

The Principle of Homologous Groups in Regulatory Affairs of Allergen Products – A Proposal

A.R. Lorenz D. Lüttkopf S. May S. Scheurer S. Vieths

Division of Allergology, Paul-Ehrlich-Institut, Langen, Germany

Key Words

Allergen extracts · Extrapolation of data · Homologous groups · Marketing authorization · Medicinal products · Regulatory affairs · Taxonomic family

Abstract

Among other legal regulations, the Note for Guidance on Allergen Products CPMP/BWP/243/96 released by the European Medicines Agency provides regulatory instructions regarding the quality of allergen extracts for diagnostic or therapeutic purposes. The current revision of this guideline intends to transform the so-called 'principle of taxonomic families' to the 'principle of homologous groups'. According to this concept, the data of one allergen extract demonstrating stability, efficacy and safety can, to a limited extent, be extrapolated to other allergen extracts belonging to the same homologous groups. The present work proposes the formation of homologous groups for pollen species and animal-derived materials on the basis of similar biochemical composition and homology/cross-reactivity of allergens or allergen sources. Some tree pollen species could be assigned to three different homologous groups, some weed pollen species to one homologous group and numerous grass pollen species to one homologous group on condition that data rely on single defined representative species. A homologous group for mites is limited to the *Dermatophagoides* species

and the grouping of vertebrate-derived materials such as dander could be possible under restrictions. The criteria for the formation of the proposed homologous groups are illustrated in detail to provide an opportunity for extending existing homologous groups by further species in case of new insights in allergens and cross-reactivity of allergen sources. In this way, the concept of homologous groups could serve as a dynamic tool in the regulation of allergen products.

Copyright © 2008 S. Karger AG, Basel

Introduction

In the field of marketing authorization of industrially manufactured allergen products in Europe, the legal requirements on quality are laid down in the European Pharmacopoeia [1]. More detailed instructions on this issue concerning allergen extracts for in vivo diagnosis and immunotherapy can be found in the Note for Guidance on Allergen Products [2], which is valid in the European countries (EU and EFTA exclusive of Switzerland). This guideline has been prepared by the Biotechnology Working Party and the present version was released in 1996 by the Committee for Proprietary Medicinal Products of the European Medicines Agency in London.

One topic of this Note for Guidance lays down the practice that under certain conditions data of one aller-

gen extract can be extrapolated to other allergen sources of the same taxonomic family. A prerequisite for the take-over of data from one extract to other extracts or mixtures thereof is that the allergen products run through identical manufacturing processes and form final products of identical formulation. Mixtures of allergen sources of different families are excluded. This concept of extrapolation of data within a taxonomic family is functional with respect to the great variety of different allergen sources and the numerous possible mixtures thereof on the one hand, and their obvious biochemical resemblance on the other hand. For example, pollen have a very similar biochemical composition with similar water content, and their low proteolytic enzymatic activity ensures the stability of extract mixtures. Last but not least, neither the long-term experience with this principle during the last years nor scientific literature disproves the practice of this principle.

Over the last decade, allergy research generated new in-depth information on allergenic molecules in allergen sources and, especially driven by recombinant DNA technology, the protein and cDNA sequences of the majority of clinically relevant allergens have been identified. It turned out that allergens belonging to identical protein families are present in different allergen sources and that these homologous allergens often show similar biochemical properties and cross-reactivity. The presence of allergens with high sequence identity as well as with cross-reactivity among different allergen sources may not necessarily be restricted to allergen sources of one taxonomic family. The classification of allergen sources in one homologous group on the basis of homology and cross-reactivity of allergens is more specific and satisfactory than mere taxonomic relationship. Therefore, it has been suggested by regulators to change the 'concept of taxonomic families' into the new 'concept of homologous groups' [3]. The Note for Guidance is currently under thorough revision in many topics and one objective is to 're-define and elaborate in detail the concept of taxonomic allergen families' [4].

We tried to bring the suggested 'concept of homologous groups' to life and therefore evaluated the scientific literature (cutoff date: April 2006) with respect to the questions of cross-reactivity and homology of allergen sources. Hence, we established a list of homologous groups which may serve as a proposal for further discussions on this issue. Allergen sources have been included in the same homologous group on the basis of two criteria: The allergen sources have comparable physicochemical and biological properties, i.e. they derive from com-

parable tissues and therefore are alike in the overall composition of proteins, carbohydrates, lipids, enzymes and water content. This criterion ensures that for example grains and pollen from cereal species are distinguished as different allergen sources because they differ in kind and content of allergens. The allergens found in wheat (*Triticum aestivum*) grains (Tri a 14, 18, 19, 25 and 26) are not present in wheat pollen (Tri a 1, 2, 3, 4, 5, 12 and 13), but the pollen allergens of wheat are homologous to pollen allergens in several grass species (table 1) [5, 6]. The second criterion applied for the formation of homologous groups has been cross-reactivity between the allergen sources shown by cross-reactivity of single allergens or the whole extracts. Cross-reactivity has mainly been shown on the basis of patients' sera supported by studies with allergen-specific polyclonal animals' sera or monoclonal antibodies in some cases. In most cases, cross-reactivity is linked with homology of allergens belonging to identical protein families with a high degree of sequence identities at protein or cDNA level.

We reviewed the scientific literature in the fields of pollen from trees, grasses and weeds, mites, insect venoms and vertebrates and could establish six homologous groups among mites and pollen from trees, grasses and weeds. It was not possible to group any allergen sources from insect venoms and vertebrates due to insufficient homology. In each homologous group, we suggest one or more species as possible representative allergen source due to sufficient scientific information on characterized allergens and cross-reactivity with other species. In regulatory practice, equally to the 'concept of taxonomic family', the required studies can be performed with a representative species of one homologous group which finally can serve as surrogate for all species within this group on two conditions: The final extracts of one homologous group firstly have to be of identical formulation and secondly have to be run through identical production processes. In several homologous groups, more than one species are qualified as representative allergen source because of equivalent substantial levels of allergen characterization.

With particular interest regarding marketing authorization applications, new scientific knowledge on the homology and cross-reactivity of allergen sources would allow to add further species to one homologous group if the extracts fulfill the four specified terms illustrated in the preceding paragraphs: (1) comparable physicochemical and biological properties of the source materials, (2) cross-reactivity/structural homology of allergens, (3) identical formulation of the final products and (4) identi-

Table 1. Known pollen allergens in grass species of the Pooideae subfamily

MW kDa	Protein family	<i>Phleum pratense</i>	<i>Lolium perenne</i>	<i>Poa pratensis</i>	<i>Dactylis glomerata</i>	<i>Antho- xanthum odoratum</i>	<i>Avena sativa</i>	<i>Festuca spp.</i>	<i>Holcus lanatus</i>	<i>Hordeum vulgare</i>	<i>Secale cereale</i>	<i>Triticum aestivum</i>
26– 32	expansin-like proteins/ group 1 allergens	Phl p 1, major [42]	Lol p 1, major	Poa p 1, major	Dac g 1, major	Ant o 1, major	(Ave s 1)	(Fes p 1), (Fes e 1), (Fes r 1)	Hol l 1, major	(Hor v 1)	(Sec c 1)	(Tri a 1)
10– 13	expansin-like proteins/ group 2 allergens	Phl p 2, borderline minor/major [42]	Lol p 2, minor [42]	(Poa p 2)	Dac g 2, minor	(Ant o 2)	(Ave s 2)		(Hol l 2)	(Hor v 2)	(Sec c 2)	(Tri a 2)
11	expansin-like proteins/ group 3 allergens	(Phl p 3)	Lol p 3, minor [42]		Dac g 3, major							(Tri a 3)
55– 60	group 4 allergens/ berberine bridge enzymes	Phl p 4, major	Lol p 4, major [125]	(Poa p 4)	Dac g 4, major	(Ant o 4)	(Ave s 4)	Fes p 4, major (Fes e 4)	(Hol l 4)	(Hor v 4)	(Sec c 4)	(Tri a 4)
31	ribonucleases/ group 5 allergens	Phl p 5, major [42]	Lol p 5, major	Poa p 5, major	Dac g 5, major	(Ant o 5)	(Ave s 5)	(Fes p 5), (Fes e 5), (Fes r 5)	Hol l 5, major	Hor v 5, ND	(Sec c 5)	(Tri a 5)
15	P particle-associated proteins/Poa IX/Phl p VI allergen family ^a	Phl p 6, major [126]		(Poa p 6)		(Ant o 6)	(Ave s 6)					
6	Ca ²⁺ -binding proteins/ polcalcins	Phl p 7, minor [42]	(Lol p CBP)	(Poa p CBP)	(Dac g CBP)	(Ant o CBP)	(Ave s CBP)	(Fes e CBP)				(Tri a CBP)
12	cytochrome C		(Lol p 10) ^b , minor [127]	(Poa p 10)								
16	trypsin inhibitors/ Ole e 1 family ^a	Phl p 11, minor [128]	Lol p 11, major [129]									
14	profilins	Phl p 12, minor [42]	(Lol p 12)	(Poa p 12)	(Dac g 12)	(Ant o 12)	(Ave s 12)			(Hor v 12)	(Sec c 12)	(Tri a 12)
50– 60	polygalacturonases/ group 13 allergens	Phl p 13, major	(Lol p 13), major [130]	(Poa p 13)	(Dac g 13)	(Ant o 13)	(Ave s 13)	(Fes p 13), (Fes e 13)		(Hor v 13)	(Sec c 13)	(Tri a 13)

Allergens shown in parentheses are not listed in the IUIS official list of allergens [6]; allergens named by the Allergome database [5]. *Festuca* spp. = *F. pratensis*, *F. elatior* L., *F. rubra*; MW = molecular weight; ND = no data available.

^a According to Swiss-Prot [36].

^b Doubtful relevance as allergen [42, 127].

cal production process of the allergen extracts. Providing in this article in detail the scientific basis for group formation as well as the criteria and the decisions made, manufacturers and regulatory authorities will be enabled to use the ‘concept of homologous groups’ in a reasoned manner and as a dynamic tool which can be adapted to the scientific progress.

Tree Pollen

Three groups of homologous tree species are suggested (table 2).

The ‘Birch Group’

The ‘birch group’ consists of five tree species belonging to the same order of Fagales, four species are members of the taxonomic family Betulaceae, while oak (*Quercus alba*) belongs to the Fagaceae family, according to the ‘National Center for Biotechnology Information (NCBI)’ taxonomy database (table 2) [7]. Table 3 illustrates that birch is the best-characterized species within this group, with six allergens identified and listed in the official ‘International Union of Immunological Societies (IUIS)’ list of allergens, including numerous isoforms and variants of Bet v 1 [6]. In all other species in this group, a Bet v 1-homologous major allergen is present as well as the Bet v

Table 2. Suggestion for homologous groups of tree species**The 'birch group'**

<i>Betula verrucosa</i> =	
<i>B. pendula</i> ^a = <i>B. alba</i>	European white birch
<i>Alnus glutinosa</i>	alder
<i>Carpinus betulus</i>	hornbeam
<i>Corylus avellana</i>	hazel
<i>Quercus alba</i>	oak
Representative allergen source: birch	

The group of Oleaceae

<i>Olea europaea</i>	olive
<i>Fraxinus excelsior</i>	ash
<i>Ligustrum vulgare</i>	privet
<i>Syringa vulgaris</i>	lilac
Representative allergen sources: olive or ash	

The group of Cupressaceae

<i>Juniperus</i> spp.	cedar
<i>Cupressus</i> spp.	cypress
Representative allergen sources: cedar or cypress	

^a Correct taxonomic name according to the NCBI taxonomic database [7].

