

6 Tree Pollen Allergens

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INTRODUCTION

IgE-mediated allergy affects more than 25% of the world's population, and proteins from trees, grasses, and mites are the most important causes of this disease (1,2). In trees, allergenic proteins can occur in pollen, fruits, or seeds. Pollen released in great abundance during the flowering season of certain trees represents a major trigger of respiratory manifestations of allergy (e.g., rhinitis and asthma), whereas tree fruits and seeds can elicit various symptoms of food allergy.

Trees recognized as major pollen allergen sources belong to certain botanical orders with defined geographical distributions and flowering periods (3). Allergenic tree species are predominantly wind pollinated, whereas insect-pollinated trees rarely elicit allergic symptoms. This is likely due to the process of allergic sensitization, which leads to the production of allergen-specific IgE antibodies (4), preferentially occurring via the respiratory mucosa (5,6) and requiring certain threshold levels of pollen (7–9).

In the temperate climate zone, thus in countries of northern and middle Europe, Northwest Africa, East Asia, from North America to the Andes, as well as in certain areas of Australia (2,3,10,11), trees belonging to the order Fagales (e.g., birch, alder, hazel) represent the predominant allergen sources. Whereas in Mediterranean countries and in areas with a Mediterranean climate (e.g., parts of North and South America, South Africa, and Australia), olive trees, which are members of the order Scrophulariales, are the most important sources of airborne allergens (12–15). Other important trees capable of inducing pollen allergy belong to two closely related plant families of the “nonflowering” plants (Gymnospermae), the Cupressaceae (e.g., cypress, cedar) and the Taxodiaceae. Cupressaceae trees represent important causes of pollinosis, especially in areas characterized by a Mediterranean climate where up to 30% of atopic individuals might be sensitized to Cupressaceae pollen (16–20). Within the Taxodiaceae, one species, the Japanese cedar (*Cryptomeria japonica*), is of increasing allergological relevance and represents the most common cause of seasonal allergy in Japan (21).

The most important allergens of these allergenic trees have been identified and characterized. Many of them have been cloned and produced as defined recombinant proteins, which can be used as tools to study the immunopathology of allergic disease (22). These defined proteins also form the basis for the development of novel strategies for diagnosis, treatment, and prevention of allergies (4,23,24). Tree pollen allergens characterized to date represent predominantly low-molecular-weight intracellular proteins or glycoproteins that are rapidly released after contact of pollen with aqueous solutions (25,26). Carbohydrate moieties may represent cross-reactive IgE epitopes occurring in tree pollen and several unrelated allergen sources, but seem to have little clinical relevance (27,28).

An exciting discovery was that the cross-reactivity observed among certain closely related species (e.g., trees belonging to the order Fagales) can be attributed to the structural and immunological similarity of relevant cross-reactive allergens (29–31). This finding implies that diagnosis and immunotherapy may be performed with a few cross-reactive marker allergens that harbor a large proportion of the cross-reactive epitopes (32–35).

TAXONOMY AND DISTRIBUTION OF ALLERGENIC TREES

Among the 250,000 well-described pollen-producing plant species, less than 100 are considered as potent causes of pollen allergy (36–39). Figure 1 displays the phylogenetic relationship between trees that are relevant sources of allergenic pollen and shows that trees of both plant divisions, the Angiospermae (commonly known as “flowering plants”) and the

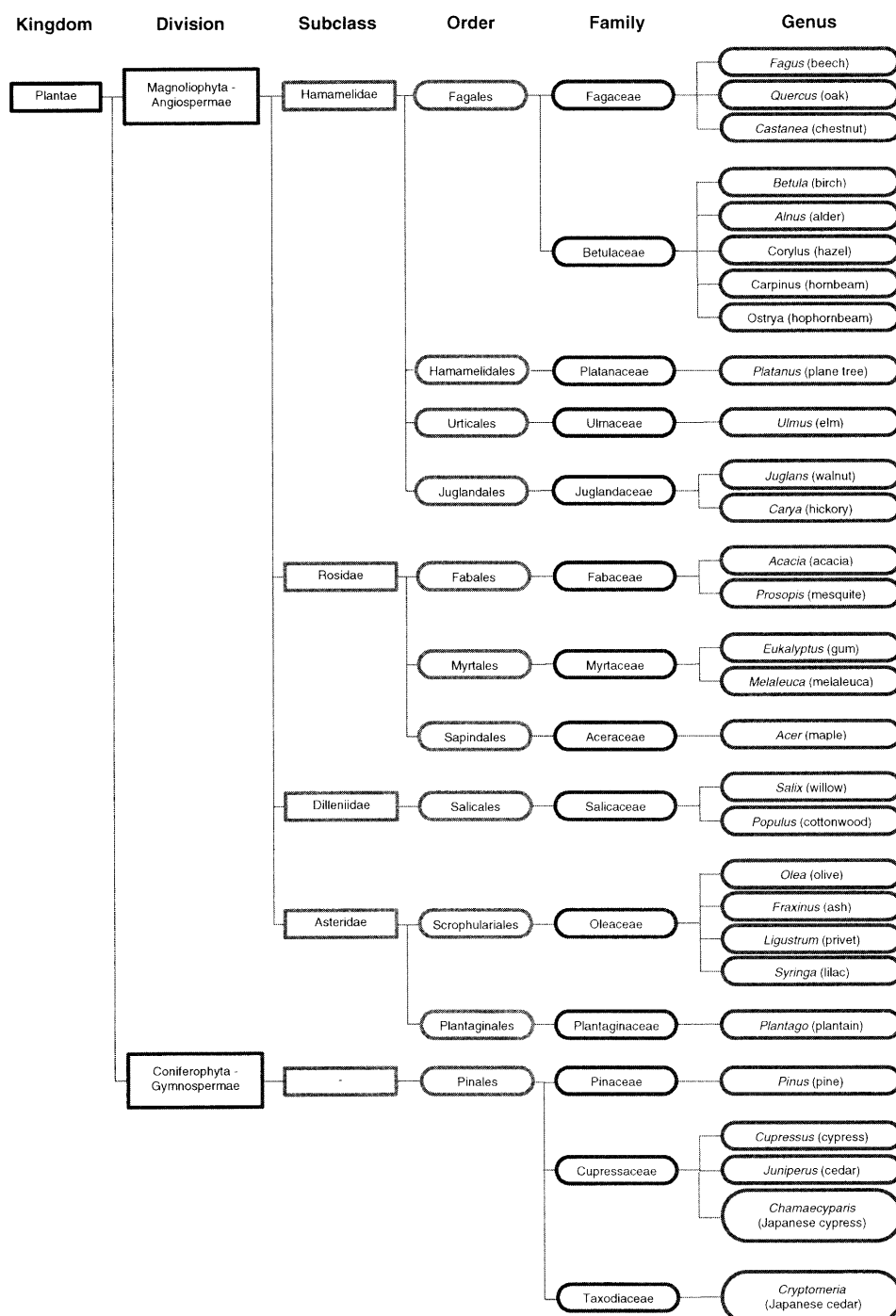


Figure 1 Taxonomy of trees producing allergenic pollen. Plants are listed following taxonomical guidelines of the Integrated Taxonomic Information System (www.itis.gov). For further information see also Ref. 172.

