase (EC 3.2.1.39) activity in rubber latex [37].

Ribonuclease

At least two allergens with ribonuclease activity have been identified and include the group 5 allergen from Timothy grass pollen [8] and the 18 kDa allergen from extracts of *Aspergillus fumigatus* (Tables 1,5) [7,49,50]. The precise role played by the allergenic grass ribonucleases in pollination is unknown at present but may be involved in self-incompatibility. In this regard, studies show that these allergens are associated with starch grains released from pollen on contact with moisture [51,52] consistent with a central role in pollination

per se. Given the homology between the Timothy group 5 allergen and similar allergens (also referred to as group 9 allergens) from other grass species, it is likely that all will possess such enzymatic activity. The fungal allergens show identity with the cytotoxins mitogillin (from *A. restrictus*) and alpha-sarcin (from *A. giganteus*) [7,49,50]. These non-glycosylated cytotoxic proteins are ribonucleases (EC 3.1.27.4) which inhibit protein synthesis and, in addition, demonstrate anti-tumour activity. They belong to the ribonuclease U₂ family and are specific for purines in single and double stranded RNA.

Non-hydrolytic enzymes

Several allergens have been reported to be (or demonstrate sufficient sequence homology to suggest such a function) non-hydrolytic enzymes. However, in comparison to the hydrolases, relatively few have been shown to be clinically important aeroallergens. The most common non-hydrolytic enzymes so far described include the fungal enzymes enolase (EC 4.2.1.11) [53–55], alcohol dehydrogenase (EC 1.1.1.1) [56–57], aldolase (EC 4.1.2.13) [55], phosphoglycerate kinase (EC 2.7.2.3) [57], cyclophilin (ubiquitous peptidylprolyl *cis trans* isomerases) (EC 5.2.1.8) [58] and aldehyde dehydrogenase (EC 1.2.) [55], soybean lipoxygenase (EC 1.13.11.12) [59], the tree and weed pollen pectin transeliminases described above (Tables 4,6), glutathione transferases from cockroach and dust mites (EC 2.5.1.18) [29,60] and the wheat enzyme acyl-CoA oxidase (EC 1.3.3.6) [61].

Enzyme inhibitors

A number of occupational and domestic allergens have been described which show enzyme inhibitory activity or demonstrate marked sequence homology to known inhibitors. They are either derived from plant seeds, pollen or from hen egg white (Tables 2,4,10) and usually inhibit protease and/or amylase activity.

Seeds

Several clinically important occupational allergens are derived from plant seeds and include wheat, barley, rice, castor bean and mustard seeds (Table 4). They are proteins associated with storage or protection from predators such as insects and may be divided into three broad groups: the storage proteins, designated the 2S albumins or napins; the 14 kDa subunit proteins known to inhibit both trypsin and α -amylase; and a third group comprising Kunitz-type protease inhibitory allergens such as soybean trypsin inhibitor.

Napins

The napins are heterodimers comprising two disulphide linked chains with apparent molecular weights of about 4 and 8 kD respectively (Table 5) [62]. These chains are encoded on the same gene, indicating that the final structure is formed by post-translational proteolytic events. They represent one of two principal storage proteins (the other being the 11S proteins) and some have been shown to possess protease inhibitory activity. They belong to the same superfamily as the protease inhibitors found in wheat and barley described below. Several allergens within the napin family, such as the mustard seed allergens Sin a 1 and Bra j 1 [63,64] and the castor bean allergen Ric c 2 [65,66], have been characterized (Table 4).

Trypsin/amylase inhibitors

The trypsin/amylase inhibitors are a heterogeneous group of proteins found in the endosperm of wheat, barley and rice. They represent important occupational allergens (Table 5) and are encoded by a family of genes [67–69]. The proteins possess the ability to inhibit both trypsin and α -amylase activities, and they may be present as glycosylated or non-glycosylated monomers, dimers and tetramers. Individual members show both intra-species and inter-species homology and the most characterized of these are the glycosylated wheat CM16 subunit protein from the wheat tetrameric amylase inhibitor (WTAI-CM16), the monomeric wheat amylase inhibitor (WMAI-1) and the glycosylated monomeric (BMAI-1), the dimeric (BD AI-1) proteins from barley and the equivalent rice allergens.