2 homologue profilin. The sequence identities of Bet v 1 homologues within this group are considerably high. For example, three Car b 1 cDNA clones from hornbeam have a deduced amino acid sequence identity of 73–75% to Bet v 1 [8]. Apart from similar sequential features, birch pollen allergens and their homologues in other tree pollen are also congruent in their importance as minor or major allergens (table 3). Additionally, cross-reactivity studies performed partially at the level of human IgE antibodies confirm the obvious relatedness of allergens from these tree species [8–15]. For example, the Bet v 1 and Bet v 2 homologues of alder, hazel, hornbeam and oak showed comparable reactivity to IgE antibodies of tree pollen-allergic subjects. By preabsorption of 102 sera with a combination of recombinant rBet v 1 and rBet v 2, the binding of IgE to whole tree pollen extracts was inhibited to a large extent (to oak: 72%, to hornbeam: 77%, to hazel: 80% and to alder: 88%) [10]. These data underline the predominance of the Bet v 1- and Bet v 2-homologous allergens in the above-mentioned Fagales extracts and illustrate why these species can be summarized within one group, although their spectrum of identified allergens may still be incomplete. The birch pollen allergen Bet v 4, a Ca²⁺-binding protein of 8 kDa, could possibly be another cross-reactive structure within the 'birch group' of tree pollen species. Up to now, cross-reactivity between Bet v 4 and Ca²⁺-binding proteins/polcalcins in pollen of

alder, hornbeam, hazel and oak has not yet been demonstrated. However, a homologous Ca²⁺-binding protein has been characterized in alder (Aln g 4). Additionally, the amino acid sequence identity of Bet v 4 is similar to several other Ca²⁺-binding proteins from botanically distant pollen allergens such as Phl p 7 from timothy grass [16, 17]. Its cross-reactivity with Phl p 7 has been demonstrated [18, 19] and can be explained by similar three-dimensional structure of both allergens [20]. The predominance of whole birch pollen extract has been confirmed by inhibition studies. Birch pollen extract completely inhibited human IgE binding to alder, hornbeam and oak, whereas the reverse inhibition of IgE binding to birch pollen extract by the mentioned extracts was incomplete [15]. The presence of luminal-binding protein Cor a 10, which was described as minor allergen in hazel pollen, has not been shown for birch or other Fagales species [21]. In the single reference [21] on this type of allergen, a probable cross-reactivity to a 70-kDa band in birch extract was indicated, which thus does not disprove the suggested group formation. Furthermore, the relevance of this protein family as allergens appears to be very limited.

Concluding from these results and since birch pollen is a very strong allergen source in Central Europe causing high sensitization rates, birch is recommended to be the representative allergen source within this group. Extrapolation of data has to be based on studies with birch pollen extracts.

The Oleaceae Group

The second group of homologous tree pollen species comprises four members of the olive family (Oleaceae): olive, ash, lilac and privet. Extracts of olive, ash, lilac and privet showed comparable IgE-binding patterns in Western blotting when probed with pooled serum of pollen-allergic subjects with positive skin prick test to olive pollen extract [22]. The major allergen Ole e 1 is a 20-kDa trypsin inhibitor and its homologues Fra e 1, Syr v 1 and Lig v 1 are present as major allergens in all Oleaceae of this group (table 4) [22, 23]. The minor olive allergen Ole e 3, a calcium-binding protein/polcalcin, is a pan-allergenic structure and homologues have been described in ash and lilac [24–26]. The β -1,3-glucanase Ole e 9 is a major allergen, and although respective allergens have not been identified in the remaining Oleaceae species, cross-reactive structures have been detected in ash, lilac and privet [27–29]. According to one reference [30], a 11-kDa allergen, named Ole e 10 by the authors, has been identified as glycosyl transferase. In ash, lilac and privet homologues have not been identified until now, but the exis-

Table 3. Known pollen allergens in tree species of the order Fagales, the ‘birch group’

MW kDa	Protein family	<i>Betula verrucosa</i>	<i>Alnus glutinosa</i>	<i>Corylus avellana</i>	<i>Carpinus betulus</i>	<i>Quercus alba</i>
17	Bet v 1 family	Bet v 1, major	Aln g 1, major	Cor a 1, major	Car b 1, major	Que a 1, major
15	profilins	Bet v 2, minor	(Aln g profilin), minor	Cor a 2, minor	(Car b 2), minor	(Que a 2), minor
23	Ca ²⁺ -binding proteins/polcalcins	Bet v 3, minor				
8	Ca ²⁺ -binding proteins/polcalcins	Bet v 4, minor	Aln g 4, minor			
34	isoflavone reductases	Bet v 6, minor				
18	cyclophilins	Bet v 7, minor				
70	luminal-binding proteins			Cor a 10, minor		

Allergens shown in parentheses are not listed in the IUIS official list of allergens [6]; allergens named by the Allergome database [5, 121]. MW = Molecular weight.

Table 4. Known pollen allergens in tree species of the Oleaceae family

MW kDa	Protein family	<i>Olea europaea</i>	<i>Fraxinus excelsior</i>	<i>Syringa vulgaris</i>	<i>Ligustrum vulgare</i>
16–20	trypsin inhibitors	Ole e 1, major	Fra e 1, major	Syr v 1, major	Lig v 1, major
13–18	profilins	Ole e 2, minor	(Fra e 2), minor		
7–10	Ca ²⁺ -binding proteins/polcalcins	Ole e 3, minor	(Fra e 3), minor [25]	Syr v 3, ND	
32	unknown	Ole e 4, major [122]			
16	superoxide dismutases	Ole e 5, minor			
5	unknown	Ole e 6, major [123]			
10	lipid transfer proteins	Ole e 7, minor			
21	Ca ²⁺ -binding proteins	Ole e 8, minor			
46	β-1,3-glucanases	Ole e 9, major	(Fra e 9), ND	(Syr v 9), ND	(Lig v 9), ND
11	glycosyl hydrolase homologues	Ole e 10, major			

Allergens shown in parentheses are not listed in the IUIS official list of allergens [6]; allergens named by the Allergome database [5]. MW = Molecular weight; ND = no data available.

tence of a cross-reactive structure can be concluded, since extracts of these pollen were able to inhibit binding of IgE to Ole e 10 [30]. In addition to the relatedness of the allergens, cross-reactivity of the extracts has widely been demonstrated with patients’ sera [25, 31, 32]: Extracts of Oleaceae mutually inhibited IgE binding [25, 31], but olive pollen extract was predominant, as it had the highest IgE-binding capacity of all Oleaceae tested in inhibition studies [32]. Therefore, olive is recommended as representative allergen source within the group of Oleaceae.

Olive trees are not present in Central and Northern Europe and consequently olive pollen is not an allergizing pollen species in allergic subjects from these regions.

From a scientific and practical point of view, it would not make sense to require studies based on olive tree pollen for applying the principle of homologous groups to extracts of other Oleaceae species which are intended for the markets in Central and Northern Europe. Therefore, we suggest ash to be another possible representative allergen source within the homologous group of Oleaceae because it is the second best-characterized species of the Oleaceae family.

The Cupressaceae Group

A third group of homologous tree species includes the *Juniperus* and *Cupressus* species of the cypress family

Table 5. Known pollen allergens in tree species of the Cupressaceae family

MW, kDa	Protein family	<i>Juniperus</i> spp.	<i>Cupressus</i> spp.
43	pectate lyases	Jun v 1, major, Jun a 1 ^a , major, (Jun c 1), ND, (Jun o 1), ND	Cup a 1, major, Cup s 1, major
43	polygalacturonases	Jun a 2, major	
30–34	thaumatin, PR-5	Jun a 3, minor [124], Jun v 3, ND	Cup s 3, major, (Cup a 3), major [34]
18	Ca ²⁺ -binding proteins/calmodulins/polcalcins	Jun o 4 (obsolete name Jun o 2), minor [9]	

Juniperus spp. = *J. virginiana*, *J. ashei*, *J. communis*, *J. oxycedrus*; *Cupressus* spp.: *C. arizonica*, *C. sempervirens*; MW = molecular weight; ND = no data available. Allergens shown in parentheses are not listed in the IUIS official list of allergens [6]; allergens named by the Allergome database [5].

^a Former name: Jun s 1 (*Juniperus sabinoides*).

(Cupressaceae). Each species of both genera can function as representative allergen source for extrapolation of data. The allergens of the cypress family (table 5) show a high degree of amino acid sequence identity. For example, the major allergen Jun a 1 shares 97% amino acid sequence identity with Jun v 1 and 95% identity with the major allergens Cup a 1 and Cup s 1. The allergens Jun a 3 and Cup a 3, which are thaumatin-like proteins, share 95% amino acid sequence identity with each other [33–36]. Therefore, the grouping of members of trees from the cypress family is justified by this remarkably high degree of sequence identity and further supported by a few cross-reactivity studies [37].

Non-Grouped Tree Species

Several tree pollen species could not be assigned to one of the above-mentioned groups (table 2) because of the absence of scientific information on allergen extracts or on identified allergens. As a consequence, the tree species *Acer* spp. (maple), *Populus* spp. (poplar), *Robinia pseudo-acacia* (locust tree), *Salix* spp. (willow) and *Ulmus* spp. (elm) have not been included in one of the groups. First results with extracts of the tree species *Fagus sylvatica* (European beech) in skin tests indicated that beech, belonging to the order Fagales, is a possible candidate for the ‘birch group’, but at the present time the information is not substantial enough to justify this classification [38]. Likewise, allergens of *Tilia* spp. (linden) have not been identified to date, and results with *Tilia* pollen extracts showing cross-reactivity with *Platanus* spp. pollen but not with olive pollen were considered insufficient for grouping [39]. Concerning *Platanus* spp. (plane tree), several allergens have been identified and characterized, but cross-reactivity studies with other tree pollen species did

not sufficiently demonstrate an immunological relationship of the allergens that would allow a group formation [9, 40, 41].

In case of the tree species *Cryptomeria japonica* (Japanese cedar) of the Cupressaceae family, high amino acid sequence identities are shared between its allergens Cry j 1 and Cry j 2 and Jun a 1 and Jun a 2, respectively, of *Juniperus ashei* (mountain cedar). Even so, it is recommended not to include this species in the homologous group of Cupressaceae (table 2) since *C. japonica* trees are rarely present in continental Europe and Northern America and therefore are without clinical relevance in these regions. Additionally, the different genetic backgrounds of the Asian, the European and the American populations may cause different sensitization patterns to cypress tree pollen in allergic subjects. Since it has not been demonstrated that the clinical relevance of homologous allergens of various cypress trees in different populations is comparable, the inclusion of *C. japonica* in the homologous group of Cupressaceae may not be justified to date.

