

Evolution in the Understanding of Cross-Reactivities of Respiratory Allergens: The Role of Recombinant Allergens

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Abstract

The aim of this review is to show the impact of the use of purified and recombinant allergens to discriminate between co- and cross-sensitization to respiratory allergens. The author describes the evolution of diagnostic tests over the last decades; the tests initially allowed the detection of simultaneously positive cutaneous tests and/or simultaneous positivity of specific IgE to different allergen extracts, but they did not differentiate cross-sensitization from co-sensitization. RAST inhibition studies with crude extracts then established cross-reactivity, but did not identify the cross-reactive allergens involved. Later, immunoblot and CRIE inhibition were able to detect multiple cross-reactive allergens and to assess their physicochemical properties. But it is only since purified and recombinant allergens have been used in the different investigations that identification of cross-reactive allergens has been made possible at a molecular level. This historical approach is illustrated by examples selected from some of the main respiratory allergen sources: tree pollen, grass pollen, weed pollen, acarids, cockroaches and mammalians. For each of these allergen sources, the author gives an updated presentation of

major and minor cross-reactive allergen molecules and refers to the last decade's major publications concerning immunochemical investigations carried out in the field of cross-reactive respiratory allergens. Emphasis is placed on the clinical applications for allergic patients: improvement in the accuracy of the diagnosis of sensitization, new concepts of immunotherapy based on genetically engineered hypoallergenic variants of cross-reactive allergens used alone or in combination, evaluation of allergen load with environmental tests using monoclonal antibodies against cross-reactive allergens.

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Better knowledge of cross-reactive respiratory allergens will lead to improved diagnosis and immunotherapy in the future. Skin tests and serological tests (e.g. CAP or radioallergosorbent test; RAST) have been common practice for many years and their results are compared to the clinical history to assess the diagnosis of inhalant allergies. Concomitant positivity to different respiratory allergen extracts is frequently observed, and detection of sensitization against purified (natural or recombinant) allergens should help to reach a more accurate diagnosis. We show with concrete examples what developments made it possible to distinguish co-sensitization from cross-reactivity. This paper is a mini-review from a clinician's point of view, based on 30 years' clinical experience.

Review of Advances in Molecular Characteristics of Respiratory Allergens and in Mechanisms of Cross-Reactivities

Among respiratory allergens, many major allergens have been fully characterized; the selection of murine monoclonal antibodies raised against purified allergens helped define allergenically relevant cross-reactive epitopes. Numerous respiratory allergens have been cloned and sequenced - thus accelerating the availability of primary structure data - and expressed as recombinant proteins [1, 2], which provided an excellent basis for the understanding of patients' IgE cross-reactivity between allergenic molecules.

Among possible techniques, the use of high-resolution electrophoresis combined with immunoblotting led to the detection of microheterogeneity within allergen groups, allowing the detection of isoallergens which have significant amino acid sequence identity, the same molecular size and the same biological function [3]. On the other hand, the use of recombinant allergen fragments and synthetic peptides has helped delineate determinants involved in T and B cell recognition; a useful step, knowing that cross-reactivity among allergens can be studied at the T and B cell level [4-7]. The study of cross-reactivity has led to a number of basic observations: cross-reactions may be limited to the presence of cross-reacting antibodies with different affinities without any clinical expression, cross-reactivity between allergens being only demonstrated by in vitro techniques under antigen excess conditions [8]. Cross-reactions correspond to allergic symptoms provoked in a patient by various offending allergen sources containing common allergen structures at the molecular (allergen molecules) or submolecular (epitope) level. However, it is not always clear whether a given cross-reactive allergen is involved in a particular inhalant-allergen-induced manifestation, and it also remains to be investigated whether the use of respiratory allergen challenges could help; the importance of a cross-reactive allergen for a given patient might in part be related to the percentage of the total IgE it has induced. The cross-reactive profiles of allergic patients are obviously variable, depending first on the individual immune response but also on environmental conditions and geographic and climatic differences which influence the number and diversity of allergen sources [9].