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Genus

- ☐ *Fagus* (beech)
- ☐ *Quercus* (oak)
- ☐ *Castanea* (chestnut)
- ☐ *Betula* (birch)
- ☐ *Alnus* (alder)
- ☐ *Corylus* (hazel)
- ☐ *Fraxinus* (hornbeam)
- ☐ *Alnus* (hornbeam)
- ☐ *Platanus* (plane tree)
- ☐ *Ulmus* (elm)
- ☐ *Juglans* (walnut)
- ☐ *Corylus* (hickory)
- ☐ *Acacia* (acacia)
- ☐ *Prosopis* (mesquite)
- ☐ *Pinus* (gum)
- ☐ *Eucalyptus* (melaleuca)
- ☐ *Acer* (maple)
- ☐ *Salix* (willow)
- ☐ *Populus* (cottonwood)
- ☐ *Olea* (olive)
- ☐ *Fraxinus* (ash)
- ☐ *Ligustrum* (privet)
- ☐ *Viburnum* (lilac)
- ☐ *Plantago* (plantain)
- ☐ *Pinus* (pine)
- ☐ *Cupressus* (cypress)
- ☐ *Juniperus* (cedar)
- ☐ *Macrocarpa* (Chinese cypress)
- ☐ *Thuja* (Chinese cedar)

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so Ref. 172.

Gymnospermae (nonflowering plants), have an impact on eliciting allergic symptoms in patients. Knowledge about taxonomical relationship between trees is of importance, since pollen of closely related trees contain cross-reactive allergen molecules (e.g., order Fagales with the major birch pollen allergen, Bet v 1), which are absent in pollen from unrelated trees. As mentioned above, overall wind pollination appears to be a prerequisite for an allergenic tree.

Looking at the plant kingdom, the orders Fagales (e.g., birch, alder, hazel, oak, beech), Scrophulariales (e.g., olive, ash), and Pinales (e.g., cypress, cedar) comprise the most potent allergenic trees. Within these orders, the trees birch, olive, and cypress are known as the most potent causes of pollen allergy, whereas plants like alder, hazel, chestnut (members of the Fagales), privet (Scrophulariales), and pine (Pinales) have lesser allergenic potential. Other plant orders contain, as a whole, predominantly trees of low or uncertain importance in eliciting allergic symptoms: these are the orders Sapindales (e.g., maple tree), Hamamelidales (e.g., plane trees), and Myrtales (e.g., gum tree, melaleuca). So far, little is also known about the sensitization potency of pollen allergens from trees belonging to the orders Juglandales (e.g., walnut), Fabales (e.g., acacia), and Salicales (e.g., willow, cottonwood). In the case of melaleuca, the low allergenic potential can be explained by the fact that this tree is not wind pollinated (40). However, other less allergenic trees, like maples, acacias, and walnuts, are wind pollinated, which indicates that the sensitization potency of a plant cannot only be explained by wind pollination.

It is interesting to note that the plant subclasses Hamamelidae and Asteridae not only comprise important allergenic trees but also the most relevant allergenic weeds: within the Hamamelidae it is the genus *Parietaria* (from the order Urticales) and within the Asteridae these are the genera *Artemisia* (mugwort, order Asterales) and *Ambrosia* (ragweed, order Asterales), which represent the most potent elicitors of weed pollen allergy. On the other hand, the subclass Rosidae includes trees (within the order Rosales) that are considered as the most relevant sources of allergenic fruits (e.g., apple, cherry).

Regarding the geographic distribution of allergenic trees, we can discriminate areas with a preferential occurrence of certain trees from areas with mixed vegetation. The geographic distribution influences the sensitization profiles of allergic patients toward certain allergenic molecules (41). Trees belonging to the order Fagales prefer the temperate climate zone and grow in Europe, Northwest Africa, East Asia, and from North America to the Andes. In contrast, olive trees, the most allergenic trees of the order Scrophulariales, occur in the Mediterranean countries and in areas with a Mediterranean climate of North and South America, South Africa, and Australia. These Mediterranean climate zones are also the preferred areas of the most allergenic trees of the order Pinales, cypress and cedar, which grow in the Mediterranean countries, Australia, New Zealand, South America, and parts of Asia (China, India). Ash, the other important pollen allergen source of the Scrophulariales, occurs in middle Europe and North America, often in the same area as the Fagales trees.

Two papers investigating the sensitization profiles of allergic patients from different parts of the world have revealed interesting differences depending on geographic areas (41,42). Birch pollen-allergic patients from the northern parts of Europe are mainly sensitized against the major birch pollen allergen, Bet v 1, which therefore may be considered as a genuine marker for birch sensitization (41). By contrast, patients from the more southern parts of Europe appear positive in a birch pollen extract-based diagnostic test, but when tested with pure recombinant allergens are more frequently positive to cross-reactive allergens (e.g., profilins, calcium-binding allergens). It is therefore likely that these patients are sensitized against other allergen sources and, due to cross-reactivity, appear positive in the birch pollen extract test. Similar results were obtained when an allergic population from Central Africa was tested with recombinant allergens, indicating that the IgE reactivity profile reflects the local pollen exposure (42). These studies and another study performed with recombinant *Parietaria* allergens (43) emphasize the importance of diagnostic testing with recombinant allergens for the accurate diagnosis of the sensitizing allergen source.

TREE POLLEN IDENTIFICATION

Pollen grains are single cells that contain within themselves the male reproductive cells, the sperm cells. Their task is to deliver these sperm cells to the female parts of the flower, where the process of fertilization takes place. Pollen grains are enclosed within an inner wall,

the intine, and an outer wall, the exine, which protect the pollen from harmful environmental influences, such as desiccation and irradiation, during distribution. The outer wall consists of very elaborate, three-dimensional patterns and is interrupted by openings called apertures. The number, distribution, and architecture of the apertures vary between plants and can be used to classify and identify tree pollen by light microscopy (36).

