

Immunological and structural similarities among allergens: Prerequisite for a specific and component-based therapy of allergy

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Summary It is known that allergic patients are frequently co-sensitized against different allergen sources. Progress made in the field of allergen characterization by molecular biological techniques has now revealed that sensitization against different allergen sources can be explained as cross-reactivity of IgE antibodies with structurally and immunologically related components present in these allergen sources. This review defines groups of cross-reactive plant allergens with significant sequence homology. The similarities among allergens may facilitate allergy diagnosis by using a few representative cross-reactive allergens to determine the patient's IgE reactivity profile (allergogram). According to that typing, a few cross-reactive allergens, carrying most of the relevant IgE epitopes, may then be selected for patient-tailored specific therapy.

Key words: allergens, cross-reactivity, IgE, therapy, type I allergy.

Introduction

Allergic symptoms are frequently associated with contact with different allergen sources. It is known that several pollen allergic patients show allergic symptoms upon ingestion of plant derived food.¹⁻³ IgE inhibition experiments indicated that cross-reactive epitopes present in pollen and plant food might be responsible for this phenomenon.^{4,5} The application of molecular biological techniques has resulted in the identification of the primary structure of several cross-reactive plant, mite, fungal and mammalian allergens.^{6,7} cDNA encoding birch profilin (Bet v 2),⁸ a highly conserved actin binding protein, Bet v 1 the major birch pollen allergen which shows significant sequence homologies to pathogenesis-related plant proteins,⁹ and those encoding major groups of grass pollen allergens¹⁰⁻¹⁶ which represent conserved monocot allergens were isolated and expressed in *Escherichia coli*. Using the recombinant allergens, cross-reactive B^{17,18} and T cell epitopes¹⁹ were identified and it could be demonstrated that sensitization with a few cross-reactive allergens induced a battery of cross-reactive IgE antibodies in experimental animal models.²⁰ This review discusses the significance of cross-reactivities among plant allergens for the classification of allergens as well as for diagnosis and therapy of allergic diseases. It is expected that a component based diagnosis of allergy, performed with recombinant allergens, will allow the selection of a few cross-reactive allergens which carry most IgE epitopes for patient-tailored immunotherapy.

Materials and methods

Expression of human IgE-Fabs (Fabs, antibody fragments) with specificity for major grass pollen allergens in Escherichia coli

Phage expressing human IgE-Fabs (Fabs, antibody fragments) with specificity for the major timothy grass pollen allergens, Phl p 1 and Phl p 5 were isolated from an IgE library which was constructed from peripheral blood mononuclear cells (PBMC) from a grass pollen allergic patient in pComb 3H by panning as previously described.²¹ Soluble recombinant human IgE-Fabs were produced in *E. coli* XL1 Blue.²²

Immunoblotting

Pollen from birch (*Betula verrucosa*), timothy grass (*Phleum pratense*), rye grass (*Lolium perenne*), rye (*Secale cereale*), Kentucky Bluegrass (*Poa pratensis*) and (*Cynodon dactylon*) were purchased from Allergon, Vålinge, Sweden. Protein extracts were prepared as previously described²³ and separated by SDS-PAGE.²⁴ They were then transferred to nitrocellulose²⁵ and the allergens were detected using recombinant human IgE-Fabs as previously described.²¹

Sequence comparison and alignments of plant allergens

The amino acid sequences of the major birch pollen allergen, Bet v 1,⁹ birch profilin, Bet v 2,⁸ the major timothy grass pollen allergens, Phl p 1,¹¹ Phl p 2,¹³ Phl p 5,¹⁶ the major ragweed allergen, Amb a 1,²⁶ a major cypress allergen, Cry j 2,²⁷ and the major olive allergen, Ole e 1²⁸ were compared with sequences from the National Institute of Health databases (GenBank, SWISS-PROT, PIR-Protein). Homologous proteins were retrieved using the BLASTP program and grouped according to sequence homology and/or cross-reactivity. The protein sequences of the allergens belonging to each group were aligned

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with each other using the GAP program (WISCONSIN PACKAGE) and the degree of homology was expressed as percentage sequence identity.