Understanding Respiratory Cross-Reactivities: A Historical Approach

Any co-sensitization to two or several crude respiratory allergen extracts raises a crucial question: are we dealing with a cross-reactivity or a cosensitization to specific allergens? It now seems evident that house dust is a mosaic of allergens; the situation was different in the early seventies, when mites were identified as the causative agents of the allergenicity of house dust. The simultaneously positive cutaneous tests and specific IgE to house dust and *Dermatophagoides* extracts and in other cases the simultaneously positive cutaneous tests and specific IgE to house dust and cat extracts were decisive in understanding that house dust extracts are a mixture of allergens [10]. RAST inhibition with either *Dermatophagoides* or cat extracts confirmed the hypothesis that IgE to house dust extract was in fact IgE to *Dermatophagoides* or cat extracts [11]. But skin tests and IgE determination also showed concomitant sensitization to allergenic extracts better defined than dust; from the beginning, in patients allergic to grass pollen, skin tests elicited responses to extracts prepared from all species of grasses, including species to which they had never been exposed. The cross-reactive allergens were demonstrated by in vitro tests using RAST inhibition [12]. Similar examples were later given for extracts prepared from different mite, cockroach and fungus species. This significant step was made in the mid-seventies, using techniques measuring allergen IgE inhibition by the allergen extract containing the cross-reactive allergen. However, the allergens involved could not be identified by this technique. Further advances came with techniques able to separate different allergens in a crude extract (immunoblot and cross-radio electrophoresis). Comparative immunoblottings of allergenic fractions of two different allergens gave the molecular weight and/or isoelectric points of potentially cross-reacting fractions. Using a large number of sera from patients allergic to different extracts allowed the identification of the number of patients showing similar IgE binding patterns. The cross-reactive allergens were usually defined by their physicochemical properties (molecular weight, isoelectric point), but their molecular nature did not appear. Only since the early nineties has the use of purified and recombinant allergens and monoclonal antibodies specific for the purified allergen in these investigations, and the use of recombinant allergen in quantified cutaneous tests allowed the identification of cross-reactive allergens at a molecular level. This is summarized in figure 1.

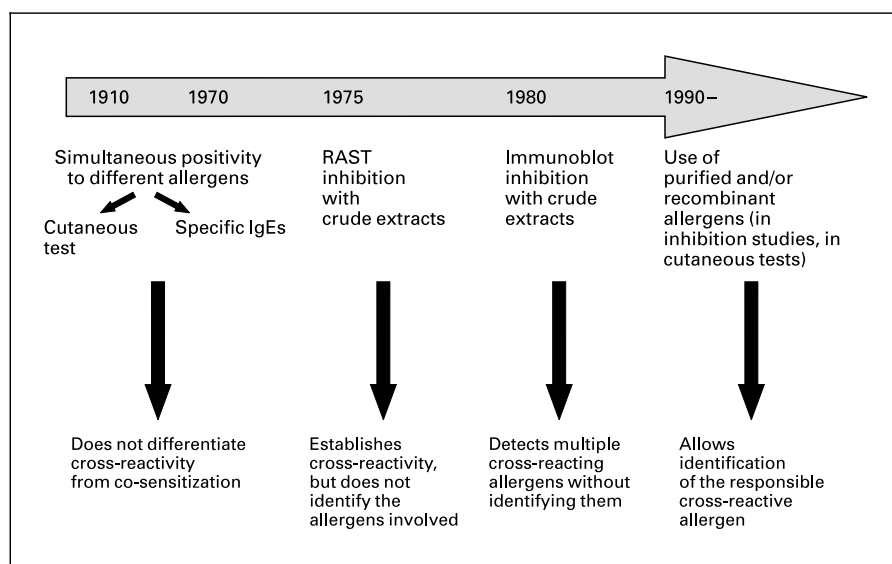


Fig. 1. Evolution of allergy diagnostic tests and immunochemical techniques in parallel with better identification of cross-reactive allergens.