Pollen of Grass Species and Cereal Species

One group of sweet grasses of the Poaceae (Gramineae) family, the subfamily Pooideae, is suggested in table 6.

Numerous allergens belonging to eleven allergen families have been identified in pollen of various grass species (table 1). For example, in *Phleum pratense* as well as in *Lolium perenne*, ten pollen allergens with molecular weights ranging from 6 to 60 kDa have been described up to now. Three allergen families are suitable as criterion

for homologous group formation of grass species: Firstly, group 1 grass pollen allergens comprise 26- to 31-kDa glycoproteins and belong to the family of expansin-like proteins. This protein family has members in all the grass species listed in table 1 and a prevalence of IgE recognition in 90–95% of grass pollen-allergic subjects [42–45]. Secondly, group 5 grass pollen allergens are major allergens with sensitization frequencies of 65–68% [42] and 80–90% [44, 46] among grass pollen-allergic subjects. They have been identified in many members of the Pooideae subfamily (and in all the members listed in table 1) but not outside this taxon. Thirdly, the group 2 grass pollen allergens consist of non-glycosylated proteins of 10–13 kDa belonging to the family of expansin-like proteins with 85–95% protein sequence identity between species. They are major or minor allergens as IgE binding to the individual members is found in 40–60% of grass pollen-allergic subjects [46]. Allergens of this family have been described for all grass species in table 1 except for *Festuca* spp.

For other allergen families such as group 3 grass pollen allergens, group 6 grass pollen allergens or group 11 grass pollen allergens, insufficient information is available for several grass species to substantially support group formation, but there are no contradictory data. The group 12 grass pollen allergens are profilins which are highly conserved pan-allergenic proteins ubiquitously found throughout the plant kingdom and thus are not useful as markers for defining homologous groups. Similarly, the group 4 grass pollen allergens are not a very distinct criterion for the formation of homologous groups, despite the high sensitization rate of up to 80% among grass pollen-sensitized individuals. Since it is likely that the IgE response to group 4 grass pollen allergens is in part directed to the glycan structures of the glycoprotein (10–15% of their molecular weight accounts for carbohydrates), IgE-binding and cross-reactivity studies may be impaired by a considerable rate of false-positive results [42].

Group formation on the basis of homologous structures is supported by IgE-binding results with patients' sera. Andersson and Lidholm [42] performed ImmunoCAP measurements in 143 grass pollen-sensitized individuals and found very similar IgE-binding capacity for *P. pratense* and all grass species listed in table 1 with the exception of *Hordeum vulgare* which was not tested. Van Ree et al. [43] determined RAST (radioallergosorbent test) values in 209 sera from grass pollen-sensitized individuals for 8 of the 11 grass species in table 1 (not tested were *Avena sativa*, *H. vulgare* and *T. aestivum*). Highest responses were observed to *Poa pratensis* followed by *Fes-*

Table 6. Suggestion for a homologous group of sweet grasses of the Poaceae (Gramineae) family, a subfamily of Pooideae

<i>Anthoxanthum odoratum</i>	sweet vernal grass
<i>Avena sativa</i>	oat
<i>Dactylis glomerata</i>	orchard grass/cocksfoot
<i>Festuca</i> spp.	meadow fescue
<i>Holcus lanatus</i>	velvet grass/Yorkshire fog
<i>Hordeum vulgare</i>	barley
<i>Lolium perenne</i>	perennial ryegrass
<i>Phleum pratense</i>	timothy grass
<i>Poa pratensis</i>	Kentucky bluegrass
<i>Secale cereale</i>	cultivated rye
<i>Triticum aestivum</i>	cultivated wheat
Representative allergen sources: timothy grass, orchard grass or Kentucky bluegrass	
Additional grass species belonging to the homologous group of Pooideae with reservations	
<i>Agropyron</i> spp.	couch grass, crested wheatgrass
<i>Agrostis</i> spp.	bent grass
<i>Alopecurus pratensis</i>	meadow foxtail
<i>Arrhenatherum elatius</i>	false oat
<i>Bromus</i> spp.	bromegrass
Representative allergen sources: timothy grass or Kentucky bluegrass	

tuca rubra, *P. pratense*, *Dactylis glomerata*, *L. perenne*, *Holcus lanatus*, *Anthoxanthum odoratum* and *Secale cereale* indicating similar IgE-binding properties among the mentioned grass species, and implying at least a partial cross-reactivity.

Several cross-reactivity studies with grass pollen allergens showing that the majority of grass species are highly cross-reactive are further indicators for the suggested group formation [43, 44, 47–59; for reviews see 42, 46, 60–64]. For example, RAST inhibition tests demonstrated that extract of *P. pratense* pollen was able to inhibit binding of IgE antibodies of subjects with grass-positive skin tests to extracts of *P. pratensis*, *L. perenne*, *Agrostis alba*, *Festuca* spp., *A. odoratum*, *Agropyron* spp. and *Bromus* spp. Similarly, extracts of *Agropyron* spp. were able to inhibit the binding to the listed extracts of grass species [58].

Five additional grass species of the Pooideae subfamily (*Agropyron* spp., *Agrostis* spp., *Alopecurus pratensis*, *Arrhenatherum elatius* and *Bromus* spp.) are separately listed in table 7. Allergenic structures in these species have been identified by IgE binding or using group-specific monoclonal or polyclonal antibodies, but detailed characterization of individual allergens has rarely been performed [47, 50, 52–54, 58, 65, 66]. These species could

Table 7. Pollen allergens in additional grass species recommended with reservations to be included in the homologous group of Pooideae

MW, kDa	Protein family	<i>Agropyron cristatum</i>	<i>Agrostis</i> spp.	<i>Alopecurus pratensis</i>	<i>Arrhenatherum elatius</i>	<i>Bromus</i> spp.
28–31	group 1 allergens		(Agr a 1, Agr ca 1)	(Alo p 1)	(Arr e 1)	(Bro a 1)
60	group 4 allergens [52]		(Agr a 60 kDa) ^a			(Bro a 4)
26–33	group 5 allergens [47]			(Alo p 5) ^b		
12	Ca ²⁺ -binding proteins/polcalcins	(Agr c CBP)				(Bro i CBP)

Agrostis spp. = *A. alba*, *A. capillaris*. *Bromus* spp. = *B. arvensis*, *B. inermis*; MW = molecular weight. Allergens shown in parentheses are not listed in the IUIS official list of allergens [6]; allergens named by the Allergome database [5]. The attribution ‘major’ or ‘minor’ allergen is not yet possible, because of lack of information.

^a Allergen neither listed in the IUIS official list of allergens nor in the Allergome database; allergen referenced in [52].

^b Allergen neither listed in the IUIS official list of allergens nor in the Allergome database; allergen referenced in [47, 52].

Table 8. Suggestion for a homologous group of weed species

<i>Ambrosia artemisiifolia</i> , <i>Ambrosia trifida</i>	ragweed
<i>Artemisia vulgaris</i>	mugwort
<i>Parietaria judaica</i> , <i>Parietaria officinalis</i>	pellitory
Representative allergen sources: ragweed or mugwort, but not pellitory	

be assigned to the homologous group of Pooideae if *P. pratense* or *P. pratensis* is chosen as representative allergen owing to the fact that cross-reactivity among its members has been shown [58].

For the homologous group of Pooideae, *D. glomerata* and *P. pratensis* especially qualify as representative reference allergens due to the high degree of IgE cross-reactivity found between these grasses and other species, as does *P. pratense*, the best-studied allergenic grass species which contains members of all relevant grass pollen allergen families.

We did not group the grass species *Cynodon dactylon* (Bermuda grass) within the homologous group of grass species because the cross-reactivity of *C. dactylon* with other grass species is not substantial. Currently, seven allergens of *C. dactylon* have been characterized and named in the official list of allergens of the IUIS (Allergen Nomenclature Subcommittee) [6]. Only two of these seven *C. dactylon* allergens are cross-reactive: Cyn d 1 and Cyn d 7, but their cross-reactive features with other grasses are not striking. Whereas the amino acid sequence identities of Cyn d 1 proteins with Lol p 1 are 62–66% and with Sor h 1 64–66% [67], cross-reactivity of Cyn d 1 with other group 1 grass allergens is contradictory: A Cyn d 1-spe-

cific rabbit serum as well as four Cyn d 1-specific monoclonal antibodies detected Cyn d 1 as well as group 1 components of the same molecular weight in pollen extracts of ten grass species and cereal species [68]. In contrast, in another test the polyclonal Cyn d 1-specific rabbit serum showed weak cross-reactivity to these ten grass species and cereal species whereas a Lol p 1-specific rabbit serum easily bound to these grass and cereal species but rarely to Cyn d 1 [68]. Another study with four Cyn d 1-specific monoclonal antibodies demonstrated a lack of cross-reactive binding to ten other grasses pollen species [69]. Additionally, eighteen Phl p 1-specific monoclonal antibodies bound to twelve different grass pollen and cereal pollen species (such as orchard grass, ryegrass, timothy grass, Kentucky bluegrass, cultivated rye or wheat and barley), whereas only four of them weakly bound to Cyn d 1 [70]. Therefore, Cyn d 1 cannot be characterized as a homologue of the group 1 grass pollen allergens. Concerning Cyn d 7, cross-reactivity has been shown to only one grass pollen allergen (Phl p 7) from timothy grass [18] as well as to birch pollen allergen Bet v 4 [71, 72] and allergen Bra r 1 from oilseed rape *Brassica rapa* (turnip) [72]. Consequently, Cyn d 7 does not belong to a grass-specific group of homologous pollen allergens. In extracts of *C. dactylon*, immunologically active members of group 2 and group 5 grass pollen allergens were absent and cross-reactivity to group 4 grass pollen allergens could not be shown [48, 52, 61]. Furthermore, *Cynodon*-positive patients’ sera were only in part detected by other grass pollen extracts such as *D. glomerata* and *P. pratensis* [43]. Therefore, the current knowledge does not justify the inclusion of *C. dactylon* within the homologous group of grass species.

Table 9. Known pollen allergens in weed species

MW, kDa	Protein family	<i>Ambrosia</i> spp.	<i>Artemisia vulgaris</i>	<i>Parietaria</i> spp.
38–42	polysaccharide lyase I family/pectate lyases	Amb a 1, major	Art v 6, minor	
38	polysaccharide lyase I family/pectate lyases	Amb a 2 ^a , major	Art v 2, major [131]/ minor [132]	
11	unknown (contains 1 plastocyanin-like domain)	Amb a 3, minor [133]		
5–8	unknown	Amb a 5, minor, Amb t 5, minor [134], Amb p 5, ND		
10–15	lipid transfer proteins	Amb a 6, minor	Art v 3, minor	Par j 1 ^b , major [74], Par o 1, major [135], Par j 2 ^b , major/minor according to region [75, 136], (Par m 1), major [137]
12	unknown	Amb a 7, minor		
14	profilins	Amb a 8, minor (Amb t profilin), ND	Art v 4, minor	Par j 3, minor
9	Ca ²⁺ -binding proteins/polcalcins	Amb a 9, minor	Art v 5, minor	Par j 4, minor [138]
18	Ca ²⁺ -binding proteins/polcalcins	Amb a 10, minor		
24–28	plant defensin family/PR-12		Art v 1, major	
10	cystatin proteinase inhibitors	(Amb a cystatin proteinase inhibitor), ND		

Ambrosia spp. = *A. artemisiifolia*, *A. trifida*, *A. psilostachya*; *Parietaria* spp., *P. judaica*, *P. officinalis*, *P. mauritanica*; MW = molecular weight; ND = no data available. Allergens shown in parentheses are not listed in the IUIS official list of allergens [6]; allergens named by the Allergome database [5].