Results

Recombinant IgE-Fabs from a grass pollen allergic patient cross-react with major allergens from different grass species

Cross-reactivities among structurally related allergens have been demonstrated by IgE-inhibition experiments, using polyclonal rabbit antisera as well as with murine mAb.²⁹⁻³² Recent advances in the expression of functional Fab fragments in *E. coli* have provided the basis for the molecular characterization of human allergen-specific IgE antibodies.²² Figure 1 shows that IgE-Fabs with specificity for the major timothy grass pollen allergens Phl p 1 and Phl p 5, cross-react with related allergens present in different grass species. The cross-reactivity of IgE antibodies with major grass pollen allergens provides the molecular basis for the clinical observation that grass-pollen-allergic individuals display symptoms upon contact with pollen from different grass species, crops and monocotyledonous plants.

Plant allergens can be divided into groups according to sequence homology and immunological similarities

Isolated allergens and related plant proteins with significant homology to birch profilin, Bet v 2,⁸ a highly conserved allergen and actin binding protein, the major birch

pollen allergen, Bet v 1,⁹ the major timothy grass pollen allergens, Phl p 1,¹¹ Phl p 2,¹³ and Phl p 5,¹⁶ the major ragweed allergen Amb a 1,²⁶ a major cypress allergen, Cry j 2,³³ and the major olive allergen, Ole e 1,²⁸ are shown in Table 1. Representative proteins from different species were included in each group. Isoallergenic variants of the same species which differ in few amino acid residues are not displayed. Members of each group have similar molecular weights and share a high degree of end to end sequence homology. Some proteins were found to cross-react with at least one other member within the groups.

Clusters of immunologically related plant allergens show a high degree of sequence homology

Profilins, which represent actin-binding and phosphoinositide (PIP)-binding proteins in eukaryotes have been identified as extremely cross-reactive allergens.^{8,17,30,34} The cDNA and deduced amino acid sequences of profilins from birch (Bet v 2),⁸ timothy grass (Phl p 11),³⁵ tobacco,³⁶ maize,³⁷ wheat³⁸ and bean³⁹ show a high degree of similarity (Table 2) and represent clinically relevant cross-reactive allergens.⁴⁰ The structural similarities among tree pollen allergens (Bet v 1,⁹ Aln g 1,⁴¹ Car b 1,⁴² Cor a 1,⁴³) and food allergens (Mal d 1,⁴⁴ Api g 1,⁴⁵) explains clinical symptoms in birch pollen allergic patients on contact with pollen from related trees as well as following ingestion of plant-derived food such as apples,²⁹ hazelnuts,³¹ celery,³⁰ (Table 3). Extensive cross-reactivities have been described among the major groups of grass pollen allergens. Homologies and significant cross-reactivities were described among the members belonging to each of the groups (group 1 allergens: Table 4;

