

Transition from a Botanical to a Molecular Classification in Tree Pollen Allergy: Implications for Diagnosis and Therapy

Nadine Mothes^a Friedrich Horak^b Rudolf Valenta^a

Center for Physiology and Pathophysiology, ^aDepartment of Pathophysiology and ^bDepartment of Otorhinolaryngology, Vienna General Hospital, Medical University of Vienna, Vienna, Austria

Key Words

Recombinant allergens · Marker allergens · Cross-reactivity · Diagnosis · Immunotherapy

Abstract

Tree pollens are among the most important allergen sources. Allergic cross-reactivity to pollens of trees from various plant orders has so far been classified according to botanical relationships. In this context, cross-reactivities to pollens of trees of the Fagales order (birch, alder, hazel, hornbeam, oak, chestnut), fruits and vegetables, between pollens of the Scrophulariales (olive, ash, plantain, privet, lilac) and pollens of the Coniferales (cedar, cypress, pine) are well established. The application of molecular biology methods for allergen characterization has revealed the molecular nature of many important tree pollen allergens. We review the spectrum of tree pollen allergens and propose a classification of tree pollen and related allergies based on major allergen molecules instead of botanical relationships among the allergenic sources. This molecular classification suggests the major birch pollen allergen, Bet v 1 as a marker for Fagales pollen and related plant food allergies, the major olive pollen allergen, Ole e 1, as a possible marker for Scrophulariales pollen allergy and the cedar allergens, Cry j 1 and Cry j 2, as potential markers for allergy to Coniferales pollens.

We exemplify for Fagales pollen allergy and Bet v 1 that major marker allergens are diagnostic tools to determine the disease-eliciting allergen source. Information obtained by diagnostic testing with marker allergens will be important for the appropriate selection of patients for allergen-specific forms of therapy.

Copyright © 2004 S. Karger AG, Basel

Introduction

Among the 250,000 well-described, pollen-producing plant species, less than 100 are significant in terms of inducing pollen allergy [1–6]. Pollens from wind-pollinated plants are the most potent allergen sources, whereas pollens from insect-pollinated plants less frequently elicit allergic symptoms. The latter may be due to the fact that allergic sensitization to pollen allergens may occur preferentially via the respiratory mucosa [7, 8] and thus requires the exposure to certain threshold levels of pollen [9, 10]. The presence of allergens in pollen and their levels of expression may vary depending on the plant species, climate, maturation stage and environmental factors [11–14]. Furthermore, there is evidence that allergens are released in varying amounts and speed as well as by different mechanisms from pollens of different plant species which may have impact on sensitization and the elicita-

tion of allergic symptoms. In this context it has been proposed that allergens which are attached to respirable particles with adjuvant properties (e.g., diesel exhaust particles) induce strong Th2 reactivity and thus may favor allergic sensitization [15, 16]. Furthermore, it has been reported that pollen allergens rapidly elute from pollen grains after contact with the respiratory mucosa explaining the fast induction of allergic symptoms [17]. On the other hand it has been demonstrated that tree pollen grains release small respirable allergen-bearing particles after hydration in rain water, which in contrast to intact pollen grains can intrude into the deep airways and thus induce allergic asthma [18, 19].

Tree pollen allergens originate from trees belonging to certain botanical orders which have rather defined geographical distributions and flowering periods [4]. Trees belonging to the order Fagales include birch, alder, hazel, hornbeam, oak and chestnut. They grow in the northern and middle parts of Europe, north-west Africa, east Asia and from North America to the area of the Andes where they represent a major component of the airborne allergen load during spring [4, 20–22]. Olive trees, belonging to the order Scrophulariales are the dominant tree pollen source in the Mediterranean areas, but can also be found in North and South America, South Africa and Australia [23–26]. Ash, belonging to the Scrophulariales has been described as another important pollen allergen source in the USA and middle Europe [25, 27, 28]. Certain other members of the angiosperms (Juglandaceae, walnut; Platanaceae, plane tree; Ulmaceae, elm; Salicaceae, willow; Fabaceae, wattle) are less important as pollen allergen sources [20, 29–31].