Kunitz protease inhibitors

In addition to 2S and trypsin/amylase inhibitors, limited data indicate that the seed derived Kunitz protease inhibitor, soybean trypsin inhibitor (Table 5), may also be allergenic [59].

Pollen

Pollens contain serine and cysteine protease inhibitors which also bind IgE. For example, ragweed pollen (Table 2) has been shown to possess a cystatin-like cysteine protease inhibitor [70]. These low molecular weight inhibitors are common in mammalian systems and are thought to be important in regulating lysosomal cysteine proteases such as the cathepsins B and H. Some pollens have also been reported to contain functionally active trypsin inhibitors which bind IgE [71]. Such inhibitors were found in

Table 7. Physicochemical and biochemical characteristics of dust mite allergens

Allergen	Frequency of reactivity (%)	Mol. weight (kDa)	Number of amino acid residues	pI	Potential glycosylation site(s)	Biochemical identity or homology
<i>D. pteronyssinus</i>						
Der p 1	>90	25	222	5.7	Yes	Cysteine protease; shows homology with Der f 1 and Eur m 1, papain, actinidin, cathepsins B and H
Der p 2	>90	14	129	6.6	No	Function unknown; shows homology with putative human epididymal protein
Der p 3	51–>90	25	?	4–<8	No	Trypsin; shows homology with Der f 3 and Der p 6 and Der f 6, mammalian and invertebrate trypsins, and other serine proteases
Der p 4	25–46	56	?	5–7	?	Amylase
Der p 5	>55	13	113	?	No	Function unknown
Der p 6	39	30	?	3.8–4.4	?	Chymotrypsin; shows homology with Der p 3 and Der f 3, and Der f 6, mammalian and invertebrate chymotrypsins, and other serine proteases
Der p 7	53	22	198	4.7	Yes	Function unknown
Der p 8	?	(11–29 kDa) 26	219	?	?	Shows homology with glutathione transferase
Der p 9	>90	28	?	4.8, 7.8, 10.5	?	Serine protease; shows homology with mite groups 3 and 6 allergens
Dpt 4	?	244	?	5	Yes	Very high density lipoprotein
<i>D. farinae</i>						
Der f 1	79	25	223	5.7	Yes	Cysteine protease; shows homology with Der p 1 and Eur m 1, papain, actinidin, cathepsins B and H
Der f 2	83	14	129	6.5	No	Function unknown; shows homology with putative epididymal protein
Der f 3	42–70	30	232	4.8	No	Trypsin; shows homology with Der p 3, and Der f 6, and Der p 6, mammalian and invertebrate trypsins, and other serine proteases
Der f 6	31	30	?	?	?	Chymotrypsin; shows homology with Der p 3 and Der f 3 and Der p 6, mammalian and invertebrate chymotrypsins, and other serine proteases
Der f 7	46	22	196	?	Yes	Function unknown
Der f 10	81	33	284	?	No	Tropomyosin
67 kDa	>10	67	?	?	?	Shows homology with heat shock protein 70 family
<i>Euroglyphus maynei</i>						
Eur m 1	?	25	?	?	Yes	Cysteine protease; shows homology with Der p 1 and Der f 1 allergens, papain, actinidin, cathepsins B and H