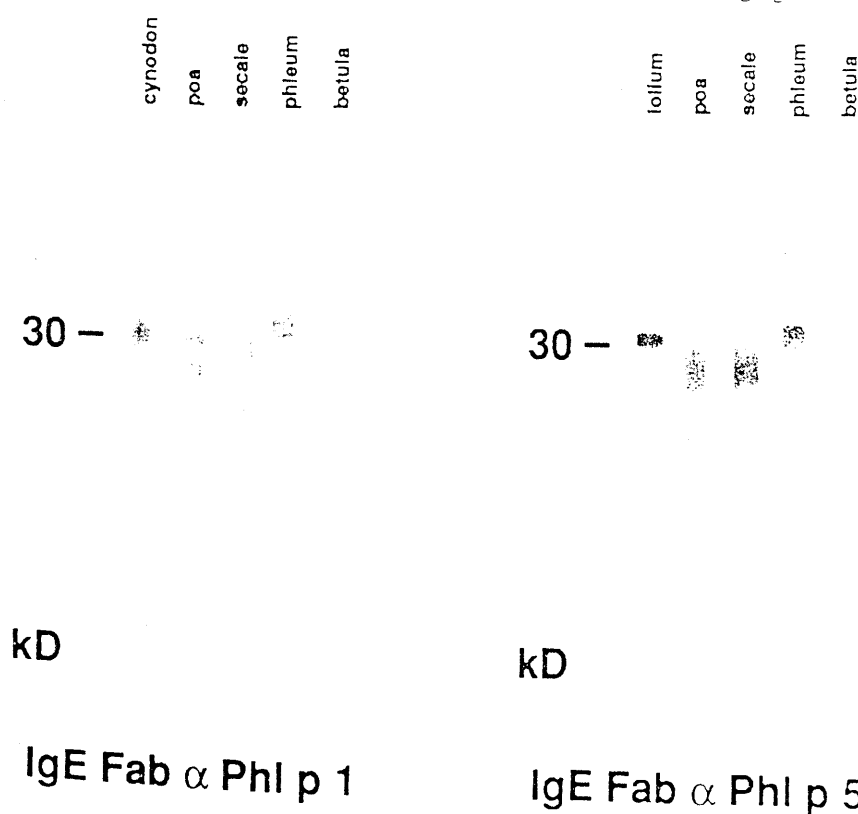


Figure 1 Cross-reactivity of recombinant human IgE-Fabs with group 1 and group 5 allergens from different grass species.

Table 1 Dividing plant allergens/proteins into families of homologous and cross-reactive allergens with structural and immunological similarities

Group	Representative members	Average molecular weight (kDa)	Reference
Profilins	Birch: Bet v 2*	14	8
	Tobacco profilin*		36
	Timothy grass: Phl p 11*		35
	Wheat profilin		38
	Maize profilin		37
	Bean profilin		39
Bet v 1	Bet v 1*	17	9
	Aln g 1*		41
	Car b 1*		42
	Cor a 1*		43
	Mal d 1*		44
	Api g 1*		45
Group 1 grass allergens	Phl p 1*	30	11
	Hol l 1		
	Lol p 1*		10
	Zea m 1		
Group 2 grass allergens	Tri s 2	11	
	Phl p 2*		13
	Lol p 2*		12
	Lol p 3		
Group 5 grass allergens	Phl p 5*	30	16
	Poa p 9		14
	Lol p 5*		15
	Hor v 5		
Amb a 1	Amb a 1	40-50	26
	Amb a 2		
	LAT56		27
	Cry j 1		
	Zm58		
Cry j 2	Cry j 2	40-50	33
	Prunus polygal		
	Malus polygal		
	Solanum polygal		
Ole e 1	Ole e 1	20	28
	Syr v 1		47
	Zm13		49
	LAT52		51
	Lol p 11		48
	riceAC		

Proteins marked with an asterisk crossreact with at least one other member within the group.

group 2 allergens: Table 5; group 5 allergens: Table 6). Despite significant sequence homology, to date cross-reactivity has not been demonstrated between Amb a 1²⁶ and the major cypress allergen Cry j 1²⁷ (Table 7). However, a high molecular weight group of grass pollen allergens, presumably representing group 4 allergens, were recently shown to share IgE epitopes with the Amb a 1 allergen family.⁴⁶ Because of the significant sequence similarities it is likely that other proteins listed in this group

share IgE epitopes with Amb a 1. The Cry j 2 homologous polygalacturonases from several fruits and vegetables listed in Table 8 were so far not tested for IgE cross-reactivity but display significant end to end sequence homology with Cry j 2.³³ The major olive allergen Ole e 1,²⁸ the major lilac allergen Syr v 1,⁴⁷ a rye grass pollen allergen designated Lol p 11⁴⁸ and a pollen-specific maize protein, Zm 13^{49,50} were shown to possess IgE binding capacity. Together with pollen-specific proteins from tomato, LAT52⁵¹ and from rice, riceAC, these proteins form a cluster of proteins with significant sequence similarity (Table 9).