The collection of air samples and the analysis and identification of pollen are of importance for physicians and patients. The allergist needs to know which species of allergenic pollen are present in the atmosphere, the number of allergenic pollen grains in a given volume of air, and the time and spatial variations of concentrations of airborne allergenic pollen. Measurements of pollen loads during certain periods of the year permit prediction of allergen exposure, and such information distributed to allergic patients can help them to avoid exposure (44). Knowledge of pollen loads during certain periods and in certain countries also allows allergic patients to plan their vacations and traveling schedules (45). However, since there may be variations of the allergen contents in pollen grains and since allergens may also be released via submicronic particles, it is important to measure and quantify not only the pollen grains but also the concentrations of the released allergenic molecules (46,47).

An interesting correlation between date of birth and sensitization against certain pollen has been described. Children who were born in early spring and summer are more frequently sensitized against birch and grass pollen, respectively (48,49). There is also compelling evidence that sensitization to certain pollen (e.g., birch) is more frequent in children exposed to heavy pollen exposure early in life than in children who have experienced mild pollen exposure (50).

A number of methods are used to collect and quantify pollen in the air (51). The most widely used technique is the Rotorod[®] Sampler, a rotating-arm impactor that recovers airborne particles on two rapidly moving plastic collector rods, which are coated with silicon grease. The Rotorod system measures the average pollen concentration during the sampling period but fails to detect variations in concentrations within this period. In contrast to this, volumetric traps allow continuous isokinetic sampling and record variations in the concentrations of pollen during the sample period. The collected samples may be counted on the basis of pollen morphology. Unfortunately, morphological counting usually does not allow discrimination between very closely related pollen species. Alternatively, collected samples can be analyzed with antibodies for quantitative determination of the allergenic molecules. As mentioned above, the latter has the advantage that, in addition to pollen-associated allergens, allergens that are released from pollen and become adsorbed to other carrier particles (e.g., aerosols) can also be measured. In this context it has been reported that pollen from birch and related trees can release allergens by a process of artificial pollen germination, which occurs when pollen are exposed to humidity (Fig. 2) (26). Furthermore, it

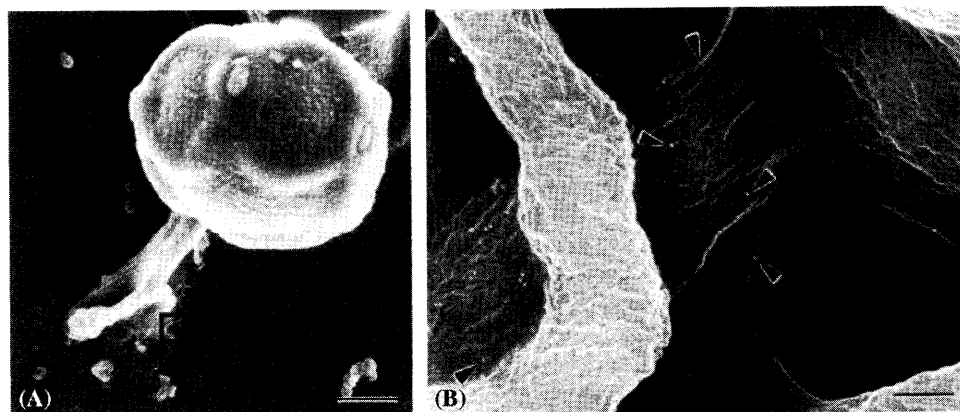


Figure 2 Humidity induced release of allergens from abortively germinated alder pollen. On contact with rain water allergen-containing particles are liberated from ruptured pollen tubes. Field emission scanning electron micrographs after immunogold labeling for Aln g 1. The rectangle in (A) marks an area comparable to the area shown in higher magnification in (B). Bound Aln g 1-specific antibodies were detected with secondary antibodies coupled to colloidal gold particles, which appear as white dots [bars represent 5 μ m in (A) and 0.25 μ m in (B)]. Source: From Ref. 26.

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was found that certain carrier molecules (diesel exhaust particles) might act as adjuvants by driving the allergen-specific immune response into a preferential TH2 pathway that is accompanied by increased production of IgE antibodies (52). On the basis of such findings, it appears that the actual measurement of allergenic molecules using antibody assays can give more accurate information about true allergen exposure than mere pollen counting. Another argument for antibody-based measurement of allergen exposure is the observation that pollen may contain greatly varying amounts of allergens depending on the maturation state of the pollen or depending on the cultivar (53,54).

CLONING OF TREE POLLEN ALLERGENS

Diagnosis and specific immunotherapy of pollinosis are currently performed with allergen extracts obtained by simple extraction procedures in aqueous buffers. Many attempts have been made to improve the quality of the extracts, since it has been recognized that extracts may lack important allergens, may contain nonallergenic materials, and may vary greatly in their composition (55,56). Furthermore, it is technically impossible to purify all of the major and minor allergens of a natural allergen source to obtain adequate, pure components for diagnostic testing. The application of molecular biology techniques to the field of allergen characterization has enabled the recombinant production of the most relevant allergens from the common allergen sources (55).

In principle, there are two strategies that can be applied to obtain cDNA coding for allergens (57). The first approach uses IgE antibodies of patients for the isolation of allergen-encoding cDNAs from expression cDNA libraries that have been constructed from the allergen source (Fig. 3). For this approach, mRNA is first isolated from the allergen source and converted into a cDNA by reverse transcription. This cDNA is then inserted into a vector (usually a phage vector) suitable for construction of an expression cDNA library. After infection of appropriate host cells (usually *Escherichia coli* cells), clones expressing allergens can be located with patients' serum IgE using immunoscreening technology. DNA from the positive clones is then isolated, purified, and subjected to sequence analysis. The second approach for the isolation of allergen-encoding cDNAs involves DNA-based screening technologies [e.g., DNA-based screening of libraries, polymerase chain reaction (PCR), or reverse transcription PCR (RT-PCR) strategies]. Once allergen-encoding cDNAs have been obtained using either approach, they can be inserted into expression vectors and recombinant allergens can be produced in large amounts and high purity (Fig. 3).