Table 7. Continued

Allergen	Frequency of reactivity (%)	Mol. weight (kDa)	Number of amino acid residues	pI	Potential glycosylation site(s)	Biochemical identity or homology
<i>Lepidoglyphus destructor</i>						
Lep d 2	87	16–18	?	7.6–7.8	?	Function unknown; shows homology with putative epididymal protein and group 2 mite allergens
79/93 kDa allergen	?	79/93	?	?	?	Function unknown
<i>Blomia tropicalis</i>						
Blo t 1	>47	11–13	?	?	?	Function unknown
Blo t 5	70	14	?	?	?	Function unknown; shows homology with mite group 5 allergens

tree, weed and grass pollens from species such as *Rumex*, *Chenopodium*, *Parietaria*, *Olea*, *Holcus* and *Poa* but they were shown to be absent from *Lolium*, *Secale*, *Dactylis* and *Cynodon* spp. The reported physicochemical properties of these allergens (a 20 kDa protein with a pI of 4.2) suggest that they may be related to the Kunitz group of protease inhibitors [72]. In this regard, the group 11 allergen from rye grass [73] and the group 1 pollen allergens from several members of the Oleaceae family, such as olive and lilac [74,75], have been shown, in part, to be structurally related to the Kunitz soybean trypsin inhibitor, although they do not appear to possess inhibitory activity, findings consistent with previously reported data [71].

Egg

Egg white has been shown to contain two proteins, either with enzyme inhibitory activity or demonstrating sequence similarity to inhibitory proteins. In this regard, the ovomucoid allergen, Gal d 1, is a known potent serine protease inhibitor (Table 10), and ovalbumin [36,76] demonstrates sequence homology with a family of proteins related to the serine protease inhibitors [77]. However, protease inhibitory activity has not been demonstrated thus far.

Proteins involved in transport

A variety of allergens demonstrate significant homology

Table 8. Physicochemical and biochemical characteristics of insect allergens

Allergen	Frequency of reactivity (%)	Mol. weight (kDa)	Number of amino acid residues	pI	Potential glycosylation site(s)	Biochemical identity or homology
Midges						
<i>Chironomus thummi thummi</i>						
Chi t 1	?	15	136	6.5	No	Haemoglobin
<i>Cladotanytarsus lewisi</i>						
17 kDa allergen	?	17	?	3.9–5.2	?	Haemoglobin
32 kDa allergen	?	32	?	3.9–5.2	?	Haemoglobin
German cockroach (<i>Blattella germanica</i>)						
Bla g 1	30	25	?	3.5	?	Function unknown
Bla g 2	58	36	328	5.2	?	Shows homology to aspartate proteases
Bla g 4	?	20	?	?	?	Shows homology with lipocalins
Bla g 5	?	?	?	?	?	Shows homology with glutathione transferase

Table 9. Physicochemical and biochemical characterization of animal and dander allergens

Allergen	Frequency of reactivity (%)	Mol. weight (kDa)	Number of amino acid residues	pI	Potential glycosylation site(s)	Biochemical identity or homology
Cat (<i>Felix domesticus</i>)						
Fel d 1 Chain 1	>80	8	70	4.3	No	Function unknown; chain shows homology with 10 kDa secretory protein from human Clara cells and rat analogue; rabbit uteroglobin
Chain 2		10	92	4.4	Yes	No known homologue
Dog (<i>Canis familiaris</i>)						
Can f 1	>70	19–25	?	5.2	?	Function unknown; expressed in skin and dander, may be analogous to the rodent lipocalin allergens
Can f 2	23	?	?	?	?	Function unknown
69 kDa allergen	40	69	?	4.5	?	Dog serum albumin
Horse (<i>Equus caballus</i>)						
Equ c 1	100	20	?	3.9–4.1	Yes	Function unknown
67 kDa allergen	?	67	583	?	Yes	Probably horse albumin
Cow (<i>Bos domesticus</i>)						
BDA20/Bos d 1	97	20–22	?	3.6	?	Function unknown; may be analogous to the rodent lipocalin allergens
AS1	31	21	190	?	?	Oligomycin sensitivity-conferring protein of the mitochondrial adenosine triphosphate synthase complex
Mouse (<i>Mus musculus</i>)						
Mus m 1	?	19	162	4.7	No	Major urinary protein; shows homology with lipocalins such as β -lactoglobulin, odorant binding proteins, Rat n 2
Rat (<i>Rattus norvegicus</i>)						
Rat n 1	60	21	?	4.5	Yes	Urinary pre-albumin, shows homology with Rat n 2
Rat n 2	90					
Liver/urine		19	162	5.3	Yes	Major urinary protein, alpha-2 μ -globulin, shows homology with lipocalins such as β -lactoglobulin, odorant binding proteins, Mus m 1
Salivary gland		18	160	4.5	Yes	