Discussion

The molecular characterization of cross-reactive plant allergens indicates that the spectrum of epitopes against which allergic patients are sensitized is less complex than originally anticipated. IgE inhibition experiments indicate that a significant percentage of specific IgE antibodies can be preadsorbed with only a few recombinant allergens. These data give rise to the hope that a panel of a few cross-reactive recombinant allergens can be used for diagnosis as well as for patient-tailored immunotherapy.⁷ Although the present contribution is focused on plant allergens, it should be emphasized that significant crossreactivities have also been described for mite, mould and mammalian allergens.⁵² We are therefore optimistic that the concept of using selected recombinant cross-reactive allergens for diagnosis and therapy may be a general strategy.

Defining allergens according to sequence homology and cross-reactivity

According to the revised allergen nomenclature, allergens are currently designated according to the accepted taxonomic name of their source and grouped according to the relation of the different genera and sources.⁵³ Using plant allergens as an example, Table 1 shows that it is also possible to group allergens according to sequence similarities and immunological relationships. We have compared sequences of representative plant allergens with the data bases. Proteins with significant sequence similarities obtained by the sequence comparisons formed groups of cross-reactive allergens and/or related proteins. The degree of sequence identities within the individual groups are displayed in Tables 2-9. Extensive cross-reactivities are known for the group of profilins which are designated 'pan-allergens'.¹⁷ Cross-reactivities are also well documented for the Bet v 1 family⁹ and for the three groups of grass pollen allergens.¹¹ Amb a 1-related allergens were found in grass pollen,⁴⁶ and a homologous cypress allergen, Cry j 1, has been described.²⁷

Co-sensitization as an explanation for allergic responses to many different allergen sources

Using IgE-absorption experiments it has been demonstrated *in vitro* that selected recombinant allergens (tree

Table 2 Sequence identities of plant profilins

Birch (sp P25816)	Tobacco (sp P41372)	Timothy grass (sp P35079)	Wheat (gp X89825)	Maize (sp P35081)	Bean (pir S49351)	
100	82	79.4	78.6	77.1	74.8	Birch
	100	81.7	79.4	77.9	74	Tobacco
		100	79.4	82.4	79.4	Timothy grass
			100	80.1	77.9	Wheat
				100	76.3	Maize
					100	Bean

The accession numbers of the profilin sequences are in parentheses.

Table 3 Sequence identities of Bet v 1 related allergens

Bet v 1 (pir S05376)	Aln g 1 (sp P38948)	Car b 1 (sp P38950)	Cor a 1 (sp Q08407)	Mal d 1 (sp P43211)	Api g 1 (gp Z48967)	
100	81.1	73	72.3	56.3	48.1	Bet v 1
	100	73.6	76.1	55.1	41.8	Aln g 1
		100	88.1	51.9	37.9	Car b 1
			100	55.1	38.6	Cor a 1
				100	38.8	Mal d 1
					100	Api g 1

The accession numbers of the sequences are in parentheses.

Table 4 Sequence identities of group 1 grass pollen allergens

Phl p 1 (sp P43213)	Hol l 1 (sp P43216)	Lol p 1 (pir S13614)	Zea m 1 (sp Q07154)	
100	91.6	87.7	57.2	Phl p 1
	100	91.6	57.2	Hol l 1
		100	54.5	Lol p 1
			100	Zea m 1

The accession numbers of the sequences are in parentheses.

Table 5 Sequence identities of group 2/3 grass pollen allergens

Tri s 2 (gp Z50867)	Phl p 2 (sp P43214)	Lol p 2 (sp p14947)	Lol p 3 (sp P14948)	
100	54.3	58.3	69.8	Tri s 2
	100	64.2	59.6	Phl p 2
		100	60	Lol p 2
			100	Lol p 3

The accession numbers of the sequences are in parentheses.