The first isolated cDNA coding for a tree pollen allergen was for Bet v 1, the major birch pollen allergen (58). The Bet v 1 cDNA was obtained following the first strategy by IgE

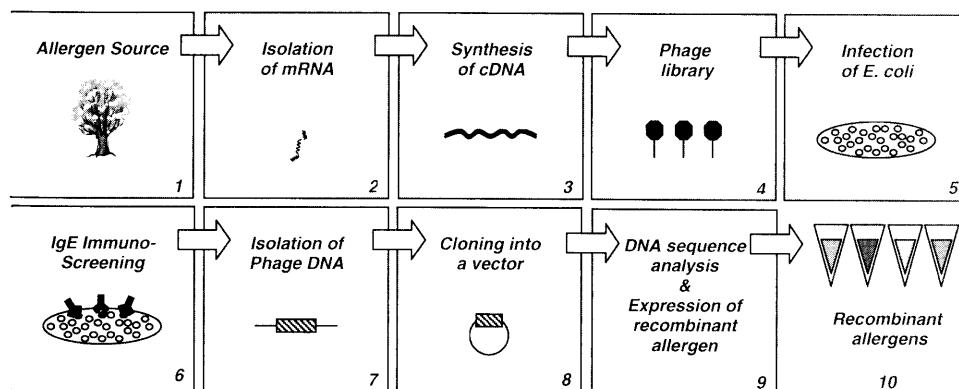


Figure 3 Cloning of tree pollen allergens and production of recombinant allergens. The different steps of the procedure, from mRNA isolation to the production of recombinant allergens, are displayed. The mRNA is isolated from the allergen source (2) and converted into a cDNA (3), which is then ligated into a phage vector (4). Expression of the inserted cDNA is achieved after infection of *Escherichia coli* cells (5) and allergen expressing phage clones can be located with IgE antibodies from allergic patients using immunoscreening technology (6). After the isolation of phage DNA, allergen-encoding cDNAs can be inserted in suitable vector systems (8) and recombinant allergens can be produced in various host organisms (e.g., prokaryotic, eukaryotic organisms).

immunoscreening of an expression cDNA library that had been constructed from mature birch pollen. Using the same approach, a series of tree cDNAs were isolated, including Bet v 2 (profilin), a highly cross-reactive birch pollen allergen and the first known plant actin-binding protein (59); Bet v 3 and Bet v 4, both calcium-binding birch pollen allergens (60–62); and Aln g 4, a calcium-binding pollen allergen from alder pollen (63).

Using the second (the DNA-based) strategy, oligonucleotides constructed according to a previously determined amino acid sequence of an allergen are applied either for PCR cloning or for screening of cDNA libraries. The PCR approach was used to clone the major pollen allergens from alder (Aln g 1), hornbeam (Car b 1), and hazel (Cor a 1) (64–66) and to isolate cDNAs coding for the major olive pollen allergen, Ole e 1; olive pollen profilin, Ole e 2; and the major privet pollen allergen, Lig v 1 (67–69). Further application of this strategy nearly completed the spectrum of olive pollen allergens (70). In contrast to this, Cry j 1 and Cry j 2, the major allergens of Japanese cedar, were obtained by DNA-based screening of cDNA libraries (71,72). On the basis of sequence similarity at the protein and nucleic acid level with the major birch pollen allergen, Bet v 1, an RT-PCR approach was used to isolate cDNAs coding for Mal d 1, the major apple allergen; Api g 1, the major celery allergen; and Pru av 1, the major cherry allergen. These important food allergens were produced as recombinant proteins and their cross-reactivity to Bet v 1 was demonstrated (73–75).

Table 1 gives an overview of tree pollen allergens, their biological functions, and characteristics. The spectrum of tree pollen and tree nut allergens has further been reviewed in several publications and there are several allergen databases that are continuously updated regarding new allergens (70,123–126). The homepage of the World Health Organisation/International Union of Immunological Societies (WHO/IUIS) Allergen Nomenclature Subcommittee (www.allergen.org) summarizes those allergens that have been submitted to the allergen nomenclature subcommittee by researchers for approval and registration. The Structural Database of Allergenic Molecules (SDAP) from the University of Texas Medical Branch offers structural data about allergens. The Allergome database (www.allergome.org) represents a frequently updated and well-kept allergen database that contains published allergen sequences and published studies using allergen molecules. A useful summary of currently available allergen databases can be found in a review article by Mari (126).

The rapid progress in the field of recombinant allergens holds promise that most of the traditional allergen raw extracts will be replaced by recombinant allergens, which cover the complete epitope repertoire of the extracts (32,127,128).

BIOLOGICAL FUNCTIONS AND STRUCTURAL CHARACTERISTICS OF TREE POLLEN ALLERGENS

Application of molecular biology techniques to allergen characterization has permitted the determination of the molecular characteristics of most common environmental allergens during the last two to three decades (127). The DNA and deduced amino acid sequences can be obtained by sequencing of the allergen-encoding cDNAs and thus allow comparisons with sequences deposited in databases. Using this approach, the biological functions of various allergens can be deduced. For example, it was found that the cDNA and the amino acid sequence of the major birch pollen allergen, Bet v 1, showed significant sequence homology with a group of proteins that were found to be upregulated when plants were wounded, infected, or subjected to stressful conditions, and accordingly these proteins were designated pathogenesis-related proteins (PR proteins) (58). Although, to date, there are no definitive experimental data to support that the family of Bet v 1-related allergens contribute to the plant defense system, it is possible that they have protective functions (129). Other functions (e.g., RNase activity, lipid carrier) have been claimed for the Bet v 1 allergen family on the basis of in vitro experiments and structural data (130–132).

Numerous Bet v 1-homologous allergens have been identified in pollen of trees belonging to the order Fagales (e.g., Aln g 1, alder; Cor a 1, hazel; Car b 1, hornbeam; Que a 1, white oak; and Cas s 1, chestnut) (see www.allergen.org). Figure 4 displays the relationship among Bet v 1-related plant allergens on the basis of sequence identities. Almost all of the proteins contain cross-reactive IgE epitopes. However, it is also known that even birch pollen contains proteins with high sequence identity to Bet v 1 but without relevant allergenic