Table 10. Physicochemical and biochemical characteristics of egg white allergens

Allergen	Frequency of reactivity (%)	Mol. weight (kDa)	Number of amino acid residues	pI	Potential glycosylation site(s)	Biochemical identity or homology
Gal d 1	34	20	186	4.6	Yes	Ovomucoid, protease inhibitor
Gal d 2	32	43	385	5.0	Yes	Ovalbumin, function unknown but protein shows homology with serine protease inhibitors
Gal d 3	47	76	686	6.7	Yes	Conalbumin, iron transport protein
Lysozyme	50	14	128	10.2	No	1,4- β -N-acetylmuramidase

to proteins involved in the transport of ligands such as lipids, pheromones, electrons, oxygen and iron, and include allergens from grass and weed pollen, animal tissue, cockroaches and hen egg white (Tables 1,2,9,10).

Lipocalins

The most important allergenic transport proteins are the 20 kDa major urinary proteins, e.g. Mus m 1 and Rat n

2, from rodents (Table 8). They are commonly found in a variety of tissues (dander, epithelium, fur, urine or saliva) [78–80]. These proteins show marked structural and sequence similarities to proteins such as odorant binding proteins, retinol binding protein and α -1-microglobulin, which are termed the lipocalins or calycins [81]. These homopolymeric proteins comprise a characteristic subunit structure, and proteins within this group are thought to play major roles in the transport of small hydrophobic molecules (e.g. polychlorinated biphenyls,

Table 11. A summary of the major groups of biochemically active allergens

Biochemical activity	Allergens
<i>Hydrolytic enzymes</i>	
<i>Proteases</i>	
Serine proteases	Group 3 mite trypsins, group 6 mite chymotrypsins, group 9 mites proteases, mammalian trypsin, mammalian chymotrypsin, bacterial subtilisins
Cysteine protease	Group 1 mite allergens, papain, bromelain
Aspartate protease	Mammalian pepsin, fungal protease, renin, Bla g 2
Metalloproteinase	Bacterial collagenase
<i>Carbohydrases</i>	Egg white lysozyme, mite group 4 amylase, plant fungal, bacterial and mammalian amylases, cellulase, xylanase, fungal pectinase, fungal glucoamylase, *Cr y j 2
<i>Ribonucleases</i>	Asp f 1, group 5 grass pollen allergens
<i>Non-hydrolytic enzymes</i>	Soybean lipoxygenase, fungal aldolase, enolase, alcohol dehydrogenase, phosphoglycerate kinase, mite and cockroach glutathione transferases, *Amb a 1, *Amb a 2, and *Cry j 1 pectate lysases
<i>Enzyme inhibitors</i>	Ric c 1, wheat CM16, wheat WMAI-1, barley CMb, BMAI-1, BDAI-1, Ory s 1, ragweed cystatin, Gal d 1, Gal d 2, soybean trypsin inhibitor, *Lol p 11, *Ole e 1
<i>Transport proteins</i>	Group 10 grass pollen cytochrome c allergens, *Amb a 3, *Amb a 6, *Amb a 7, Mus m 1, Rat n 2, Fel d 1, Gal d 3, midge haemoglobins, Bla g 4, animal serum albumins
<i>Regulatory proteins</i>	Grass, weed and tree pollen profilins, tree pollen calmodulin, mite and fungal tropomyosins, mite and fungal heat shock proteins, ribosomal p2 proteins, bovine oligomycin sensitivity-conferring protein
<i>Miscellaneous</i>	Wheat lectin, pollen lectin, latex lectin (prohevein)

* Indicates that allergens possess activity or show homology to proteins that do so.

pheromones, vitamins) through hydrophilic environments. With regard to the rodent allergens it has been proposed they play a major role in disseminating pheromones, a suggestion supported by recent crystallographic data [82]. More recently, cockroach allergen designated Bla g 4 has been cloned and the resulting sequence shown to demonstrate homology with lipocalins including the rodent urinary proteins [29].