Table 7 Sequence identities of Amb a 1 homologous allergens/proteins

Amb a 1 (sp P27759)	Amb a 2 (pir E53240)	P56 (gp X15500)	Cry j 1 (gp D34639)	Zm58 (gp L20139)	
100	67.2	47.6	45.4	39.8	Amb a 1
	100	47.1	47	41.7	Amb a 2
		100	44.1	45.6	P56
			100	34.9	Cry j 1
				100	Zm58

The accession numbers of the sequences are in parentheses.

Table 6 Sequence identities of group 5 grass pollen allergens

Phl p 5 (pir S37400)	Poa p 9 (sp P22285)	Lol p 5 (pir JT0756)	Hov v 5 (gp U06640)	
100	85	78.9	74.8	Phl p 5
	100	68.3	73.7	Poa p 9
		100	70.4	Lol p 5
			100	Hov v 5

The accession numbers of the sequences are in parentheses.

pollen allergens, grass pollen allergens) carry most of the IgE epitopes of natural isoallergens,^{54,55} as well as of related allergens present in other sources.^{11,16,30,31,32} It may therefore be speculated that sensitization against a few cross-reactive allergens may be responsible for the induction of IgE antibodies against many different allergen sources. Using rhesus monkeys it has been demonstrated that immunization with recombinant birch pollen allergens (Bet v 1, Bet v 2) induces IgE antibodies which cross-react with Bet v 1 and Bet v 2-homologous allergens in

Table 8 Sequence identities of Cry j 2 homologous proteins

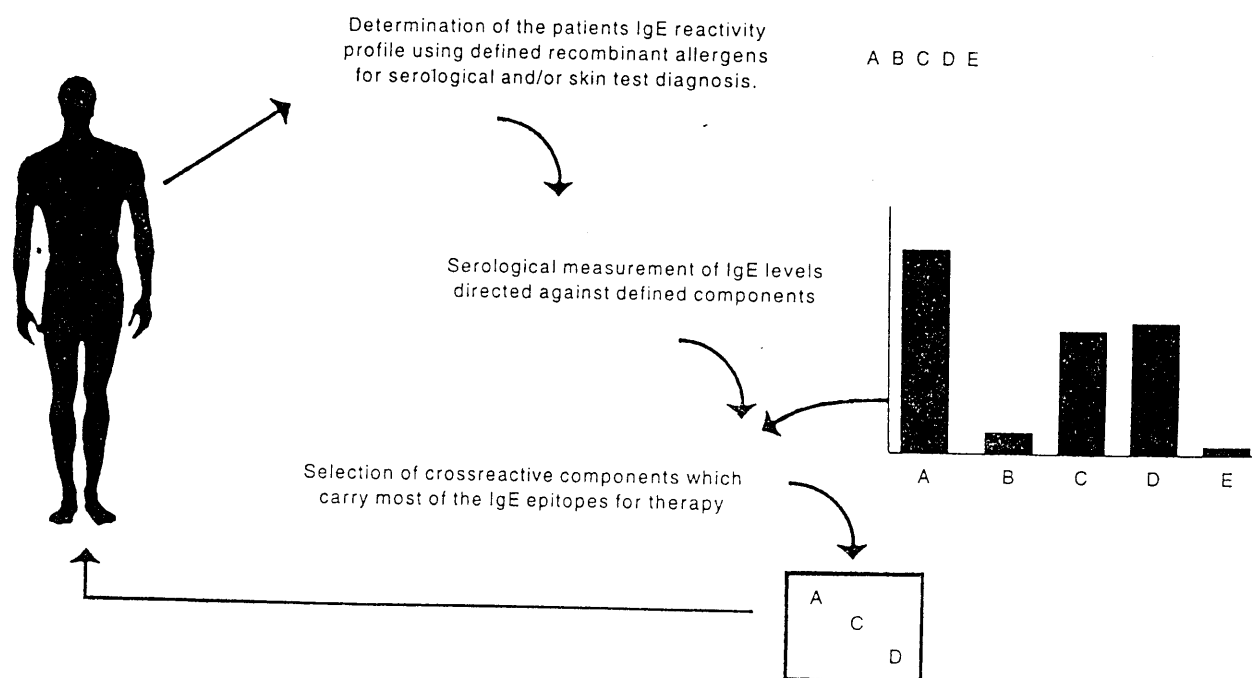
Cry j 2 (pir JC2498)	Prunus polygal (gp X77231)	Malus polygal (gp L27743)	Solanum polygal (gp U23053)	
100	41.4	40.1	40.5	
	100	81.6	39.9	Cry j 2
		100	40.4	Prunus
			100	Malus
				Solanum