Table 1 Tr

Species

Fagales

Betula verru

Alnus glutin

Corylus ave

Carpinus be

Castanea s

Quercus al

Hamamelid

Platanus ac

Scrophulari

Olea europ

Fraxinus e

Ligustrum

Syringa vu

Plantagin

Plantago la

Pinales

Cryptomer

japonica

Chamaecy

obtusa

Cupressus

arizonica

Cupressus

semperv

Table 1 Tree Pollen Allergens Cloned and/or Characterized to Date

Species	Common name	Allergen	Function and similarity	MW (kDa)	References
<u>Fagales</u>					
<i>Betula verrucosa</i>	Birch	Bet v 1	Pathogenesis-related protein (PR10)	17	58
		Bet v 2	Profilin	15	59
		Bet v 3	Ca ²⁺ -binding protein	23.7	60
		Bet v 4	Ca ²⁺ -binding protein	9.3	61,62
		Bet v 5	Isoflavone reductase	35	76
		Bet v 6	Isoflavone reductase	33.5	77
		Bet v 7	Cyclophilin	18	78,79
		Bet v 8	Pectin esterase	66	80
<i>Alnus glutinosa</i>	Alder	Aln g 1	PR10 ^a ; Bet v 1 related	17	64
		Aln g 4	Ca ²⁺ -binding protein; Bet v 4 related		63
<i>Corylus avellana</i>	Hazel	Cor a 1	PR10; Bet v 1 related		66
		Cor a 2	Profilin; Bet v 2 related	14	b
		Cor a 10	Luminal-binding protein	70	81
<i>Carpinus betulus</i>	Hornbeam	Car b 1	PR10; Bet v 1 related	17	65
<i>Castanea sativa</i>	Chestnut	Cas s 1	PR10; Bet v 1 related	22	82
<i>Quercus alba</i>	White oak	Que a 1	PR10; Bet v 1 related	17	83
<u>Hamamelidales</u>					
<i>Platanus acerifolia</i>	London plane tree	Pla a 1	Invertase inhibitor	18	84,85
		Pla a 2	Polymethylgalacturonase	43	86
		Pla a 3	Lipid transfer protein	10	87
<u>Scrophulariales</u>					
<i>Olea europea</i>	Olive tree	Ole e 1		16	67
		Ole e 2	Profilin, Bet v 2 related	15–18	68
		Ole e 3	Ca ²⁺ -binding protein; Bet v 4 related	9.2	88,89
		Ole e 4		32	90
		Ole e 5	Superoxide dismutase	16	90,91
		Ole e 6		5.8	92,93
		Ole e 7			94
		Ole e 8	Ca ²⁺ -binding protein	21	95
		Ole e 9	β -1,3-Glucanase	46.4	96,97
		Ole e 10	Glycosyl hydrolase	10.8	98
<i>Fraxinus excelsior</i>	Ash	Fra e 1	Ole e 1 related	20	99,100,101
<i>Ligustrum vulgare</i>	Privet	Lig v 1	Ole e 1 related	20	69
<i>Syringa vulgaris</i>	Lilac	Syr v 1	Ole e 1 related	20	102
		Syr v 3	Ca ²⁺ -binding protein; Bet v 4 related	8.9	103
<u>Plantaginales</u>					
<i>Plantago lanceolata</i>	English plantain	Pla l 1		18	104
<u>Pinales</u>					
<i>Cryptomeria japonica</i>	Japanese cedar	Cry j 1	Pectate lyase	41–45	71,105,106
		Cry j 2	Polymethylgalacturonase	46.6	72,107,108
		Cry j 3	PR5; thaumatin-like protein	27.3	109,110
<i>Chamaecyparis obtusa</i>	Japanese cypress	Cha o 1	Pectate lyase; Cry j 1 related	40.2	111
		Cha o 2	Polymethylgalacturonase; Cry j 2 related	46	112
<i>Cupressus arizonica</i>	Arizona cypress	Cup a 1	Pectate lyase; Cry j 1 related	43	113
		Cup a 3	PR5; thaumatin-like protein	21	114
<i>Cupressus sempervirens</i>	Italian cypress	Cup s 1	Pectate lyase; Cry j 1 related	43	AAF72629 ^c
		Cup s 3	PR5; thaumatin-like protein	34	115

(Continued)

Table 1 Tree Pollen Allergens Cloned and/or Characterized to Date (Continued)

Species	Common name	Allergen	Function and similarity	MW (kDa)	References
<i>Juniperus ashei</i>	Mountain cedar	Jun a 1	Pectate lyase; Cry j 1 related	43	116,117,121
		Jun a 2	Polymethylgalacturonase; Cry j 2 related	55.7	118
		Jun a 3	PR5; thaumatin-like protein	30	119
<i>Juniperus oxycedrus</i>	Prickly juniper	Jun o 4	Ca ²⁺ -binding protein; Bet v 4 related	18	120
<i>Juniperus virginiana</i>	Eastern red cedar	Jun v 1	Pectate lyase; Cry j 1 related	43	122
		Jun v 3	PR5; thaumatin-like protein		122

Allergenic molecules are listed according to their taxonomical orders (underlined). Allergen sources (species and common name), designations according to the World Health Organization/International Union of Immunological Societies Allergen Nomenclature Subcommittee (WHO/IUIS), functions and similarities, molecular weights (in kDa), and references or accession numbers are displayed.

^aPR, pathogenesis-related protein.

^bWHO/IUIS (www.allergen.org) database.

^cAllergenOnline (FARRP) (www.allergenonline.com) database.

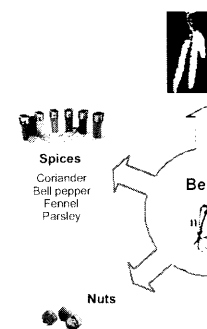
	Asparagus	Carrot	Celery	Tomato	Soybean	Pea	Medicago	Pear	Apricot	Cherry	Apple	Beech	Hornbeam	Alder	Hazel	Birch
Birch	39	38	40	47	49	54	55	58	60	59	66	69	79	81	83	100
Hazel	40	41	40	46	53	53	55	59	63	59	67	69	92	82	100	
Alder	41	39	40	40	50	50	52	55	61	57	64	67	79	100		
Hornbeam	39	42	40	46	51	51	56	56	62	58	66	68	100			
Beech	39	41	42	49	57	54	55	70	74	74	71	100				
Apple	39	40	45	47	50	51	54	70	79	76	100					
Cherry	37	40	43	49	55	51	51	84	76	100						
Apricot	37	44	50	50	53	51	52	73	100							
Pear	35	39	40	43	54	50	50	100								
Medicago	33	36	40	42	66	83	100									
Pea	29	33	39	39	70	100										
Soybean	34	37	40	41	100											
Tomato	43	32	40	100												
Celery	32	51	100													
Carrot	33	100														
Asparagus	100															

Figure 4 Sequence identities (%) between Bet v 1-homologous proteins from different sources. The percentage of sequence identity between Bet v 1-related allergens from various sources is displayed.

activity (133). The existence of these hypoallergenic Bet v 1 isoforms and of nonallergenic proteins with high sequence homology to Bet v 1 (134) demonstrates that sequence homology per se cannot predict with certainty whether a protein is allergenic or not. The latter aspect is also important because it is impossible to predict with certainty the allergenic potential of genetically modified plants exclusively on the basis of sequence homologies of the transgene with genes coding for known allergens (135).