Examples Selected among the Main Respiratory Allergens

Plant Allergens

Cross-reactions involving plant allergens can occur between taxonomically closely related (within a family or across family lines) or distantly related plant allergens [13]. Cross-reactions are also common between pollen allergens and food allergens (especially food allergens from the families Rosaceae, Umbelliferae, Solanaceae and Cucurbitaceae) [14]. Moreover cross-reactions can involve nonpollenic plant aeroallergens, i.e., allergens from *Ficus benjamina* (Moraceae), *Hevea brasiliensis* (Euphorbiaceae), and food vegetable allergens [15–17].

Tree Pollen Allergens Cross-Reactivity (tables 1, 2). Tree pollen allergen cross-reactivity can be observed for major allergens, but also for minor allergens which elicit IgE antibodies in a smaller proportion of patients. The major allergen of birch was described in 1983 [18] and was the first tree pollen allergen cloned [19], followed by major allergens of other trees from the order Fagales (Aln g 1 for alnus, Cor a 1 for hazel, Car b 1 for hornbeam). These allergens exhibit a high degree of sequence identity (>70%) and their cross-reactivity was demonstrated by using recombinant Bet v 1, which inhibits IgE binding to related allergens present in trees belonging to the Fagales [20, 21]. A high degree of cross-reactivity was also demonstrated for the Oleaceae of the tree family Astaridae (table 2) and in the Coniferales between the two major allergens of the Cupressaceae and Taxodiaceae. Examples of

minor cross-reactive allergens are given by profilin and calcium-binding allergens present in different tree families and also in more distant botanical families between tree, grasses and weeds (table 3). These minor cross-reactive allergens also induce cross-reactivity between different groups of trees. Concomitant sensitization to birch and ash (Fagales and Oleaceae) belonging, respectively, to the families Betulaceae and Oleaceae has been observed recently in temperate climates [35, 36]. We have been able to demonstrate by inhibition experiments performed with recombinant allergens that profilin or a calcium-binding allergen could be a cross-reactive allergen [36].

Grass Pollen Cross-Reactivities. Since grass pollen immunotherapy was initiated by Freeman and Noon [37], clinical evidence of cross-reactivity among grass pollens has suggested that diagnosis and immunotherapy could be achieved with a limited number of grasses, the selection of species being based upon their regional prevalence. Later on, grass pollen cross-reactivity was investigated by inhibition techniques and revealed extensive allergen cross-reactivity among taxonomically related grasses, suggesting that sensitization to one grass pollen species led to multiple grass sensitivities [12, 38] with the exception of Bermuda grass [39]. Several inhibition studies performed with sera from North American and European subjects confirmed that there is little or no allergen cross-reactivity between the Pooids (to which timothy grass belongs) and the Chloridoideae (to which Bermuda grass belongs) [40]. The availability of purified grass pollen allergens and recombinant allergens has allowed the detection of cross-

Table 1. Cross-reactive allergens among major tree pollen allergens

Plant species	Allergens	Refer-ences	Molecular weight, kD	Prevalence of sensitization, %	Sequence identities with other allergens
<i>Fagales</i>					
Betulaceae					
Birch	Bet v 1	18	17	95	Mal d 1 (apple)
Alder	Aln g 1				Api g 1 (celery)
Corylaceae					
Hazel	Cor a 1	20, 21			Dau c 1 (carrot)
Hornbean	Car b 1				
Fagaceae					
Oak	Que a 1				
<i>Astariidae</i>					
Oleaceae					
Olea	Ole e 1	22, 23	19	>70	Maize pollen Lol p 11
Fraxinus exc.	Fra e 1				Soybean I.P.
Ligustrum vul.	Lig v 1	24, 25			Tomato
Syringa vul.	Syr v 1				
<i>Coniferales</i>					
Cupressaceae					
Japanese cedar	Cry j 1	26, 27	41–45	>80	Amb a 1
Taxodiaceae					
Japanese cypress	Cha o 1				Amb a 2
					Phl p 4
	Cry j 2	28	45	>80	
	Cha o 2				