The accession numbers of the sequences are in parentheses. Polygal, polygalacturonase.

Table 9 Sequence identities of Ole e 1-related allergens and proteins

Ole e 1 (sp P19963)	Syr v 1 (pir S43242)	Zm 13 (sp P33050)	LAT52 (sp P13447)	Lol p 11 (pir A54002)	riceAC (pir S31710)	
100	88.9	35.4	35.4	31.6	30.1	Ole e 1
	100	35.4	36.8	31.6	29.4	Syr v 1
		100	34.2	44.4	72.2	Zm 13
			100	39.9	35.2	LAT52
				100	46.2	Lol p 11
					100	riceAC

The accession numbers of the above sequences are in parentheses.

**Figure 2** Component-based diagnosis and patient-tailored therapy based on recombinant allergens.

pollen and plant-derived food.²⁰ These data suggest that a few relevant cross-reactive allergens and epitopes can induce a battery of cross-reactive IgE antibodies which lead to clinical symptoms on contact with different allergen sources.

Recombinant allergens permit a component-based diagnosis of type I allergy: The allergogram

To date type I allergy is diagnosed using extracts prepared directly from allergen sources. Depending on the extraction procedure and the choice of raw material these extracts contained an ill-defined mixture of allergens and non-allergenic components. *In vitro* and *in vivo* diagnosis therefore only allowed to determine whether an individual is sensitized against a particular allergen source. Using defined recombinant allergens it has now become possible to dissect the polyclonal IgE response of allergic patients.^{56,57} For many allergen sources (plant pollen, mites) it is now possible to determine what components patients are sensitized to and estimate the percentage of IgE directed against each component. It is conceivable that allergic patients will suffer on contact with those allergens against which high levels of specific IgE have been produced. Measurement of IgE levels directed against recombinant allergens may therefore be useful to identify those components which have induced high levels of IgE.

The allergogram as a basis to select recombinant allergens for specific patient-tailored therapy

Hyposensitization treatment of type I allergy has been established since 1911.⁵⁸ Currently hyposensitization is performed by injecting increasing doses of crude allergen extracts. The allergen extracts used for injection contain, in addition to allergens against which patients are sensitized, other allergens which are not recognized and non-allergenic components. Figure 2 shows that, based on the determination of the patients IgE reactivity pattern (allergogram), components can be selected for hyposensitization therapy against which significant levels of IgE are produced. Regardless of the form of immunotherapy (injection of T cell or B cell epitopes, complete and/or modified allergens) that will be used, it is desirable to establish the patients' reactivity profile prior to therapy to avoid application of material against which a patient is not sensitized. Selection of allergens according to the patients' reactivity profile (allergogram) might improve hyposensitization therapy by avoiding *de novo* sensitization against unwanted components.

To perform such a component-based form of specific immunotherapy it will, however, be necessary that a reasonable panel of allergens/epitopes can be defined. The extensive cross-reactivities and structural similarities among the plant allergens summarized in this review were also found for many mite, fungal and mammalian allergens, and give rise to the hope that immunotherapy of type I allergies may indeed be performed with a patient-tailored cocktail of cross-reactive allergens/epitopes. The

prospect of performing specific immunotherapy with a few defined components may therefore represent the ultimate clinical relevance of cross-reactivity among allergens.

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