^a Amb a 2 shares 65% amino acid sequence identity with Amb a 1.

^b Par j 1 and Par j 2 allergens share sequence identity [136, 139].

Weed Pollen

We suggest to form one group of weed species (table 8).

In *Ambrosia* and *Artemisia*, allergens belonging to the same protein families are pectate lyases with the major allergen Amb a 1 (ragweed) and the minor allergen Art v 6 (mugwort), the lipid transfer proteins represented by the minor allergens Amb a 6 and Art v 3, and the calcium-binding proteins/polcalcins Amb a 9 and Art v 5, which are both minor allergens as well. Cross-reactivity between pollen extracts of the weeds *Ambrosia* (ragweed) and *Artemisia* (mugwort) was shown by ragweed extract inhibiting 80% of the IgE binding to mugwort extract with sera from mugwort-allergic subjects [73]. Concluding from these data, the listing of *Ambrosia* and *Artemisia* as homologous weeds in one group appears to be justified.

In *Parietaria* (pellitory), the spectrum of identified pollen allergens is incomplete in comparison to that of *Ambrosia* and *Artemisia* (table 9), but the most important and best-characterized allergens of *Parietaria*, Par j/o/m 1, are lipid transfer proteins and therefore belong to the

same protein family as the allergens Amb a 6 from ragweed and Art v 3 from mugwort [74]. Moreover, weed pollen-allergic subjects are often co-sensitized to all three weeds *Ambrosia*, *Artemisia* and *Parietaria*. Even in regions where *Parietaria* is a rare weed, the sensitization of weed-allergic subjects to *Parietaria* is highly associated (93–100%) with concomitant sensitization to ragweed and mugwort [75]. Therefore, we propose the inclusion of *Ambrosia*, *Artemisia* and *Parietaria* in one group of homologous weed species with the restriction of *Ambrosia* or *Artemisia* but not *Parietaria* being representative allergen sources for all three weeds.

To date, the *Plantago* species (plantain) cannot be assigned to any homologous group because of insufficient information on plantain allergens and their cross-reactivity [76, 77]. The only identified pollen allergen of *Plantago lanceolata* in the IUIS list, Pla l 1, is a member of the Ole e 1 family with an amino acid sequence identity of 40% with Ole e 1, but natural Pla a 1 is to a negligible degree cross-reactive to natural Ole e 1 [78].

Table 10. Allergens in house dust mites of the genus *Dermatophagoides*

MW, kDa	Protein family	<i>Dermatophagoides pteronyssinus</i>	<i>Dermatophagoides farinae</i>
25	cysteine proteases	Der p 1, major	Der f 1, major
14	group 2 mite allergens/NPC2 family ^a	Der p 2, major	Der f 2, major
25	serine proteases: trypsins	Der p 3, minor/major	Der f 3, minor/major
57	amylases	Der p 4, minor	(Der f 4), minor [140]
15	unknown	Der p 5, major	(Der f 5), minor [141]
25	serine proteases: chymotrypsins	Der p 6, minor [142]	Der f 6, minor [142]
25–31	unknown	Der p 7, major	Der f 7, major
26	glutathione S transferases	Der p 8, minor	(Der f 8), ND
30	collagenolytic serine proteases	Der p 9, major	(Der f 9), ND
37	tropomyosins	Der p 10, major	Der f 10, major
96	paramyosins	Der p 11, major	Der f 11, major
15	fatty acid-binding proteins	(Der p 13), ND	Der f 13, ND
177	apolipoprotein-like proteins	Der p 14, major	Der f 14, major
63–105	chitinases (95 kDa)	(Der p 15), major [143]	Der f 15, major
55	gelsolins/villins		Der f 16, minor
53	Ca ²⁺ -binding proteins		Der f 17, minor
60	chitinases (60 kDa)	(Der p 18), major [143]	Der f 18, major
40	arginine kinases	Der p 20, ND	
16	unknown	Der p 21, ND	
17 ^a	similar to mite group 2 allergen ^b		Der f 22, ND [6]

Allergens shown in parentheses are not listed in the IUIS official list of allergens [6]; allergens named by the Allergome database [5]. MW = Molecular weight; ND = no data available. All prevalences according to Thomas et al. [145], if not indicated by a reference.

^a According to Swiss-Prot [36].

^b According to Entrez Protein Accession No. ABG35122 [144].

House Dust Mites

The broadest and most complex spectrum of allergens among allergen sources discussed in this article has been identified in the house dust mite genus *Dermatophagoides* (table 10). In the two common species *D. farinae* (American house dust mite) and *D. pteronyssinus* (European house dust mite), 20 allergens have been identified to date, with molecular weights ranging from 14 to 177 kDa. Of note, 15 of the 20 allergens are present as homologues in both species. Consequently, the grouping of these two mite species solely on the basis of the presence of related allergens appears to be justified, and contradictory results from cross-reactivity studies are not expected.

We did not assign the following species of mites to the homologous group of house dust mites because of the absence of identified allergens as well as insufficient information on cross-reactivity of allergen extracts: *Acarus siro* (flour mite), *Glycyphagus domesticus* (house mite),

Lepidoglyphus destructor (house mite), *Tyrophagus putrescentiae* (storage mite) and *Thyreophagus entomophagus* (flour mite).

Insect Venoms

Until now, exclusively the insect venoms of honeybee (*Apis mellifera*) and yellow jacket species (*Vespula*) are relevant as diagnostic and therapeutic extracts, and double sensitization to both venoms is rather frequent [79–81]. Within groups of *Vespula*-sensitized subjects according to RAST results, the rate of subjects also sensitized to honeybee venom ranges from 45% (278 of 611) to 89% (41 of 46) and, correspondingly, in honeybee-positive subjects co-sensitization to *Vespula* species ranges from 45% (278 of 622) to 84% (41 of 49) [79, 80]. Reasons for double sensitization to honeybee and yellow jacket could be independent sensitization to both insect venoms or cross-

Table 11. Known allergens of insect venoms

MW kDa	Protein family	<i>Apis mellifera</i>	<i>Vespula</i> spp.	<i>Dolicho- vespula</i> spp.	<i>Polistes</i> spp.	<i>Vespa</i> spp.	<i>Bombus</i> spp.
16	phospholipases A2	Api m 1, major					Bom p 1, Bom t 1
34	phospholipases A1B		Ves m 1, major, Ves v 1, major, Ves s 1, major, (Ves g 1)	Dol m 1	Pol a 1, Pol d 1, Pol e 1, Pol g 1	Vesp c 1, Vesp m 1	
46	hyaluronidases	Api m 2, major	Ves m 2, major [91], Ves v 2, major [146], (Ves f 2), (Ves g 2), (Ves p 2), (Ves s 2), major [147]	Dol m 2	Pol a 2, (Pol e 2), (Pol g 2)	(Vesp c 2)	(Bom p hyaluronidase)
49	acid phosphatases	Api m 3, major					(Bom p acid phosphatase)
3	melittin	Api m 4, minor					
30–34	serine proteases				Pol d 4, Pol e 4, (Pol g 4)		Bom p 4, Bom t 4
23	unknown/antigen 5		Ves f 5, ND, Ves g 5, ND, Ves m 5, major [91], Ves p 5, ND, Ves s 5, major [147], Ves vi 5, ND, Ves v 5, major [146]	Dol m 5, Dol a 5	Pol a 5, Pol d 5, Pol e 5, Pol f 5, Pol g 5, Pol m 5	Vesp c 5, Vesp m 5	
8	allergen C	Api m 6, minor					
39	CUB serine protease	Api m 7, major [148]					

Vespula spp. = *V. flavopilosa*, *V. germanica*, *V. maculifrons*, *V. pensylvanica*, *V. squamosa*, *V. vidua*, *V. vulgaris*; *Dolichovespula* spp. = *D. maculata*, *D. arenaria*; *Polistes* spp. = *P. annularis*, *P. dominulus*, *P. exclamans*, *P. fuscatus*, *P. gallicus*, *P. metricus*; *Vespa* spp. = *V. crabro*, *V. mandarinia*; *Bombus* spp. = *B. pensylvanicus*, *B. terrestris*; MW = molecular weight; ND = no data available. Allergens shown in parentheses are not listed in the IUIS official list of allergens [6]; allergens named by the Allergome database [5].

reactivity between the two venom extracts. According to studies by Reisman et al. [82] and Straumann et al. [83], 17 of 25 and 12 of 22 double-positive sera, respectively, showed cross-reactivity in inhibition studies, thus assuming cross-reactivity as the predominant reason for double sensitization to honeybee venom and yellow jacket venom. However, latest data of inhibition studies with glycan-rich pollen extracts and synthetic neoglycoproteins revealed ‘cross-reactive carbohydrate determinants’ (CCD) as a major cause of double sensitization to extracts of honeybee venom and yellow jacket venom [81, 84, 85]. Hemmer et al. [84] showed that in 10 of 15 double-positive sera the cross-reactivity was solely caused by CCD. Jappe et al. [85] identified IgE reactivity to CCD in 123 of 147 (75%) double-positive sera. In honeybee venom, the glycosylated allergens currently known are phospholipase A2 (Api m 1), hyaluronidase (Api m 2) and acid phosphatase (Api m 3) [86, 87], whereof the glycan parts of phospholipase A2 and hyaluronidase were characterized as IgE-binding CCD [84, 88, 89]. In yellow jacket venom, solely the major allergen hyaluronidase (Ves m/v/f/g/p/s 2) has been shown to be a glycoprotein containing CCD [84, 90] but further, yet unidentified IgE-binding glycoproteins are present in yellow jacket as well as in honeybee venoms [84, 91]. The only homologous aller-

genic structure in honeybee venom and yellow jacket venom identified so far is hyaluronidase, with an amino acid sequence identity of 53% between Api m 2 (Swiss-Prot Q08169) and Ves v 2 of *Vespula vulgaris* (Swiss-Prot P49370; table 11) [92, 93]. The comparison of the three-dimensional structures of the two hyaluronidases Api m 2 and Ves v 2 showed a high resemblance, but differences in the surface topology and the charge distribution on the surface were appraised as reasons for the absence of common IgE epitopes and cross-reactivity [94, 95]. Concluding from these data, the venoms of honeybee and yellow jacket cannot form one homologous group.