Non-lipocalin transport proteins

Several allergens have been found to demonstrate homology with proteins known to transport a variety of ligands such as electrons, iron, oxygen and lipid (Tables 1,2,10). For example, grass pollens contain 12 kDa electron transport related pollen allergens such as the haeme containing cytochrome c (group 10) allergens from grass pollens [83], whereas weeds such as ragweed contain plastocyanin and stellacyanin (blue copper proteins) related allergens designated Amb a 3 and Amb a 7 respectively [84,85]. The former proteins are characteristic of mitochondria, whereas the latter are associated with chloroplasts but it is not clear whether each of these proteins plays a direct role in electron transport *per se* [86]. In addition to potential electron transport allergens, several other weed pollen allergens, namely Amb a 6, Par o 1 and Par j 1, show marked homology with known plant lipid transfer proteins [87–89]. Other transport allergens include the iron transport protein allergen from egg white (Gal d 3, conalbumin) [36], haemoglobin allergens from midges [90,91] and animal serum albumins [92,93].

Regulatory proteins

Several allergens have been shown to possess regulatory properties. Interestingly, several of these proteins are often encountered as antigens in food allergy, parasite infection and autoimmunity. They include tree, weed and grass pollen profilins [94], tree pollen calmodulin [95] (Table 3), the cytoskeletal protein tropomyosin from mites [96] (Table 7), fungal and mite heat shock proteins [97,98] (Tables 5,7), the fungal ribosomal acidic phosphoproteins [99] (Table 5) and the bovine oligomycin sensitivity-conferring protein of the mitochondrial adenosine triphosphate synthase complex [100] (Table 9).

The regulatory properties of these proteins are varied, although several appear to be associated with actin. For example, plant profilins are thought to play a role in actin streaming and pollen tube growth due to their association with actin polymerization, and tropomyosins are involved in actin binding and muscle contraction

[94,101–103]. In this regard, this latter protein has been shown to be an important shellfish food allergen and autoantigen [102,103]. Calmodulins also play a regulatory role via calcium modulation. In this regard, the dietary parvalbumin allergen, Gad c 1, from codfish [101] also possesses calcium binding properties but is regarded as a transport rather than modulatory protein. The heat shock proteins are thought to perform a number of regulatory functions, including binding to actin and various receptors. The ribosomal acidic phosphoproteins and bovine oligomycin sensitivity-conferring protein of the mitochondrial adenosine triphosphate synthase complex are important regulatory proteins and the former represents an important parasite and autoantigen [104].

Other allergens

Other allergens have been characterized which do not, as yet, fall within the groupings described above. They include the lectin allergens from wheat [105], latex [37] and pollen [106].

Allergens with unknown biochemical activities

It is clear that despite the above descriptions, the biochemical properties of some of the major aeroallergens remain to be elucidated. They include allergens from grass, tree and weed pollens, fungi, animal danders and dust mites. However, it is likely that many will fall within the groups described above with further study. In the following sections, we highlight those allergens whose biochemical activities remain to be determined. In addition, we review appropriate data pertaining to these allergens and speculate on their possible functions.