Table 2. Cross-reactive allergens among minor tree pollen allergens

Plant species	Allergens	References	Molecular weight, kD	Prevalence of sensitization, %	Sequence identities with other allergens
<i>Profilin</i>					
Betulaceae					
Birch	Bet v 2	28	14	12 50	Phl p 12 Bermuda grass Tomato profilin
Oleaceae					
Olea	Ole 2	30		22	Wheat profilin RGly m 3
Other trees					
Aulus		31			
Platanus					
Populus					
Ulmus					
<i>Calcium-binding allergens</i>					
Betulaceae					
Birch	Bet v 4	32, 33	9.3	21	Cyn d 7
Alder	Aln g 4				Phl p 7 Bra r 1
Oleaceae					
Olea	Ole 3	34			

Table 3. Cross-reactivities among grass pollen allergens

Grass species	Allergens	Refer-ences	Molecular weight, kD	Prevalence of sensitization, %	Sequence identities with other allergens
Group I Pooideae	<i>L. perenne</i> (rye grass) <i>P. pratense</i> (timothy grass) <i>P. pratensis</i> (blue grass) <i>D. glomerata</i> (orchard grass)	Lol p 1 Phl p 1 Poa p 1 Dac g 1	42, 43, 44 45 46 47	30 95	Lol p 2 Lol p 3
Group II	<i>L. perenne</i> (rye grass) <i>D. glomerata</i> (orchard grass)	Lol p 2 Dac g 2	48 49	11 35–50	Lol p 3
Group III	<i>L. perenne</i> (rye grass) <i>D. glomerata</i> (orchard grass)	Lol p 3 Dac g 3	50 51	11–14 35–70	
Group IV	<i>L. perenne</i> (rye grass) <i>D. glomerata</i> (orchard grass) <i>P. pratense</i> (timothy grass)	Lol p 4 Dac g 4 Phl p 4	52 53 54	50–60 50–75	Amb a 1 Amb a 2 carbohydrate moiety?
Group V	<i>P. pratense</i> (timothy grass) <i>P. pratensis</i> (blue grass) <i>L. perenne</i> (rye grass)	Phl p 5 Poa p 5 Lol p 5	55, 56 41 57	27–38 many isoforms 70–80	
Group VI	<i>P. pratense</i> (timothy grass)	Phl p 6	58	12 75	group V
Group VII Chloridoideae	<i>C. dactylon</i> (Bermuda grass) <i>P. pratense</i> (timothy grass)	Cyn d 7 Phl p 7	59 60	12 35	Bet v 1
Group X	<i>P. pratensis</i> (blue grass) <i>P. pratense</i> (timothy grass) <i>L. perenne</i> (rye grass)	Cytochrom C	61	11	
Group XI	<i>L. perenne</i> (rye grass) <i>P. pratense</i> (timothy grass)	Lol p 11 Phl p 11	62	16–18 65	maize tomato olive
Group XII	<i>P. pratense</i> (timothy grass)	Profilin	63, 64	14 20–50	birch profilin

reactivity among grass pollen allergens at the molecular level. The first cloning reported was group 5 from Kentucky blue grass [41] which has high sequence identity (80–90%) with rye and timothy. For several other grass pollen allergens, a high degree of similarity between the various groups from different species was observed: Lol p 1, Lol p 2, Lol p 3, Lol p 5 from *Lolium perenne* have been studied in detail together with Phl p 1, Phl p 5 and Phl p 6 from *Phleum pratense*, Poa p 1, Poa p 5 from *Poa pratensis*, Dac g 2 Dac g 3, Dac g 4 from *Dactylis glomerata*, Cyn d 1 from *Cynodon dactylon*, profilin and others (table 3). Laffer et al. [45] have demonstrated that Phl p 1 inhibits IgE binding to group I allergens from 8 different grass species. Using a mix of recombinant grass pollen allergens, it was possible to establish a positive diagnosis of grass allergy in 87% of the cases [65].