However, scientific literature provides hints that honeybee venom could be homologous with bumblebee venom. Homologues of the major allergens phospholipase A2, hyaluronidase and acid phosphatase from honeybee venom are also present in bumblebee venom, as noted in table 11. The amino acid sequence identity between the phospholipases Api m 1 of honeybee venom and Bom p 1 and Bom t 1 of bumblebee venom are 54 and 53%, respectively [96]. A high percentage of 75 and 85% of double-positive sera in honeybee- and bumblebee-allergic subjects, respectively, could indicate cross-reactivity between the two venoms [97]. Six of 7 bumblebee-allergic patients reported amelioration of their

Table 12. Known allergens from dog (*Canis familiaris*) and cat (*Felis domesticus*)

MW kDa	Protein family	<i>Canis familiaris</i>		<i>Felis domesticus</i>	
		allergen	origin	allergen	origin
23–25	lipocalins	Can f 1, major [149]	salivary protein		
19	lipocalins	Can f 2, minor [149]	salivary protein		
69	albumins	Can f 3, minor [149]	serum protein, salivary protein	Fel d 2, minor [149]	serum protein
18	unknown	Can f 4, major [150]	epithelial protein		
18	secretoglobulin/ uteroglobulin			Fel d 1, major [149]	salivary protein, epithelial protein
11	cystatins			Fel d 3, minor [149]/, major [151]	epithelial protein
21	lipocalins			Fel d 4, major [117]	salivary protein
400	IgA			Fel d 5w, major [152]	serum protein
800–1,000	IgM			Fel d 6w, borderline minor/major [152]	serum protein
150	IgG			(Fel d 7), ND	serum protein

Fel d 3 contains cysteine protease inhibitor motif also present in Can f 1 and Can f 2 and two of three lipocalin motifs [151].

Allergen shown in parentheses is not listed in the IUIS official list of allergens [6]; allergen named by the Allergome database [5]. MW = Molecular weight; ND = no data available.

bumblebee allergy after immunotherapy with honeybee venom [98].

The vespids of the genera *Vespula* (yellow jackets), *Dolichovespula* (white face hornet and yellow hornet), *Polistes* (wasps) and *Vespa* (European hornet) seem to be candidates for another homologous group of insect venoms. Cross-reactivity was demonstrated between the two yellow jacket species *Vespula maculifrons* and *Vespula squamosa* as well as between *Vespula maculifrons* and *Vespa crabro* (European hornet) on the basis of inhibition studies with human sera [99]. A mixture of venoms of *Vespula germanica*, *V. maculifrons* and *Vespula vulgaris* inhibited IgE binding to *Dolichovespula maculata* (white face hornet) and vice versa and antigenic relatedness could be possible between *Vespula* spp. and *Polistes* spp. (wasps) due to RAST correlation studies [100, 101]. A possible cross-reactivity within the vespids could among others be based on phospholipase A1B as common structure (table 11), as demonstrated by studies with phospholipases of *Vespula*, *D. maculata* and *Polistes exclamans* and supported by a considerable amino acid sequence identity of 69% of Ves m 1 with Dol m 1.01 [93, 102, 103]. Antigen 5 (Ag5) allergens display another common and potentially cross-reactive structure within the vespids sharing amino acid sequence identities of 57–67% of Ves v 5 (*V. vulgaris*) with the Ag5 allergens of the *Dolichovespula*, *Polistes* and *Vespa* species and identities of 71–98% within diverse *Vespula* species [104–106].

Allergen Extracts Derived from Vertebrates

For the establishment of a homologous group, we considered the following animal species: cat (*Felis domesticus*), dog (*Canis familiaris*), horse (*Equus caballus*), mouse (*Mus musculus*), rat (*Rattus* spp.), guinea pig (*Cavia porcellus*), hamster (*Cricetus cricetus*) and rabbit (*Oryctolagus cuniculus*).

The known allergens of these animals are present in different body compartments such as pelt, fur, dander, sweat, saliva, urine and serum showing varying types and contents of allergens [107–112]. In addition to the tissue source, the production method influences the allergen composition of an extract [113]. Consequently, the establishment of a homologous group of animals is, basically, impossible. However, allergens belonging to the same protein family and showing cross-reactivity are present in taxonomically distant animal species. One example is a 67-kDa albumin, which is present as allergen in nearly all of the above-mentioned species and which has been demonstrated as cross-reactive structure in cats, dogs, horses, mice, rats and guinea pigs [114–116]. Another example is the 21-kDa allergen lipocalin, which is cross-reactive between cat, horse and dog [117]. The 21-kDa lipocalins of cat (Fel d 4), mouse (Mus m 1) and rat (Rat n 1) share considerable amino acid sequence identities with horse allergen Equ c 1 (70, 49 and 51%). However, in specific cases, allergen extracts from different animal species may be included in one homologous group and proof of quality for

one extract can be extrapolated to the other extracts of the group. Prerequisites could be (1) identical source material used for extract preparation, (2) known presence of cross-reactive allergens and (3) sufficient cross-reactivity shown by inhibition studies. For example, under these conditions dog and cat extracts could be grouped. Only one allergen, the 69-kDa albumin, is present in both species and is known as a cross-reactive allergen (table 12) [118]. Nevertheless, inhibition studies surprisingly showed cross-reactivity of cat and dog extracts under certain conditions [108, 118–120]. Data demonstrated cross-reactivity for cat hair and dog hair but not for cat saliva and dog saliva or cat urine and dog urine, respectively [108]. Thus, subject to the quality of individual extracts and the demonstration of the presence of homologous allergens, grouping may be justified on the basis of detailed data provided by the manufacturer.

Conclusion

For allergen sources sharing a similar biochemical matrix composition, the ‘concept of taxonomic families’ could be replaced by the ‘concept of homologous groups’ forming groups of antigenically related allergen sources with well-characterized allergen sources as ‘representa-

tive allergens’. We present a proposal of homologous groups consisting of tree pollen species (‘birch group’, Oleaceae, Cupressaceae), grass and cereal pollen species (Pooideae), weeds and house dust mites (*Dermatophagoides* spp.). In most of the cases, the homologous groups reflect a close taxonomic relatedness. The concept of homologous groups is dynamic and groups could be complemented by additional species depending on the availability of new information on identified allergens, homologous allergen families and cross-reactivities.

Acknowledgments

We thank Marie-Christine Annequin [AFSSAPS (Agence française de sécurité sanitaire des produits de santé), Saint-Denis, France], Carl Doleman [NIBSC (National Institute for Biological Standards and Control), Potters Bar, UK], Marcel Hoefnagel [RIVM (Rijksinstituut voor Volksgezondheid en Milieu), Bilthoven, The Netherlands], Fritz Lackner [AGES (Österreichische Agentur für Gesundheit und Ernährungssicherheit), Vienna, Austria], Sandra Lopes [AFSSAPS, Saint-Denis, France], Margarida Menezes Ferreira [INFARMED (Instituto Nacional de Farmácia e do Medicamento), Lisbon, Portugal], Carlo Pini [ISS (Istituto Superiore di Sanità), Rome, Italy] and Peter Stjärnkvist [MPA (Läkemedelsverket), Uppsala, Sweden] for fruitful discussions.

References

- 1 Monograph: Allergen Products – Producta allergenica; in European Directorate for the Quality of Medicines (EDQM) (ed): European Pharmacopoeia, ed 6. Strasbourg, Council of Europe, 2008, pp 679–680.
- 2 Committee for Proprietary Medicinal Products (CPMP) and Biotechnology Working Party (BWP): Note for Guidance on Allergen Products. 1996;CPMP/BWP/243/96.
- 3 Pini C: Summary of the regulators’ session. Arb Paul Ehrlich Inst Bundesamt Sera Impfstoffe Frankf AM 2006;95:45–48.
- 4 Committee for Medicinal Products for Human Use (CHMP) and Biologics Working Party (BWP): Concept Paper on the revision of the Note for Guidance on Allergen Products (CPMP/BWP/243/96): Production and quality issues. 2005; CHMP/BWP/229472/2005.
- 5 Allergome – A database of allergenic molecules. <http://www.allergome.org/>.
- 6 International Union of Immunological Societies (IUIS) – Allergen Nomenclature Subcommittee: The official ‘List of Allergens’. Update March 10, 2008. <http://www.allergen.org/Allergen.aspx>.
- 7 The National Center for Biotechnology Information (NCBI) Taxonomy Database. <http://www.ncbi.nlm.nih.gov/Taxonomy/taxonomyhome.html/index.cgi>.
- 8 Larsen JN, Stroman P, Ipsen H: PCR based cloning and sequencing of isogenes encoding the tree pollen major allergen Car b I from *Carpinus betulus*, hornbeam. Mol Immunol 1992;29:703–711.
- 9 Tinghino R, Twardosz A, Barletta B, Puggioni EM, Iacovacci P, Butteroni C, Afferni C, Mari A, Hayek B, di Felice G, Focke M, Westritschnig K, Valenta R, Pini C: Molecular, structural, and immunologic relationships between different families of recombinant calcium-binding pollen allergens. J Allergy Clin Immunol 2002;109:314–320.
- 10 Niederberger V, Pauli G, Gronlund H, Froschl R, Rumpold H, Kraft D, Valenta R, Spitzauer S: Recombinant birch pollen allergens (rBet v 1 and rBet v 2) contain most of the IgE epitopes present in birch, alder, hornbeam, hazel, and oak pollen: a quantitative IgE inhibition study with sera from different populations. J Allergy Clin Immunol 1998;102:579–591.
- 11 Ipsen H, Hansen OC: The NH₂-terminal amino acid sequence of the immunochemically partial identical major allergens of alder (*Alnus glutinosa*) Aln g I, birch (*Betula verrucosa*) Bet v I, hornbeam (*Carpinus betulus*) Car b I and oak (*Quercus alba*) Que a I pollens. Mol Immunol 1991;28:1279–1288.
- 12 Rohac M, Birkner T, Reimitzer I, Bohle B, Steiner R, Breitenbach M, Kraft D, Scheiner O, Gabl F, Rumpold H: The immunological relationship of epitopes on major tree pollen allergens. Mol Immunol 1991;28:897–906.
- 13 Valenta R, Breiteneder H, Petternburger K, Breitenbach M, Rumpold H, Kraft D, Scheiner O: Homology of the major birch-pollen allergen, Bet v I, with the major pollen allergens of alder, hazel, and hornbeam at the nucleic acid level as determined by cross-hybridization. J Allergy Clin Immunol 1991;87:677–682.
- 14 Wiedemann P, Giehl K, Almo SC, Fedorov AA, Girvin M, Steinberger P, Rudiger M, Ortner M, Sippl M, Dolecek C, Kraft D, Joekusch B, Valenta R: Molecular and structural analysis of a continuous birch profilin epitope defined by a monoclonal antibody. J Biol Chem 1996;271:29915–29921.