Grass and weed pollen allergens

With regard to grass pollen allergens, the biochemical identity of the groups 1 [107–109], 2 [110], 3 [111], 4 [112] and 6 [113] remains to be determined (Table 2). However, despite this, the prototype group 1 allergen from rye grass has been shown to be located in the outer wall and cytoplasm of pollen grains, and around the starch granules, in contrast to the ribonuclease group 5(9) allergen located in the granule [51,52]. Given the variety of enzymes found in pollen, it is feasible that these allergens will also be enzymatically active. With regard to the weed pollen allergens (Table 2), the biochemical identities of the ragweed groups 1 [42], 2 [42] and 5 [114] remain to be determined, although the sequence studies described earlier suggest that the groups 1 and 2 allergens will be pectate lyases. A

number of allergens from weed species other than ragweed [115,116] have also been described but again, their identity and/or relationship with ragweed allergens remains to be determined.

Tree pollen allergens

The biochemical functions of some of the major allergens present in pollens produced by trees also remain unknown, particularly the 21 kDa group 1 allergens from the trees belonging to the order Fagales, such as birch, hazel and oak (Table 3). However, sequence homology searches show that they are similar to a variety of pathogenesis-related (PR) proteins of unknown function from peas and beans [117–119] produced in response to stress or infection by bacteria, fungi and viruses. In this regard, mRNA coding for the group 1 tree pollen allergens can be induced in isolated birch cell suspension cultures by co-cultivation with bacteria and fungi, but not by stressing the cells with heat or metal ions [120] treatments. A number of unrelated PR proteins have also been described which are enzymatic or enzyme-inhibitory. They include proteins such as chitinases, β -1,3-glucanases and invertases, and ribosome inactivating proteins and protease inhibitors [121–123]. Whether the group 1 tree pollen allergens possess any of these activities remains to be determined. Interestingly, several of the allergens from rubber tree latex possess some of the above properties and are classified as PR proteins.

In addition to the group 1 allergens, the identities of several other tree pollen allergens remains to be determined but it is likely that some, for example the allergens from the Cupressaceae, will correspond to the pectin degrading enzymes reported for Japanese cedar. Other tree pollen allergens may be related to the Kunitz trypsin inhibitors, given the reported similarity between these proteins and the olive tree pollen allergen, Ole e 1 described above.

Fungal allergens

The functions of most of the major allergens from the clinically important fungal species, with the exception of the hydrolytic and non-hydrolytic enzymes, and the heat shock protein described above, are unknown (Table 5). These include allergens from *Alternaria*, *Cladosporium*, and *Rhizopus* [124–129]. For example, the major allergen from *Alternaria alternata*, Alt a 1, has been shown to be a heat stable, cytoplasmic acidic glycoprotein comprising 2 disulphide linked chains of apparent molecular weight of 15 kDa [124,125]. However, despite the availability of N-terminal amino

acid sequence data, the identity of this protein remains unclear. In addition, a major acidic 70 kDa glycoprotein allergen has also been isolated from *A. alternata* [126] but its identity has also to be ascertained. Other allergens of unknown biochemical activity from fungi include the *Cladosporium* allergens Cla h 1 and 2, the 64 kDa allergen from *Penicillium notatum* and the Rhiz IIIb and Rhiz VIb allergens from *Rhizopus nigricans* (Table 5) [127–129].

Animal allergens

Despite knowing much about the structure of rodent lipocalin allergens present in epithelium and urine, the biochemical identities of the other major, non-rodent animal allergens from these sources remain to be determined (Table 9). However, given the similarity in physicochemical characteristics between the low molecular weight dander and urine allergens from rabbit, dog, horse, guinea pig and cow allergens, it is feasible that they too will be shown to be lipocalins and play a role in transport. At present, it is not clear whether the 35 kDa acidic glycoprotein cat allergen Fel d 1 will turn out to be a lipocalin, despite knowing much about its structure [130–132]. In this regard, it has been demonstrated that Fel d 1 (Table 9) comprises a non-covalently bound dimeric glycoprotein, where each monomer comprises two distinct disulphide linked peptides designated chains 1 and 2. Chain 1 comprises a non-glycosylated peptide of 70 amino acid residues whereas chain 2 is a glycosylated 92 residue peptide.