Weed Pollen Cross-Reactivity. Weeds include several botanical families: Compositae, Amaranthaceae, Urticaceae, Brassicaceae, Plantaginaceae. Ragweed is an important pollen source worldwide. Clinical studies have shown that patients with positive skin tests for ragweed also test positive for pollen proteins derived from several distinct plant families [66]. Cross-reactivity of IgE antibodies to several species of ragweed was also demonstrated by Leifermann et al. [67], who measured their inhibitory capacities by the RAST. Common allergenic structures with comparable molecular weight were identified in ragweed and mugwort, and profilin is one of the cross-reactive allergens [68]. The results of cloning studies of the two major allergens Amb a 1 and Amb a 2 showed both intra-species and interspecies cross-reactivity. Moreover, Amb a 1 and Amb a 2 each belong to a family of isoforms and are the molecular basis for cross-reactivity with grass and tree pollens [69] (see also tables 1, 3).

Cross-Reactions Involving Acarid Allergens

Cross-reactions involving acarid allergens can occur between closely related species within a family (i.e. *Dermatophagoides*) or between subfamilies like the Pyroglyphidae (i.e. *Dermatophagoides* and *Euroglyphus*) or the Glyciphagidae (i.e. *Lepidoglyphus* and *Blomia tropicalis*). But cross-reactivity was also observed between different families (Pyroglyphidae and storage mites [70], even between acarids and insects (i.e. cockroaches). Clinical experience has shown that cutaneous tests are positive for two species of *Dermatophagoides* even if one species is rarely present in the environment. For Pyroglyphide mites, ELISA inhibition using purified allergens demonstrated the existence of cross-reactive allergens between Der p 1 and Der f 1 [71, 72] and between Der p 2 and Der f 2 [73].

For Der p 3 and Der f 3, the interspecies reactivity does not correlate as well as the interspecies reactivity of groups I and II [74]. Studies of molecular properties of 12 groups of mite allergens have allowed the demonstration of the homologies (sequence identity) within each group of allergens [75]. A usual degree of polymorphism of the alleles was observed, which could explain variations and regional differences in *Dermatophagoides* allergen extracts according to the sources of the allergenic extracts. Table 4 summarizes the main data concerning each group of mite allergens. For the two Pyroglyphide mites, *D. pteronyssinus* and *D. farinae*, great homologies are also found for group 7 [83] and nearly full amino acid sequence identity for group 10 [85]. The commonly observed cosensitization to *Dermatophagoides* and *Euroglyphus maynei* [87] can be explained in part by the sequence identity of group I and group II allergens [88]. Simultaneous positive IgE binding to *Dermatophagoides* and *Glyciphagidae* (to which *B. tropicalis* and *Lepidoglyphus destructor* belong) occurred at a relatively high frequency in different studies [89, 90]. However, RAST inhibition showed moderate cross-reactivity between these mite species [90, 91]. Using the immunoblotting inhibition technique, Johansson et al. [92] confirmed the existence of distinct as well as common allergenic determinants in *L. destructor* and *D. pteronyssinus* (especially components of 16 and 21 kD). The same authors also assessed the cross-reactivity between *B. tropicalis* and *L. destructor* (two species which have different geographical distributions) using immunoblot inhibition techniques with the recombinant Lep d 2 allergen [93]; a component of 14.5 kD in *B. tropicalis* similar to Lep d 2, is one of the cross-reacting allergens. A recent study gives a good illustration of the low cross-reactivity between two purified allergens of *D. pteronyssinus* (Der p 5) and *B. tropicalis* (Blo t 5) despite a 43% sequence identity between the two allergens [94]. RAST inhibitions and dot blot inhibitions with rDer p 5 and rBlo t 5 showed only moderate cross-reactivity. Moreover, these findings were also supported by skin tests: titration end-points of intradermal skin tests showed lower concentration end-points with rBlo t 5 as compared to rDer p 5 in atopic patients exposed mainly to *B. tropicalis* allergens in Singapore. Opposite results were observed in temperate regions where patients are essentially exposed to *D. pteronyssinus* [81]. The existence of cross-reactivity between mites from different families was pointed out by Miyamoto et al. [95]. Johansson et al. [96] showed cross-reactivity between *D. pteronyssinus* and *Acarus siro*, and *Tyrophagus putrescentiae*, cross-reactivity between *L. destructor* and *A. siro* and *T. putrescentiae*. A high correlation coefficient