Table 1 gives an overview of tree pollen allergens grouped according to botanical classifications. Each of the different trees contains a spectrum of allergens. However, it appears that certain allergenic molecules occur in different trees as proteins with significant sequence homology and cross-reactive epitopes. In general, it is possible to identify certain groups of

(A)

**Figure 5** (See color version of this figure in the online edition of this journal.)

cross-reactive all... Car b 1, and Ca... These allergens... unrelated trees b... reactivity might

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The birch p... Ole e 3 (88) and... analysis of the... binding motifs

MW (kDa)	References
43	116,117,121
55.7	118
30	119
18	120
43	122
	122

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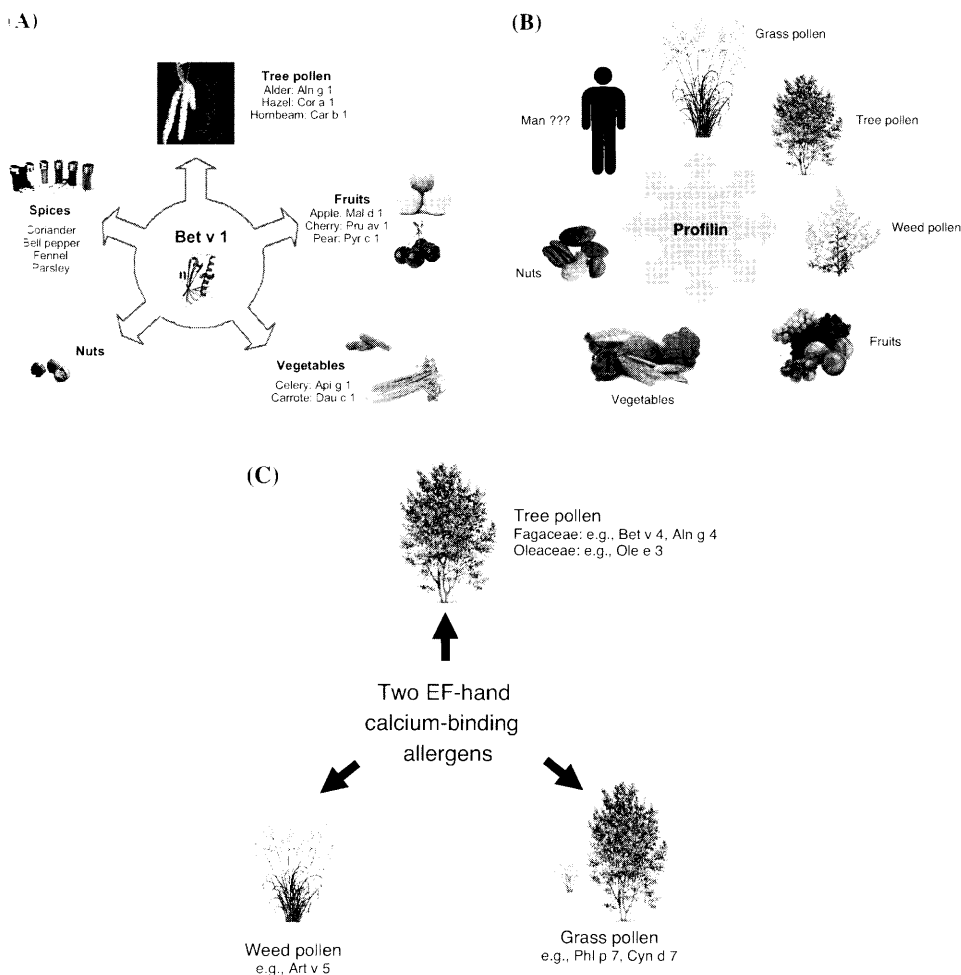


Figure 5 (See color insert.) (A) Bet v 1 cross-reactive allergens can be found in pollen of trees belonging to the order Fagales as well as in fruits, vegetables, nuts, and spices. (B) Profilins, the most cross-reactive allergens described to date, occur in pollen of botanically unrelated plants (trees, grasses, weeds), in plant-derived food (fruits, vegetables, nuts), and even in man. (C) Two EF-hand calcium-binding allergens can be found in pollen from trees, grasses, and weeds.

cross-reactive allergens. For example, there are the Bet v 1-related allergens, Aln g 1, Cor a 1, Car b 1, and Cas s 1, which can be found in pollen of trees belonging to the order Fagales. These allergens are also expressed in nuts of trees of the order Fagales and in fruits of unrelated trees belonging to the order Rosales (within the subclass Rosidae), and due to cross-reactivity might elicit symptoms of food allergy in pollen-allergic patients (Fig. 5A) (136).

A second group of highly cross-reactive allergens are the profilins. These are actin-binding proteins, expressed in all eukaryotic cells, which link signal transduction processes with the reassembling of the cytoskeleton (59,137–141). They are structurally conserved low-molecular-weight (12–15 kDa) proteins and represent probably the most widely distributed and conserved allergens described so far (59). They include proteins from pollen of botanically unrelated plants (trees, grasses, weeds), for instance, birch pollen profilin (Bet v 2) or olive pollen profilin (Ole e 2), proteins of plant-derived foods (fruits, vegetables, nuts), and even human proteins (Table 1 and Fig. 5B).

The birch pollen allergens Bet v 3 and Bet v 4 (60–62) as well as the olive pollen allergens Ole e 3 (88) and Ole e 8 (95) belong to the group of calcium-binding proteins (142). Sequence analysis of the cDNA coding for these allergens revealed the presence of typical calcium-binding motifs (i.e., binding sites for calcium), termed EF-hands (142). Bet v 3, an allergen

highly expressed in mature pollen (60), contains three EF-hands, the olive pollen allergen Ole e 8 and the cypress pollen allergen Jun o 4 contain four EF-hands (95,120), whereas Bet v 4 and Ole e 3 contain two EF-hand calcium-binding motifs (61,62). The calcium-binding allergens with two EF-hands have been found in a variety of pollen from botanically unrelated trees, grasses, and weeds, and represent another family of highly cross-reactive allergens (Fig. 5C) (143). It is important to note that calcium-binding allergens are predominantly expressed in pollen but not in other plant tissues and are therefore responsible only for pollen, but not for food cross-reactivity. IgE inhibition experiments indicate that there is also extensive IgE cross-reactivity between members with different numbers of EF-hands (143). IgE recognition of calcium-binding allergens is enhanced in the presence of calcium, and calcium binding causes a conformational change resulting in higher thermal stability of the allergens (144). The calcium dependence of IgE binding suggests that patients are preferentially sensitized against the calcium-bound allergens (144). The first three-dimensional structure of a two EF-hand allergen, namely of the two EF-hand allergen from timothy grass (Phl p 7), has been resolved by X-ray crystallography. This three-dimensional analysis provides further insight into the structure and conformational changes of these highly cross-reactive allergens and suggests a ligand-binding function (145).

Another group of pollen allergens is represented by the major olive pollen allergen, Ole e 1, which shares high sequence identity and cross-reactive epitopes with allergens from closely related trees of the Oleaceae family, ash (Fra e 1) (99–101), privet (Lig v 1) (69), and lilac (Syr v 1) (102), but lacks cross-reactivity with homologous allergens from other plants. The Ole e 1 pollen allergens are glycosylated proteins and their glycan moieties seem to be involved in the antigenic and allergenic properties of these allergens. However, so far no functional role is assigned to these allergens (70).