Sequence searches have revealed that chain 1 shows homology with rabbit uteroglobin, the 10–13 kDa secretory protein from human and rat Clara cells and the alpha chain of the androgen binding protein from mouse saliva [133]. The precise function of these proteins is unclear but both the salivary and Clara cell proteins possess ligand binding activity [133] and it is thus feasible that the cat allergen will also be a transport protein. It has been suggested that the mouse protein plays a role in sex recognition and it is possible that the function of the cat allergen may be similar.

Dust mite allergens

The biochemical identities of several of the major mite allergens, including the group 2 [134,135], 5 [136] and 7 [137] allergens, remain to be determined despite the availability of a diverse array of data (Table 7). The group 2 allergens are neutral to basic 14 kDa non-glycosylated allergens recognized by the majority of mite allergic individuals, and the complete amino acid sequences of the allergens from *D. pteronyssinus*,

D. farinae and the storage mite *Lepidoglyphus destructor* have been determined. Sequence homology searches have indicated that the allergens are similar to a putative human epididymal protein of unknown function and it has been argued that the mite protein plays a role in reproduction [138], although confocal microscopy suggests that the allergens may also be involved in digestion [139]. The 14 kDA group 5 and the 22 kDA group 7 allergens are recognized by about 50% of mite allergic individuals and are produced by several mite species. In both instances, sequence data have failed to reveal their biochemical identities. A number of other mite allergens have been described but their biochemical activities are unknown [140–141].

Biochemical properties and immunogenicity

Clinical and experimental observations suggest the capacity of an individual to produce IgE antibody in response to allergen is under the influence of a number of genes which probably operate at the level of the immunoregulatory T-cell system. However, possession of these genes does not guarantee IgE expression and it has been proposed that environmental exposure to allergens, together with other factors, is critical in the induction process [142,143]. The respiratory mucosa is the obvious primary site of interaction and it is likely that any physicochemical property associated with an allergen, or situations facilitating contact with immunocompetent cells such as the MHC Class II⁺ dendritic cells below the epithelium, is likely to enhance IgE production in genetically susceptible individuals. In this regard, studies from our laboratory have shown that the protease allergens from dust mites have a direct effect on epithelial integrity and permeability [144], cause mast cell degranulation in a non-IgE dependent manner [145] and induce cytokine release from respiratory epithelium [146].

Molecular size, solubility and absolute concentration will undoubtedly contribute to this process [147,148], as will mucosal inflammation induced by irritant gases and respiratory tract infections. However, it is possible that inherent biochemical properties, for example enzymatic activity, specific physicochemical attributes (high pI) or post-translational modifications (glycosylation), will contribute. Interestingly, many of the biochemical properties described for allergens are often associated with pathogens and exploited by cells comprising the innate arm of the immune defence system, such as macrophages and monocytes via a range of receptors [2,149]. We have proposed [2] that these cells may recognize allergens in a similar fashion and be rendered immunogenic. Given that the amount of antigen necessary to activate

immunocompetent cells is several orders of magnitude lower when they are complexed to receptors [150,151], such observations may account for the relatively strong immunogenic signals resulting from exposure to minute quantities of allergen (ng per day) in the respiratory tree.

Conclusions

The data presented in this review reveal the fruits of many studies and reflect the significant impact of modern technology on protein characterization. Most of the clinically important allergens have now been identified and the physicochemical data obtained offer hope of better and more reproducible diagnosis, and the development of novel immunotherapeutic approaches to allergic disease. The allergens can be classified into discrete groups based on biochemical activities, and include hydrolytic and non-hydrolytic enzymes, inhibitors, and transport and regulatory proteins (Table 11). Of these, hydrolytic enzymes appear prominent allergens in both occupational and domestic settings, both numerically and with regard to their frequency of recognition. Whether this reflects their abundance or relative concentration within a particular source or, alternatively, that biochemical activity is of particular significance, remains to be established. In addition to the potential clinical gains accruing from a scientific study of allergens from disparate sources, the data generated have had, and will continue to have, significant impact on our understanding of the biochemistry of many organisms which might otherwise have been overlooked.

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