Table 4. Cross-reactivities among acarid allergens – pyroglyphidae include *D. pteronyssinus*, *D. farinae* and *E. maynei* (Der p, Der f, Eur m); Glyciphagidae include *B. tropicalis* and *L. destructor* (Blo t, Lep d)

	Allergens	References	Molecular weight, kD	Prevalence of sensitization, %	Degree of sequence identity
Group 1 (cystein proteases)	Der p 1 Der f 1 Eur m 1	77 76, 77, 78	25	75–90	80% for Der p 1, Der f 1 80% for Der p 1, Eur m 1 80% for Der f 1, Eur m 1 76% for the 3 species 25–30% with other cyst. prot.
Group 2 (epididymal proteins)	Der p 2 Der f 2 Eur m 2 Lep d 2 (anc. Lep d 1) Tyr p 2	79 80 81	14	70–90	88% for Der p 2, Der f 2 40% for Der p 2, Lep d 2, Tyr p 2
Group 3	Der p 3 Der f 3 Eur m 3	74	30	70–90	81% for Der p 3, Der f 3 75% between 3 species
Group 5	Der p 5	82	14	40	43% between Blo t 5/Der p 5
Group 6	Der p 6 Der f 6	74	25	40	37% for Der p 3, Der p 6
Group 7	Der p 7 Der f 7	83	22–28	50	86% for Der p 7, Der f 7
Group 8 (glutathion S transferase)	Der p 8	84	26	40	25% for Der p 8, Bla g 5
Group 10 (tropomyosin)	Der p 10 Der f 10	85	37 29	50–95	98.5% Der p 10, Der f 10 75% with other arthropod tropomyosins 60% with mammalian tropomyosins
Group 11	Der p 11	86	95–100	80	Der p 2

was found between rLep d 2 and rTyr p 2 with respect to skin tests and RAST, which is in favor of similar IgE epitopes to group 2 allergens for these two mite species [97]. A practical aspect derived from the existence of common epitopes present in different species of storage mites is the development of an ELISA inhibition test allowing the detection of these mites by using a monoclonal antibody against the 39-kD allergen of *L. destructor* which recognizes common epitopes in *Glycyphagus domesticus*, *T. putrescentiae* and *A. siro* [98]. *Dermatophagoides*-sensitized allergic individuals may experience allergic symptoms after consumption of crustaceans and mollusks [99]. This has been attributed in part to the cross-reactivity of group 10 allergens to vertebrate tropomyosins [85]. Glutathione-S-transferase, Der p 8, reacting with IgE in 40% of sera from mite-allergic patients, has 25% sequence identi-

ty with an important allergen of cockroaches, Bla g 5, though actual cross-reactivity cannot be established [84].

Cross-Reactions Involving Cockroach Allergens

Interspecies cross-reactivity was demonstrated between different species of cockroaches, and so was extra-species cross-reactivity with other Insecta, Arachnida and Crustacea families. RAST and blot inhibition studies have shown cross-reactivity between crude extracts for *Blattella germanica*, *Blattella asahinai*, *Periplaneta americana* and *Blatta orientalis* [100]. The first cockroach allergen to be identified using immunochemistry was Bla g 1; only *B. germanica* allergen has a structural homologue in *P. americana* [101]. Other sequenced allergens, i.e. Bla g 2, Bla g 4, Bla g 5 for *B. germanica* and Per a 2, Per a 3, Per a 7 for *P. americana*, are species specific. The only

cross-reactive allergens from *B. germanica* and *P. americana* are the group 1 allergens Bla g 1 and Per a 1, to which 30–50% of cockroach-allergic patients are sensitized.