In the division of Gymnospermae, two separate groups of pollen allergens have been identified (124): the pectate lyases and the polymethylgalacturonases. The major Japanese cedar pollen allergen Cry j 1, the most thoroughly studied member of the pectate lyases (105,106), displays high sequence homology and IgE cross-reactivity with major allergens of other trees from the order Pinales (e.g., Japanese cypress, Arizona cypress, Italian cypress, mountain cedar). Interestingly, sequence identities of nearly 50% were also found with the major ragweed allergens, the pectate lyases Amb a 1 and Amb a 2 (124,146), but no cross-reactivity with these allergens was described. The second major allergen from Japanese cedar pollen, Cry j 2, has been classified as a polymethylgalacturonase, with cross-reactivity to homologous pollen allergens from other members of the Pinales (107,108). Database searches further revealed significant sequence homologies (~40%) of Cry j 2 with polygalacturonases known as grass pollen allergens (Phl p 13) (147) and with polygalacturonases associated with fruit ripening in tomato (124), but no relevant IgE cross-reactivity seems to exist between Cry j 2 and these enzymes.

Table 1 gives an overview of so far characterized tree pollen allergens and provides information about their sources, biological functions, molecular weights (kDa), and references about their description. Features that all tree pollen allergens have in common are that they represent low-molecular-weight proteins or glycoproteins that rapidly elute from pollen after contact with aqueous solutions (148). The use of immunogold electron microscopy reveals that these allergens are mainly intracellular proteins, which either elute from pollen or, under certain conditions, are expelled from pollen by rupture or abortive pollen germination (25,26). Analysis of the three-dimensional structures of important pollen allergens does not identify structural motifs that are common among all allergens. However, these studies show that cross-reactivity between allergens is based on structural similarities (149).

CROSS-REACTIVITY BETWEEN TREE POLLEN ALLERGENS

During the last decade the most common allergens have been identified by molecular cloning and produced as recombinant allergens (22). In this context, IgE inhibition studies performed with purified recombinant allergens have greatly enhanced our understanding of cross-reactivity at the molecular level (22).

Figure 5A illustrates as an example the cross-reactivity within the group of Bet v 1-related allergens. Allergens containing cross-reactive IgE epitopes have been described in pollen,

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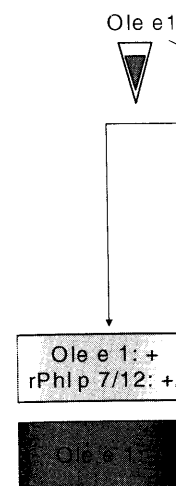


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pollen allergen Ole e 1, whereas Bet v 4 and timothy grass pollen allergens are completely unrelated trees, grass pollen allergens (Fig. 5C) are predominantly expressed in grass pollen, but not for timothy grass. Extensive IgE cross-reactivity of IgE recognition of timothy grass pollen binding causes cross-reactivity with allergens (144). The major timothy grass pollen allergens (e.g., Phl p 1, Phl p 2, Phl p 5 from timothy grass) cross-react with allergens from other grasses, and certain weed allergens (e.g., Pari j 2 from *Parietaria*) cross-react with allergens only present in weeds (143). On the basis of this observation it has been proposed to use such species-specific marker allergens to confirm sensitization to certain allergen sources. These marker allergens can thus be used as diagnostic gatekeepers to confirm suitability of patients for immunotherapy with a given allergen extract (Fig. 6) (35,153). Another argument for using major species-specific marker allergens as an inclusion criterion for immunotherapy is that the currently used allergen extracts are mainly standardized regarding these major allergens.

Major pollen allergen, timothy grass pollen, cross-reacts with allergens from timothy grass (e.g., v 1) (69), and lilac pollen from other plants. The timothy grass pollen seems to be a major allergen. However, so far no

allergens have been identified. The major Japanese cedar pollen allergen, the pectate lyases, are major allergens of Japanese cedar. Italian cypress, also found with the Japanese cedar (146), but no cross-reactivity from Japanese cedar pollen to cypress pollen. Database searches for polygalacturonases associated with pollen exist between Cry j

allergens and provides information (Da), and references are common that they are from pollen after immunoscopy reveals that pollen or, under immunoscopy (25,26). Immunoscopy does not identify pollen. Studies show that

molecular cloning studies performed to understand of cross-

of Bet v 1-related allergens described in pollen,

fruits, vegetables, nuts, and seeds (136). Accordingly, Bet v 1-sensitized patients frequently suffer from an oral allergy syndrome caused by ingestion of food containing cross-reactive allergens. Because of extensive cross-reactivity among the Bet v 1-related allergens, it is not surprising that immunotherapy with birch pollen vaccine alone also improves allergy to pollens of related trees and food allergy (150-152).

It appears that cross-reactivity has in principle two facets that can be applied to diagnosis and therapy. Certain allergens/epitopes are restricted to certain allergen sources and thus can be used as marker molecules to confirm sensitization to these sources (153). For example, Bet v 1 cross-reacts mainly with pollen allergens of trees belonging to the Fagales order. The major olive pollen allergen, Ole e 1, cross-reacts with pollen allergens of trees belonging to the Oleaceae order, including ash (35,154). The major timothy grass pollen allergens (e.g., Phl p 1, Phl p 2, Phl p 5 from timothy grass) cross-react with allergens from other grasses, and certain weed allergens (e.g., Pari j 2 from *Parietaria*) cross-react with allergens only present in weeds (143). On the basis of this observation it has been proposed to use such species-specific marker allergens to confirm sensitization to certain allergen sources. These marker allergens can thus be used as diagnostic gatekeepers to confirm suitability of patients for immunotherapy with a given allergen extract (Fig. 6) (35,153). Another argument for using major species-specific marker allergens as an inclusion criterion for immunotherapy is that the currently used allergen extracts are mainly standardized regarding these major allergens.

However, allergens have been identified that exhibit very broad cross-reactivity and thus indicate polysensitization. These allergens include for example the group of profilins (Fig. 5B) and two EF-hand calcium-binding (Fig. 5C) allergens. Patients who are sensitized to profilin (e.g., Bet v 2 or Phl p 12, the timothy grass pollen profilin) cross-react in most cases with profilins from various unrelated plants and suffer from pollen and plant food polysensitization (Fig. 5B) (153). Patients who are sensitized to calcium-binding allergens (e.g., Bet v 4, Phl p 7, the two EF-hand calcium-binding allergen from timothy grass) suffer in most cases from multiple pollen sensitization to trees, grasses, and weeds (Fig. 5C) (153). Such patients may benefit less from allergen vaccine-based immunotherapy because the currently used therapeutic vaccines are not standardized regarding these molecules, and patients with polysensitization seem to benefit less from allergen-specific immunotherapy (155).