- 15 Jung K, Schlenvoigt G, Jäger L: Allergologic-immunochemical study of tree and bush pollen. III. Cross reactions of human IgE antibodies with various tree pollen allergens (in German). *Allerg Immunol (Leipz)* 1987;33: 223–230.
- 16 Engel E, Richter K, Obermeyer G, Briza P, Kungl AJ, Simon B, Auer M, Ebner C, Rheinberger HJ, Breitenbach M, Ferreira F: Immunological and biological properties of Bet v 4, a novel birch pollen allergen with two EF-hand calcium-binding domains. *J Biol Chem* 1997;272:28630–28637.
- 17 Verdino P, Barderas R, Villalba M, Westritschnig K, Valenta R, Rodriguez R, Keller W: Three-dimensional structure of the cross-reactive pollen allergen Che a 3: visualizing cross-reactivity on the molecular surfaces of weed, grass, and tree pollen allergens. *J Immunol* 2008;180:2313–2321.
- 18 Niederberger V, Hayek B, Vrtala S, Laffer S, Twardosz A, Vangelista L, Sperr WR, Valent P, Rumpold H, Kraft D, Ehrenberger K, Valenta R, Spitzauer S: Calcium-dependent immunoglobulin E recognition of the apo- and calcium-bound form of a cross-reactive two EF-hand timothy grass pollen allergen, Phl p 7. *FASEB J* 1999;13:843–856.
- 19 Twardosz A, Hayek B, Seiberler S, Vangelista L, Elfman L, Gronlund H, Kraft D, Valenta R: Molecular characterization, expression in *Escherichia coli*, and epitope analysis of a two EF-hand calcium-binding birch pollen allergen, Bet v 4. *Biochem Biophys Res Commun* 1997;239:197–204.
- 20 Neudecker P, Nerkamp J, Eisenmann A, Nourse A, Lauber T, Schweimer K, Lehmann K, Schwarzwinger S, Ferreira F, Rosch P: Solution structure, dynamics, and hydrodynamics of the calcium-bound cross-reactive birch pollen allergen Bet v 4 reveal a canonical monomeric two EF-hand assembly with a regulatory function. *J Mol Biol* 2004;336:1141–1157.
- 21 Gruenh S, Suphioglu C, O'Hehir RE, Volkman D: Molecular cloning and characterization of hazel pollen protein (70 kD) as a luminal binding protein (BiP): a novel cross-reactive plant allergen. *Int Arch Allergy Immunol* 2003;131:91–100.
- 22 Martin-Orozco E, Cardaba B, del Pozo V, de Andres B, Villalba M, Gallardo S, Rodriguez-Garcia MI, Fernandez MC, Alche JD, Rodriguez R: Ole e I: epitope mapping, cross-reactivity with other Oleaceae pollens and ultrastructural localization. *Int Arch Allergy Immunol* 1994;104:160–170.
- 23 Palomares O, Swoboda I, Villalba M, Balic N, Spitzauer S, Rodriguez R, Valenta R: The major allergen of olive pollen Ole e 1 is a diagnostic marker for sensitization to Oleaceae. *Int Arch Allergy Immunol* 2006;141:110–118.
- 24 Ledesma A, Villalba M, Batanero E, Rodriguez R: Molecular cloning and expression of active Ole e 3, a major allergen from olive-tree pollen and member of a novel family of Ca²⁺-binding proteins (polcalcins) involved in allergy. *Eur J Biochem* 1998;258:454–459.
- 25 Niederberger V, Purohit A, Oster JP, Spitzauer S, Valenta R, Pauli G: The allergen profile of ash (*Fraxinus excelsior*) pollen: cross-reactivity with allergens from various plant species. *Clin Exp Allergy* 2002;32:933–941.
- 26 Ledesma A, Barderas R, Westritschnig K, Quiralte J, Pascual CY, Valenta R, Villalba M, Rodriguez R: A comparative analysis of the cross-reactivity in the polcalcin family including Syr v 3, a new member from lilac pollen. *Allergy* 2006;61:477–484.
- 27 Duffort O, Palomares O, Lombardero M, Villalba M, Barber D, Rodriguez R, Polo F: Variability of Ole e 9 allergen in olive pollen extracts: relevance of minor allergens in immunotherapy treatments. *Int Arch Allergy Immunol* 2006;140:131–138.
- 28 Huecas S, Villalba M, Rodriguez R: Ole e 9, a major olive pollen allergen is a 1,3-beta-glucanase. Isolation, characterization, amino acid sequence, and tissue specificity. *J Biol Chem* 2001;276:27959–27966.
- 29 Palomares O, Villalba M, Quiralte J, Polo F, Rodriguez R: 1,3-β-glucanases as candidates in latex-pollen-vegetable food cross-reactivity. *Clin Exp Allergy* 2005;35:345–351.
- 30 Barral P, Batanero E, Palomares O, Quiralte J, Villalba M, Rodriguez R: A major allergen from pollen defines a novel family of plant proteins and shows intra- and interspecies cross-reactivity. *J Immunol* 2004;172:3644–3651.
- 31 Kernerman SM, McCullough J, Green J, Ownby DR: Evidence of cross-reactivity between olive, ash, privet, and Russian olive tree pollen allergens. *Ann Allergy* 1992;69: 493–496.
- 32 Bousquet J, Guerin B, Hewitt BE, Lim S, Michel FB: Allergy in the Mediterranean area. III: cross reactivity among Oleaceae pollens. *Clin Allergy* 1985;15:439–448.
- 33 Midoro-Horiuti T, Mathura V, Schein CH, Braun W, Yu S, Watanabe M, Lee JC, Brooks EG, Goldblum RM: Major linear IgE epitopes of mountain cedar pollen allergen Jun a 1 map to the pectate lyase catalytic site. *Mol Immunol* 2003;40:555–562.
- 34 Cortegano I, Civantos E, Aceituno E, del Moral A, Lopez E, Lombardero M, del Pozo V, Lahoz C: Cloning and expression of a major allergen from *Cupressus arizonica* pollen, Cup a 3, a PR-5 protein expressed under polluted environment. *Allergy* 2004;59:485–490.
- 35 GenBank – National Institutes of Health (NIH) genetic sequence database. <http://www.ncbi.nlm.nih.gov/Genbank/>.
- 36 Swiss-Prot – Protein knowledgebase. <http://www.expasy.org/sprot/>.
- 37 Tinghino R, Barletta B, Palumbo S, Afferni C, Iacovacci P, Mari A, di Felice G, Pini C: Molecular characterization of a cross-reactive *Juniperus oxycedrus* pollen allergen, Jun o 2: a novel calcium-binding allergen. *J Allergy Clin Immunol* 1998;101:772–777.
- 38 Mari A, Wallner M, Ferreira F: Fagales pollen sensitization in a birch-free area: a respiratory cohort survey using Fagales pollen extracts and birch recombinant allergens (rBet v 1, rBet v 2, rBet v 4). *Clin Exp Allergy* 2003; 33:1419–1428.
- 39 Mur P, Feo Brito F, Lombardero M, Barber D, Galindo PA, Gomez E, Borja J: Allergy to linden pollen (*Tilia cordata*). *Allergy* 2001;56: 457–458.
- 40 Asturias JA, Ibarrola I, Eraso E, Arilla MC, Martinez A: The major *Platanus acerifolia* pollen allergen Pla a 1 has sequence homology to invertase inhibitors. *Clin Exp Allergy* 2003;33:978–985.
- 41 Ibarrola I, Arilla MC, Martinez A, Asturias JA: Identification of a polygalacturonase as a major allergen (Pla a 2) from *Platanus acerifolia* pollen. *J Allergy Clin Immunol* 2004; 113:1185–1191.
- 42 Andersson K, Lidholm J: Characteristics and immunobiology of grass pollen allergens. *Int Arch Allergy Immunol* 2003;130:87–107.
- 43 van Ree R, van Leeuwen WA, Aalberse RC: How far can we simplify in vitro diagnostics for grass pollen allergy? A study with 17 whole pollen extracts and purified natural and recombinant major allergens. *J Allergy Clin Immunol* 1998;102:184–190.
- 44 van Ree R, Clemens JG, Aalbers M, Stapel SO, Aalberse RC: Characterization with monoclonal and polyclonal antibodies of a new major allergen from grass pollen in the group I molecular weight range. *J Allergy Clin Immunol* 1989;83:144–151.
- 45 Freidhoff LR, Ehrlich-Kautzky E, Grant JH, Meyers DA, Marsh DG: A study of the human immune response to *Lolium perenne* (rye) pollen and its components, Lol p I and Lol p II (rye I and rye II). I. Prevalence of reactivity to the allergens and correlations among skin test, IgE antibody, and IgG antibody data. *J Allergy Clin Immunol* 1986;78: 1190–1201.
- 46 Stewart GA, Thompson PJ: The biochemistry of common aeroallergens. *Clin Exp Allergy* 1996;26:1020–1044.
- 47 Matthiesen F, Lowenstein H: Group V allergens in grass pollens. II. Investigation of group V allergens in pollens from 10 grasses. *Clin Exp Allergy* 1991;21:309–320.
- 48 Niederberger V, Laffer S, Froschl R, Kraft D, Rumpold H, Kapiotis S, Valenta R, Spitzauer S: IgE antibodies to recombinant pollen allergens (Phl p 1, Phl p 2, Phl p 5, and Bet v 2) account for a high percentage of grass pollen-specific IgE. *J Allergy Clin Immunol* 1998;101:258–264.