Cross-reactivity of cockroaches with other arthropods (Insecta and Arachnidae) was also assessed. Witteman et al. [103] demonstrated that IgE antibodies of 30% of patients allergic to house dust mite react with silver fish, cockroaches and chironomid extracts. RAST inhibition studies showed cross-reactivity between *D. pteronyssinus* and silver fish, cockroach and chironomid extracts. Pascual et al. [104], using CAP inhibition and immunoblot inhibition, demonstrated several common IgE-binding components between German cockroaches and chironomids. Moreover, by using immunoelectrophoretic techniques and RAST inhibition, O'Neil et al. [105] demonstrated that cockroach antigens cross-react with several species of Crustaceae. But only recently was it shown that a major cockroach allergen, Per a 7 from *P. Americana*, had a high degree of sequence identity with tropomyosins from invertebrates, especially dust mites, *D. pteronyssinus* and *D. farinae* (80% identity) and shrimps (82% identity) [106]. ELISA and Western blot inhibition experiments with natural and recombinant purified tropomyosins from shrimps, mites and cockroaches, demonstrated the IgE cross-reactivity of these tropomyosin allergens. The clinical relevance of cross-sensitization via tropomyosin appeared more clearly with shrimp RAST changing from negative to positive and oral symptoms occurring after eating shrimp during mite immunotherapy [107]. Another study suggested that induction of sensitization to mite and German cockroaches in unexposed subjects could be subsequent to their sensitization to Crustaceae: this was observed in Martinique, an island with a great consumption of Crustaceae [108].

Cross-Reactions Involving Mammalian Allergens

Studies to date suggest that taxonomic groupings may be helpful in predicting the degree of allergenic cross-reactivity among different mammalian animal species. Cross-reactivity between dog and cat extracts has been previously reported by several authors using different approaches, including the RAST inhibition technique [109–111]. These data were confirmed by immunoblot analysis showing different allergenic components, especially one of 69 kD (presumed to be albumin) and recognized by sera from patients allergic to both cats and dogs [112]. Other clinical studies demonstrated that allergy to pets was a high risk factor for the development of occupational allergy in laboratory animal workers [113]; allergen components recognized by IgE from fur farmers exposed to

fox, mink, racoon dog and pitchew were also recognized by sera from patients allergic to dogs and cats [114]. The molecular characterization of animal allergens has led to the elucidation of the molecular nature of cross-reactive allergens. Among them albumin represents the major component with a variety of cross-reactive epitopes, to which patients allergic to different animals can preferentially react. The prevalences of sensitization to the albumin of the animal species to which patients are allergic, ranged from 15 to 20% for cat-allergic individuals [115–118], from 10 to 35% for dog-allergic individuals [119, 120]. One study found 18% of subjects sensitized to albumin among 43 horse-allergic individuals [121].

Using a panel of 200 sera from patients allergic to animals, Spitzauer et al. [122] showed that, among 30% of sera reacting against albumin, there were variabilities of IgE binding to different mammal albumins despite a high degree of sequence homology. This was also confirmed by RAST inhibition studies [117, 121, 122]. However, the results obtained are not all consistent with each other; this could be explained by differences in selection criteria of sera and the relatively small number of sera used. Dog albumin probably contains most of the IgE epitopes present in albumin from other species [120]. Using peptides of horse albumin, it was also demonstrated that horse IgE-binding epitopes were destroyed after reduction and alkylation, suggesting that intrachain disulfide links are essential for the allergenic activity of albumin. Recently, complete sequence analysis of dog albumin was performed and comparison with other albumin sequences showed a high degree of sequence identity with cat and human serum albumin (86 and 79%, respectively) [122]. Recombinant dog albumin expressed in *Escherichia coli* may be useful in the future for the diagnosis of allergies [123]. The clinical importance of cross-reactions between cat and pig serum albumin was pointed out by Hilger et al. [118], who reported anaphylactic reactions in a patient sensitized to cats after eating pork. Severe anaphylactic reactions were also observed in mammalian-sensitized subjects who had contact with bovine serum albumin fluid during a first artificial insemination [124, 125]. Earlier studies also demonstrated the cross-reactive potential of albumin from rodents [126].