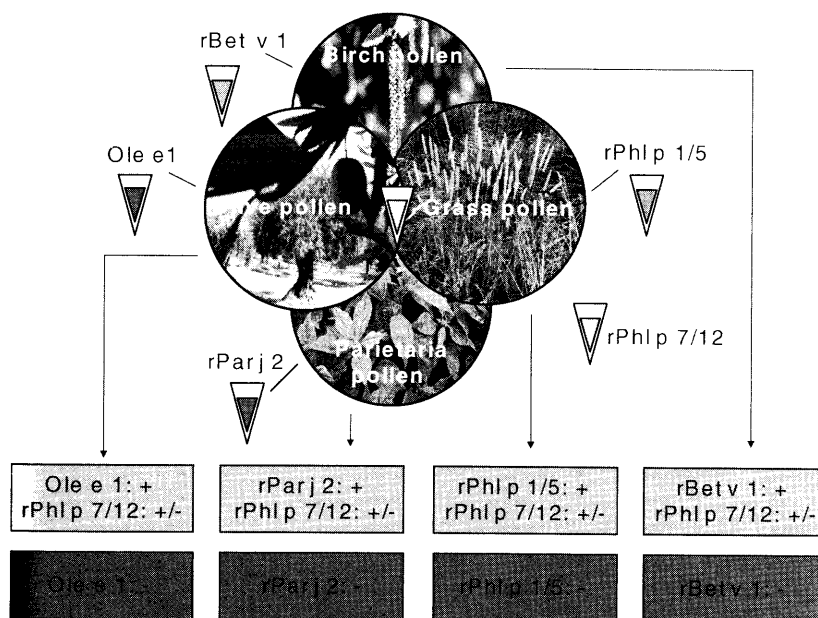


Figure 6 Marker allergens for the diagnosis of pollen allergies. Marker allergens for genuine sensitization to birch pollen, olive and ash pollen, *Parietaria* pollen, and grass pollen are indicated. The marker allergens that occur as cross-reactive allergens in each of these pollen sources are Phl p 7 and Phl p 12. A suggestion for prescription of immunotherapy based on test results obtained with the marker allergens is given at the bottom of the figure. Source: From Ref. 35.

In vitro diagnostic tests equipped with recombinant marker allergens to facilitate the selection of patients for immunotherapy with birch pollen and grass pollen extracts are available from diagnostic companies and can currently be used by clinicians (www.meduniwien.ac.at/allergy-research-christian-doppler; Publications).

TRANSITION FROM ALLERGEN EXTRACT-BASED DIAGNOSIS AND THERAPY TO RECOMBINANT ALLERGEN-BASED DIAGNOSIS AND THERAPY

The rapid progress of allergen characterization through the application of molecular cloning techniques has provided recombinant allergens covering most allergen sources, including trees. Recombinant allergens allow determination of the individual sensitization profiles of allergic patients, a process that has been designated component-resolved diagnosis (CRD) (33). The diagnostic information obtained by CRD is more precise than diagnosis based on extract-based methodology. Extract-based diagnosis will only identify potential allergen sources but does not provide any information regarding the disease-eliciting allergens within the given allergen source. In order to utilize the full spectrum of recombinant allergens for allergy diagnosis, novel forms of multiallergen tests are under development (156). Some of the new tests combine chip and microarray technology whereas others simply utilize nitrocellulose-based test systems for the elucidation of a patient's reactivity profile in a single test (156–158). In addition, recombinant allergens have been incorporated into established quantitative and automated *in vitro* allergy test systems, where they allow a more precise quantitative measurement of specific IgE and IgG.

Using these recombinant allergen-based tests, it has been possible to dissect the sensitization profiles of patients from various populations (41,42,159), to monitor the development of allergies from early childhood to adulthood (160), to investigate the development of IgE profiles during the natural course of allergic disease (161), and to study the effects of allergen-specific immunotherapy (161-164).

Recombinant allergen-based tests and CRD have resulted in several interesting observations regarding the pathogenesis of allergic diseases and the potential mechanisms for allergen-specific immunotherapy. The monitoring of IgE and IgG responses during allergen-specific immunotherapy has reemphasized the importance of specific blocking antibodies for the success of allergen-specific immunotherapy (161,162,164). The finding that allergen vaccines induce a highly heterogeneous immune response against the individual components in the vaccine has underlined the need for improvement of therapeutic allergen preparations (164). Moreover, it appears that injection of allergen vaccines may induce IgE reactivity against new allergens in treated patients (163,165). Although the clinical relevance of these findings has not been confirmed, these data support the idea that patients would benefit from treatment according to their individual sensitization profiles.

The concept of treating allergic patients according to their sensitization profile with purified recombinant allergens, termed component-resolved immunotherapy (CRIT), has therefore been proposed (33). During the last few years, several candidate molecules have been developed by recombinant DNA technology (22,166). These molecules are characterized by strongly reduced allergenic activity, while T-cell epitopes and immunogenicity (i.e., capacity to induce protective IgG responses) are maintained (166–168). The recombinant hypoallergenic allergen derivatives have been evaluated *in vitro* in experimental animal models and *in vivo* provocation testing in patients (22,166). The human work verifies reduced allergenic activity. The first immunotherapy study with recombinant allergen derivatives was performed with hypoallergenic derivatives of the major birch pollen allergen, Bet v 1 (151,152,169–171), and subsequently several other successful immunotherapy studies have been performed with recombinant allergens (24).

SALIENT POINTS

- The most relevant tree pollen allergens are derived from wind-pollinated trees belonging to the order Fagales (e.g., birch), Scrophulariales (e.g., olive), and Pinales (e.g., cedar and cypress).

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- The most common and important tree pollen allergens have been produced as recombinant allergens. Panels of recombinant allergens resembling the epitope complexity of natural allergen extracts are becoming available.
- The molecular characterization of tree pollen allergens reveals that there are families of cross-reactive allergens that are characterized by high sequence homology and immunological cross-reactivity.
- Recombinant allergen-based diagnostic tests are available or will be available in the near future for clinical use to determine the sensitization profiles of patients and to improve the selection of the most accurate treatment forms. This technique has been used successfully for research to establish sensitization profiles of patients, to reveal pathogenesis underlying allergic diseases and to study the effects of allergen-specific immunotherapy.
- Recombinant allergen derivatives with reduced allergenic activity have been developed and evaluated. The first immunotherapy trials are underway with the new molecules to study the mechanisms, efficacy, and safety of CRIT with recombinant allergen molecules.

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