- 49 Rossi RE, Monasterolo G, Monasterolo S: Measurement of IgE antibodies against purified grass-pollen allergens (Phl p 1, 2, 3, 4, 5, 6, 7, 11, and 12) in sera of patients allergic to grass pollen. *Allergy* 2001;56:1180–1185.
- 50 Esch RE, Klapper DG: Cross-reactive and unique grass group I antigenic determinants defined by monoclonal antibodies. *J Allergy Clin Immunol* 1987;79:489–495.
- 51 Laffer S, Valenta R, Vrtala S, Susani M, van Ree R, Kraft D, Scheiner O, Duchene M: Complementary DNA cloning of the major allergen Phl p I from timothy grass (*Phleum pratense*); recombinant Phl p I inhibits IgE binding to group I allergens from eight different grass species. *J Allergy Clin Immunol* 1994;94:689–698.
- 52 Leduc-Brodard V, Inacio F, Jaquinod M, Forest E, David B, Peltre G: Characterization of Dac g 4, a major basic allergen from *Dactylis glomerata* pollen. *J Allergy Clin Immunol* 1996;98:1065–1072.
- 53 Esch RE, Klapper DG: Identification and localization of allergenic determinants on grass group I antigens using monoclonal antibodies. *J Immunol* 1989;142:179–184.
- 54 Strandberg R, Spackman V, Porter SJ: Distribution of a major allergen of rye grass (*Lolium perenne*) pollen between other grass species. *Int Arch Allergy Appl Immunol* 1987;83:96–103.
- 55 van Ree R, Driessen MN, van Leeuwen WA, Stapel SO, Aalberse RC: Variability of cross-reactivity of IgE antibodies to group I and V allergens in eight grass pollen species. *Clin Exp Allergy* 1992;22:611–617.
- 56 Leiferman KM, Gleich GJ: The cross-reactivity of IgE antibodies with pollen allergens. I. Analyses of various species of grass pollens. *J Allergy Clin Immunol* 1976;58:129–139.
- 57 Leiferman KM, Gleich GJ, Jones RT: The cross-reactivity of IgE antibodies with pollen allergens. II. Analyses of various species of ragweed and other fall weed pollens. *J Allergy Clin Immunol* 1976;58:140–148.
- 58 Martin BG, Mansfield LE, Nelson HS: Cross-allergenicity among the grasses. *Ann Allergy* 1985;54:99–104.
- 59 Esch RE, Klapper DG: Isolation and characterization of a major cross-reactive grass group I allergenic determinant. *Mol Immunol* 1989;26:557–561.
- 60 Smith PM, Ong EK, Knox RB, Singh MB: Immunological relationships among group I and group V allergens from grass pollen. *Mol Immunol* 1994;31:491–498.
- 61 Weber RW: Patterns of pollen cross-allergenicity. *J Allergy Clin Immunol* 2003;112:229–239.
- 62 Weber RW: Cross-reactivity of pollen allergens. *Curr Allergy Asthma Rep* 2004;4:401–408.
- 63 Mohapatra SS, Lockey RF, Shirley S: Immunobiology of grass pollen allergens. *Curr Allergy Asthma Rep* 2005;5:381–387.
- 64 Esch RE: Grass pollen allergens. *Clin Allergy Immunol* 2004;18:185–205.
- 65 Kahn CR, Marsh DG: Monoclonal antibodies to the major *Lolium perenne* (rye grass) pollen allergen Lol p I (Rye I). *Mol Immunol* 1986;23:1281–1288.
- 66 Arilla MC, Ibarrola I, Eraso E, Aguirre M, Martinez A, Asturias JA: Quantification in mass units of group 1 grass allergens by a monoclonal antibody-based sandwich ELISA. *Clin Exp Allergy* 2001;31:1271–1278.
- 67 Smith PM, Suphioglu C, Griffith IJ, Theriault K, Knox RB, Singh MB: Cloning and expression in yeast *Pichia pastoris* of a biologically active form of Cyn d 1, the major allergen of Bermuda grass pollen. *J Allergy Clin Immunol* 1996;98:331–343.
- 68 Matthiesen F, Schumacher MJ, Lowenstein H: Characterization of the major allergen of *Cynodon dactylon* (Bermuda grass) pollen, Cyn d I. *J Allergy Clin Immunol* 1991;88:763–774.
- 69 Han SH, Chang ZN, Chi CW, Perng HJ, Liu CC, Tsai JJ, Tam MF: Use of monoclonal antibodies to isolate and characterize Cyn d I, the major allergen of Bermuda grass pollen. *J Allergy Clin Immunol* 1993;92:549–558.
- 70 Duffort O, Quintana J, Ipsen H, Barber D, Polo F: Antigenic similarity among group I allergens from grasses and quantitation ELISA using monoclonal antibodies to Phl p 1. *Int Arch Allergy Immunol* 2008;145:283–290.
- 71 Suphioglu C, Ferreira F, Knox RB: Molecular cloning and immunological characterization of Cyn d 7, a novel calcium-binding allergen from Bermuda grass pollen. *FEBS Lett* 1997;402:167–172.
- 72 Smith PM, Xu H, Swoboda I, Singh MB: Identification of a Ca²⁺ binding protein as a new Bermuda grass pollen allergen Cyn d 7: IgE cross-reactivity with oilseed rape pollen allergen Bra r 1. *Int Arch Allergy Immunol* 1997;114:265–271.
- 73 Hirschwehr R, Heppner C, Spitzauer S, Sperr WR, Valent P, Berger U, Horak F, Jäger S, Kraft D, Valenta R: Identification of common allergenic structures in mugwort and ragweed pollen. *J Allergy Clin Immunol* 1998;101:196–206.
- 74 Ayuso R, Carreira J, Lombardero M, Duffort O, Peris A, Basomba A, Polo F: Isolation by mAb based affinity chromatography of two Par j I isoallergens. Comparison of their physicochemical, immunochemical and allergenic properties. *Mol Immunol* 1993;30:1347–1354.
- 75 Stumvoll S, Westritschnig K, Lidholm J, Spitzauer S, Colombo P, Duro G, Kraft D, Geraci D, Valenta R: Identification of cross-reactive and genuine *Parietaria judaica* pollen allergens. *J Allergy Clin Immunol* 2003;111:974–979.
- 76 Asero R, Mistrello G, Roncarolo D, Casarini M: Detection of allergens in plantain (*Plantago lanceolata*) pollen. *Allergy* 2000;55:1059–1062.
- 77 Garcia Ortiz JC, Ventas P, Cosmes P, Lopez-Asunsolo A: An immunoblotting analysis of cross-reactivity between melon, and plantago and grass pollens. *J Invest Allergol Clin Immunol* 1996;6:378–382.
- 78 Calabozo B, Diaz-Perales A, Salcedo G, Barber D, Polo F: Cloning and expression of biologically active *Plantago lanceolata* pollen allergen Pla l 1 in the yeast *Pichia pastoris*. *Biochem J* 2003;372:889–896.
- 79 Hoffman DR, Miller JS, Sutton JL: *Hymenoptera* venom allergy: a geographic study. *Ann Allergy* 1980;45:276–279.
- 80 Mueller U, Roth A, Yman L, Patrizzi R: Use of RAST technique in wasp sting hypersensitivity. Cross-reactions between various insect antigens are specially considered. *Allergy* 1978;33:197–202.
- 81 Hemmer W, Focke M, Kolarich D, Wilson IB, Altmann F, Wohrl S, Gotz M, Jarisch R: Antibody binding to venom carbohydrates is a frequent cause for double positivity to honeybee and yellow jacket venom in patients with stinging-insect allergy. *J Allergy Clin Immunol* 2001;108:1045–1052.
- 82 Reisman RE, Wypych JI, Lazell MI: Further studies of patients with both honeybee- and yellow-jacket-venom-specific IgE. *Int Arch Allergy Appl Immunol* 1987;82:190–194.
- 83 Straumann F, Bucher C, Wüthrich B: Double sensitization to honeybee and wasp venom: immunotherapy with one or with both venoms? Value of FEIA inhibition for the identification of the cross-reacting IgE antibodies in double-sensitized patients to honeybee and wasp venom. *Int Arch Allergy Immunol* 2000;123:268–274.
- 84 Hemmer W, Focke M, Kolarich D, Dalik I, Gotz M, Jarisch R: Identification by immunoblot of venom glycoproteins displaying immunoglobulin E-binding N-glycans as cross-reactive allergens in honeybee and yellow jacket venom. *Clin Exp Allergy* 2004;34:460–469.
- 85 Jappe U, Raulf-Heimsoth M, Hoffmann M, Burow G, Hubsch-Muller C, Enk A: In vitro *Hymenoptera* venom allergy diagnosis: improved by screening for cross-reactive carbohydrate determinants and reciprocal inhibition. *Allergy* 2006;61:1220–1229.
- 86 Marz L, Kuhne C, Michl H: The glycoprotein nature of phospholipase A2, hyaluronidase and acid phosphatase from honey-bee venom. *Toxicon* 1983;21:893–896.
- 87 Kubelka V, Altmann F, Marz L: The asparagine-linked carbohydrate of honeybee venom hyaluronidase. *Glycoconj J* 1995;12:77–83.
- 88 Weber A, Schroder H, Thalberg K, Marz L: Specific interaction of IgE antibodies with a carbohydrate epitope of honey bee venom phospholipase A2. *Allergy* 1987;42:464–470.