Besides albumin, which is highly cross-reactive, major cat and dog allergens can also share common epitopes, as was demonstrated by inhibition studies [117, 127]. Other cross-reactivities have been suggested for wild animal allergens, but the results are generally based on a limited number of cases and sera. Simultaneous sensitizations were observed by skin tests to extracts of mammal aller-

gens of the same order (deer, sheep, goat, cow and camel). Both inhibition studies and clinical symptoms confirmed cross-reactivities between deer and cow allergens in 6 out of 8 patients [128]. Inhibition studies with sera from fur-exposed workers showed that dog and cat extracts are cross-reactive with other Canidae (fox), Procyonidae (raccoon) and Mustelidae (minks and ferrets); these species all belong to the same Carnivora order [114]. The major mouse allergen Mus n 1 shows an approximately 64% sequence homology with the rat urine allergens Rat n 1 [129]; the two allergens belong to the lipocalin family of proteins and have about 25% homology with Bos d 2, the major allergen from cow dander.

Conclusions

The aim of this review was to show the advances in our knowledge of the principal inhalant allergens, except moulds, which were not dealt with in this review. Considering each group of allergens, we tried to draw the main lines of development directly linked to scientific progress over the last 3 decades and the availability of new immunochemical techniques. Awareness of the fact that IgE recognizes defined cross-reactive allergen molecules helps to interpret allergy symptoms. Precautions must indeed be taken when considering positive cutaneous sensitization and positive RAST findings as evidence for exposure (a prudent attitude which has clinical importance when keeping in mind that modern life often means changes in environment and increased mobility). A positive reaction both in vivo and in vitro tests to an allergen with cross-reactive potential may explain allergic reactions to other allergen sources which contain immunologically related molecules. Diagnostic investigations will increasingly use recombinant or purified natural cross-reactive allergens: first results in skin testing with cross-reactive pollen allergens [130, 131] and recombinant storage mite allergens [97] are promising. In vitro testing is beginning to show the same satisfactory results [9, 132–134]. Though the predictive value of positive RAST findings and cutaneous tests to an aeroallergen for the occurrence of respiratory allergic symptoms is not clearly known, detection of sensitization to an important cross-reactive allergen is of interest; for instance, positive reactions to recombinant dog albumin in atopic individuals can identify those individuals who are at risk of developing allergic reactions to other mammalian albumin sources. For the same reason, it is interesting to detect individuals sensitized to cross-reactive pollen allergens, such as

Bet v 1 or profilin, which have homologues in vegetable food allergens, as there is a risk for them to develop clinical food allergy. The evaluation of allergen load in different environments has practical implications as well. Immunoassays using monoclonal antibodies against cross-reactive allergens, can, for instance, measure mite allergen content in dust samples [98, 135]. But most importantly, recombinant respiratory cross-reactive allergens will not only be a new diagnostic concept, they will also constitute a new method of immunotherapy. Modified recombinant allergens have reduced IgE-binding capacities and thus improve immunotherapy. This has been successfully achieved by producing oligomers and fragments for Bet v 1 [136], and by site-directed mutagenesis for Bos d 2 [137] and for group 2 mite allergens [138, 139]. It has also been recently shown in two clinical studies including, respectively, 30 and 23 subjects that hypoallergenic derivatives of Bet v 1 (fragments and trimers) showed at least a hundred-fold reduced capacity to induce immediate skin reactions [140, 141]; genetically modified rBet v 1 may thus be a candidate molecule for birch pollen immunotherapy. In the future, a panel of genetically engineered hypoallergenic variants of cross-reactive allergens, used alone or in combination with others, administered in high doses, could be a new form of treatment if they appear safe and effective in clinical trials.

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