- 89 Tretter V, Altmann F, Kubelka V, Marz L, Becker WM: Fucose alpha 1,3-linked to the core region of glycoprotein N-glycans creates an important epitope for IgE from honeybee venom allergic individuals. *Int Arch Allergy Immunol* 1993;102:259–266.
- 90 King TP, Alagon AC, Kuan J, Sobotka AK, Lichtenstein LM: Immunochemical studies of yellowjacket venom proteins. *Mol Immunol* 1983;20:297–308.
- 91 Hoffman DR, Wood CL: Allergens in *Hymenoptera* venom. XI. Isolation of protein allergens from *Vespa maculifrons* (yellow jacket) venom. *J Allergy Clin Immunol* 1984;74:93–103.
- 92 Gmachl M, Kreil G: Bee venom hyaluronidase is homologous to a membrane protein of mammalian sperm. *Proc Natl Acad Sci USA* 1993;90:3569–3573.
- 93 King TP, Lu G, Gonzalez M, Qian N, Soldatova L: Yellow jacket venom allergens, hyaluronidase and phospholipase: sequence similarity and antigenic cross-reactivity with their hornet and wasp homologs and possible implications for clinical allergy. *J Allergy Clin Immunol* 1996;98:588–600.
- 94 Skov LK, Seppala U, Coen JJ, Crickmore N, King TP, Monsalve RI, Kastrup JS, Spangfort MD, Gajhede M: Structure of recombinant Ves v 2 at 2.0 Ångstrom resolution: structural analysis of an allergenic hyaluronidase from wasp venom. *Acta Crystallogr D Biol Crystallogr* 2006;62:595–604.
- 95 Markovic-Housley Z, Miglierini G, Soldatova L, Rizkallah PJ, Mueller U, Schirmer T: Crystal structure of hyaluronidase, a major allergen of bee venom. *Structure* 2000;8:1025–1035.
- 96 Hoffman DR, El Choufani SE, Smith MM, de Groot H: Occupational allergy to bumblebees: allergens of *Bombus terrestris*. *J Allergy Clin Immunol* 2001;108:855–860.
- 97 Hoffman DR, Jacobson RS: Allergens in *Hymenoptera* venom. XXVII: bumblebee venom allergy and allergens. *J Allergy Clin Immunol* 1996;97:812–821.
- 98 Kochuyt AM, van Hoeyveld EM, Stevens EA: Occupational allergy to bumble bee venom. *Clin Exp Allergy* 1993;23:190–195.
- 99 Hoffman DR, McDonald CA: Allergens in *Hymenoptera* venom. VII. Species specific reactivity to yellow jacket venoms. *Ann Allergy* 1981;47:23–27.
- 100 Reisman RE, Mueller U, Wypych JI, Elliott W, Arbesman CE: Comparison of the allergenicity and antigenicity of yellow jacket and hornet venoms. *J Allergy Clin Immunol* 1982;69:268–274.
- 101 Reisman RE, Wypych JI, Mueller UR, Grant JA: Comparison of the allergenicity and antigenicity of *Polistes* venom and other vespid venoms. *J Allergy Clin Immunol* 1982;70:281–287.
- 102 Hoffman DR: Allergens in *Hymenoptera* venom. XVI. Studies of the structures and cross-reactivities of vespid venom phospholipases. *J Allergy Clin Immunol* 1986;78:337–343.
- 103 Hoffman DR: Allergens in *Hymenoptera* venom. XXVI. The complete amino acid sequences of two vespid venom phospholipases. *Int Arch Allergy Immunol* 1994;104:184–190.
- 104 Hoffman DR: Allergens in *Hymenoptera* venom. XXV. The amino acid sequences of antigen 5 molecules and the structural basis of antigenic cross-reactivity. *J Allergy Clin Immunol* 1993;92:707–716.
- 105 Lu G, Villalba M, Coscia MR, Hoffman DR, King TP: Sequence analysis and antigenic cross-reactivity of a venom allergen, antigen 5, from hornets, wasps, and yellow jackets. *J Immunol* 1993;150:2823–2830.
- 106 Henriksen A, King TP, Mirza O, Monsalve RI, Meno K, Ipsen H, Larsen JN, Gajhede M, Spangfort MD: Major venom allergen of yellow jackets, Ves v 5: structural characterization of a pathogenesis-related protein superfamily. *Proteins* 2001;45:438–448.
- 107 Didierlaurent A, Foglietti MJ, Guerin B, Hewitt BE, Percheron F: Comparative study on cat allergens from fur and saliva. *Int Arch Allergy Appl Immunol* 1984;73:27–31.
- 108 Viander M, Valovirta E, Vanto T, Koivikko A: Cross-reactivity of cat and dog allergen extracts. RAST inhibition studies with special reference to the allergenic activity in saliva and urine. *Int Arch Allergy Appl Immunol* 1983;71:252–260.
- 109 Guerin B, Hewitt BE: A comparative study of allergen extracts from cat fur, cat pelt and cat saliva. *Ann Allergy* 1981;46:127–131.
- 110 Brown PR, Leitermann K, Ohman L Jr: Distribution of cat allergen 1 in cat tissues and fluids. *Int Arch Allergy Appl Immunol* 1984;74:67–70.
- 111 Hoffman DR: Dog and cat allergens: urinary proteins or dander proteins? *Ann Allergy* 1980;45:205–206.
- 112 Wallenbeck I, Einarsson R: Identification of dander-specific and serum-specific allergens in cat dandruff extract. *Ann Allergy* 1987;59:131–134.
- 113 Martinez A, Martinez J, Sanz ML, Bartolome B, Palacios R: Dander is the best epithelial source for dog allergenic extract preparations. *Allergy* 1994;49:664–667.
- 114 Spitzauer S, Schweiger C, Sperr WR, Pandjaitan B, Valent P, Muhl S, Ebner C, Scheiner O, Kraft D, Rumpold H: Molecular characterization of dog albumin as a cross-reactive allergen. *J Allergy Clin Immunol* 1994;93:614–627.
- 115 Spitzauer S, Pandjaitan B, Muhl S, Ebner C, Kraft D, Valenta R, Rumpold H: Major cat and dog allergens share IgE epitopes. *J Allergy Clin Immunol* 1997;99:100–106.
- 116 Goubran Botros H, Gregoire C, Rabillon J, David B, Dandeu JP: Cross-antigenicity of horse serum albumin with dog and cat albumins: study of three short peptides with significant inhibitory activity towards specific human IgE and IgG antibodies. *Immunology* 1996;88:340–347.
- 117 Smith WA, Butler AJ, Hazell LA, Chapman MD, Pomes A, Nickels DG, Thomas WR: Fel d 4, a cat lipocalin allergen. *Clin Exp Allergy* 2004;34:1732–1738.
- 118 Boutin Y, Hebert H, Vrancken ER, Mourad W: Allergenicity and cross-reactivity of cat and dog allergenic extracts. *Clin Allergy* 1988;18:287–293.
- 119 Wüthrich B, Guerin B, Hewitt BE: Cross-allergenicity between extracts of hair from different dog breeds and cat fur. *Clin Allergy* 1985;15:87–93.
- 120 Ohman L Jr, Bloch KJ, Kendall S, Lowell FC: Allergens of mammalian origin. IV. Evidence for common allergens in cat and dog serum. *J Allergy Clin Immunol* 1976;57:560–568.
- 121 Mari A: Importance of databases in experimental and clinical allergology. *Int Arch Allergy Immunol* 2005;138:88–96.
- 122 Boluda L, Alonso C, Fernandez-Caldas E: Purification, characterization, and partial sequencing of two new allergens of *Olea europaea*. *J Allergy Clin Immunol* 1998;101:210–216.
- 123 Batanero E, Ledesma A, Villalba M, Rodriguez R: Purification, amino acid sequence and immunological characterization of Ole e 6, a cysteine-enriched allergen from olive tree pollen. *FEBS Lett* 1997;410:293–296.
- 124 Midoro-Horiuti T, Goldblum RM, Kurosky A, Wood TG, Brooks EG: Variable expression of pathogenesis-related protein allergen in mountain cedar (*Juniperus ashei*) pollen. *J Immunol* 2000;164:2188–2192.
- 125 Bose R, Ekramoddoullah AK, Kisil FT, Sehon AH: Human and murine antibodies to rye grass pollen allergen Lol p IV share a common idiotope. *Immunology* 1988;63:579–584.
- 126 Vrtala S, Fischer S, Grote M, Vangelista L, Pastore A, Sperr WR, Valent P, Reichelt R, Kraft D, Valenta R: Molecular, immunological, and structural characterization of Phl p 6, a major allergen and P-particle-associated protein from Timothy grass (*Phleum pratense*) pollen. *J Immunol* 1999;163:5489–5496.
- 127 Ansari AA, Killoran EA, Marsh DG: An investigation of human immune response to perennial ryegrass (*Lolium perenne*) pollen cytochrome c (Lol p X). *J Allergy Clin Immunol* 1987;80:229–235.
- 128 Marknell DeWitt A, Niederberger V, Lehtonen P, Spitzauer S, Sperr WR, Valent P, Valenta R, Lidholm J: Molecular and immunological characterization of a novel timothy grass (*Phleum pratense*) pollen allergen, Phl p 11. *Clin Exp Allergy* 2002;32:1329–1340.

- 129 van Ree R, Hoffman DR, van Dijk W, Brodard V, Mahieu K, Koeleman CA, Grande M, van Leeuwen WA, Aalberse RC: Lol p XI, a new major grass pollen allergen, is a member of a family of soybean trypsin inhibitor-related proteins. *J Allergy Clin Immunol* 1995;95:970–978.
- 130 Petersen A, Suck R, Hagen S, Cromwell O, Fiebig H, Becker WM: Group 13 grass allergens: structural variability between different grass species and analysis of proteolytic stability. *J Allergy Clin Immunol* 2001;107:856–862.
- 131 Nilsen BM, Grimsoen A, Paulsen BS: Identification and characterization of important allergens from mugwort pollen by IEF, SDS-PAGE and immunoblotting. *Mol Immunol* 1991;28:733–742.
- 132 Nilsen BM, Paulsen BS: Isolation and characterization of a glycoprotein allergen, Art v II, from pollen of mugwort (*Artemisia vulgaris* L.). *Mol Immunol* 1990;27:1047–1056.
- 133 Atassi H, Atassi MZ: Antibody recognition of ragweed allergen Ra3: localization of the full profile of the continuous antigenic sites by synthetic overlapping peptides representing the entire protein chain. *Eur J Immunol* 1986;16:229–235.
- 134 Goodfriend L, Choudhury AM, Klapper DG, Coulter KM, Dorval G, del Carpio J, Osterland CK: Ra5G, a homologue of Ra5 in giant ragweed pollen: isolation, HLA-DR-associated activity and amino acid sequence. *Mol Immunol* 1985;22:899–906.
- 135 Giuliani A, Pini C, Bonini S, Mucci N, Ferroni L, Vicari G: Isolation and purification of a major allergen from *Parietaria officinalis* pollen. *Allergy* 1987;42:434–440.
- 136 Duro G, Colombo P, Costa MA, Izzo V, Porcasi R, di Fiore R, Locorotondo G, Mirisola MG, Cocchiara R, Geraci D: cDNA cloning, sequence analysis and allergological characterization of Par j 2.0101, a new major allergen of the *Parietaria judaica* pollen. *FEBS Lett* 1996;399:295–298.
- 137 Ayuso R, Carreira J, Polo F: Quantitation of the major allergen of several *Parietaria* pollens by an anti-Par 1 monoclonal antibody-based ELISA. Analysis of crossreactivity among purified Par j 1, Par o 1 and Par m 1 allergens. *Clin Exp Allergy* 1995;25:993–999.
- 138 Bonura A, Gulino L, Trapani A, di Felice G, Tinghino R, Amoroso S, Geraci D, Valenta R, Westritschnig K, Scala E, Mari A, Colombo P: Isolation, expression and immunological characterization of a calcium-binding protein from *Parietaria* pollen. *Mol Immunol* 2008;45:2465–2473.
- 139 Costa MA, Colombo P, Izzo V, Kennedy H, Venturella S, Cocchiara R, Mistrello G, Falagiani P, Geraci D: cDNA cloning, expression and primary structure of Par j I, a major allergen of *Parietaria judaica* pollen. *FEBS Lett* 1994;341:182–186.
- 140 Lake FR, Ward LD, Simpson RJ, Thompson PJ, Stewart GA: House dust mite-derived amylase: allergenicity and physicochemical characterization. *J Allergy Clin Immunol* 1991;87:1035–1042.
- 141 Le Mao J, Mayer CE, Peltre G, Desvaux FX, David B, Weyer A, Senechal H: Mapping of *Dermatophagoides farinae* mite allergens by two-dimensional immunoblotting. *J Allergy Clin Immunol* 1998;102:631–636.
- 142 Yasueda H, Mita H, Akiyama K, Shida T, Ando T, Sugiyama S, Yamakawa H: Allergens from *Dermatophagoides* mites with chymotryptic activity. *Clin Exp Allergy* 1993;23:384–390.
- 143 O'Neil SE, Heinrich TK, Hales BJ, Hazell LA, Holt DC, Fischer K, Thomas WR: The chitinase allergens Der p 15 and Der p 18 from *Dermatophagoides pteronyssinus*. *Clin Exp Allergy* 2006;36:831–839.
- 144 Entrez Protein – National Institutes of Health (NIH) protein sequence database. <http://www.ncbi.nlm.nih.gov/sites/entrez?db=protein>.
- 145 Thomas WR, Smith WA, Hales BJ, Mills KL, O'Brien RM: Characterization and immunobiology of house dust mite allergens. *Int Arch Allergy Immunol* 2002;129:1–18.
- 146 Binder M, Fierlbeck G, King TP, Valent P, Bühring HJ: Individual *Hymenoptera* venom compounds induce upregulation of the basophil activation marker ectonucleotide pyrophosphatase/phosphodiesterase 3 (CD203c) in sensitized patients. *Int Arch Allergy Immunol* 2002;129:160–168.
- 147 Hoffman DR: Allergens in *Hymenoptera* venom XIV: IgE binding activities of venom proteins from three species of vespids. *J Allergy Clin Immunol* 1985;75:606–610.
- 148 Winningham KM, Fitch CD, Schmidt M, Hoffman DR: *Hymenoptera* venom protease allergens. *J Allergy Clin Immunol* 2004;114:928–933.
- 149 Virtanen T, Jarvi RM: Mammalian allergens. *Clin Allergy Immunol* 2004;18:297–317.
- 150 Saarelainen S, Taivainen A, Rytönen-Nissinen M, Auriola S, Immonen A, Mantyjarvi R, Rautiainen J, Kinnunen T, Virtanen T: Assessment of recombinant dog allergens Can f 1 and Can f 2 for the diagnosis of dog allergy. *Clin Exp Allergy* 2004;34:1576–1582.
- 151 Ichikawa K, Vailes LD, Pomes A, Chapman MD: Molecular cloning, expression and modelling of cat allergen, cystatin (Fel d 3), a cysteine protease inhibitor. *Clin Exp Allergy* 2001;31:1279–1286.
- 152 Adedoyin J, Johansson SG, Gronlund H, van Hage M: Interference in immunoassays by human IgM with specificity for the carbohydrate moiety of animal proteins. *J Immunol Methods* 2006;310:117–125.