Other important tree families capable of inducing pollen allergy, belong to the non-flowering plants (Gymnosperms). The Coniferales order includes several families not all with the same allergological relevance. They comprise the families of the Pinaceae (pine) and Cupressaceae (cypress, cedar and juniper) which grow in the Mediterranean areas, Australia, New Zealand, North America, South America and certain parts of Asia (e.g., China, India) [32–35]. The Pinaceae family seems to be of scarce allergological relevance, and rare reported cases do not correlate with sensitization to other Coniferales genera [36], whereas the Cupressaceae family are of great allergological relevance. The Taxodiaceae (Japanese cedar), often considered as part of the Cupressaceae family, is another allergologically relevant and representative genera of the Coniferales order. Taxodiaceae dominates in Japan and grows to a lesser degree in China, Mediterranean areas and North America [37].

Botanical Relationships among Trees

As has already been noted and is now well established, there is allergic cross-reactivity to pollens of trees belonging to certain botanical orders [25, 38–42]. For this reason, it was found useful to group pollen allergen sources according to botanical aspects, a classification which also correlated well with the degree of allergic cross-reactivity [42, 43]. Figure 1 gives an overview of the botanical classification of plants and important tree pollen allergen sources. The order Fagales which belongs to the Hamamelides is one of the most important allergen sources and its members contain major cross-reactive allergens (table 1).

Birch is the most important tree pollen allergen source in northern Europe. It grows in the cooler parts of the continent and is usually not found in the Mediterranean area. Depending on the area, climate and weather conditions, the flowering season may be variable starting from early March to May [4]. *Alnus* is closely related to birch, however, the allergenicity of alder pollen is generally lower than that of birch. The distribution area of alder is similar to that of birch but alder usually flowers earlier in the year than birch, depending on the weather [4]. The Corylaceae family is closely related to the Betulaceae family. Hazel is a cultivated plant generally grown in mountainous areas of Europe. It frequently extends further south, reaching Mediterranean areas including southern Italy [4]. Hornbeam (*Carpinus betulus*, also called wild hazel) generally grows in hilly/mountainous areas. Chestnut grows in areas influenced by the Atlantic climate such as France, the southern rim of the Alps, and mountainous areas of the Mediterranean. The flowering period of the chestnut tree is generally in June and July.

Of comparable allergenicity are pollens of the other members of the subclass *Hamamelides*, including the Platanaceae family (e.g., London plane tree). The plane tree is present almost everywhere in Europe and allergenic molecules from its pollen have been identified (table 1).

Besides the order Fagales, the order Scrophulariales, which comprises the Oleaceae (fig. 1B), represents an important source of tree pollen allergens [4]. The family of the Oleaceae with its allergens is well documented and dominates as an allergen source predominantly the Mediterranean area. This family consists of the genera *Olea* (olive tree), *Fraxinus* (ash), *Ligustrum* (privet) and *Syringa* (lilac) and flowers in spring [4].

The third important cluster of allergenic trees is represented by the division of the non-flowering plants (Co-

niferophyta-Gymnosperms; fig. 1C). They include the order Coniferales which comprises the plant families of the Pinaceae (pine), the Cupressaceae/Taxodiaceae (cypress, cedar, juniper and Japanese cedar). Allergy to coniferous trees is a well-established pollinosis [44]. In some Mediterranean areas it is as relevant as other major pollinoses, ranking second only to grasses [36] (fig. 1C).

Spectrum of Tree Pollen Allergens according to Botanical Classifications

In tables 1–3 we provide a list and description of allergens in pollens of trees belonging to the order Fagales, Scrophulariales and Pinales. The allergens have been

grouped according to allergen sources and are abbreviated according to the international allergen nomenclature. We further display the molecular weight, information about the determined and/or assumed biological function of the allergens as well as about cross-reactivity with other allergens and allergen sources.

Allergens of the Order Fagales with Emphasis on Birch Pollen

The cDNA coding for the major birch pollen allergen, Bet v 1, was one of the first allergen-encoding cDNAs that could be isolated [45]. Subsequently, recombinant Bet v 1, a 17-kDa protein, was expressed as biologically active

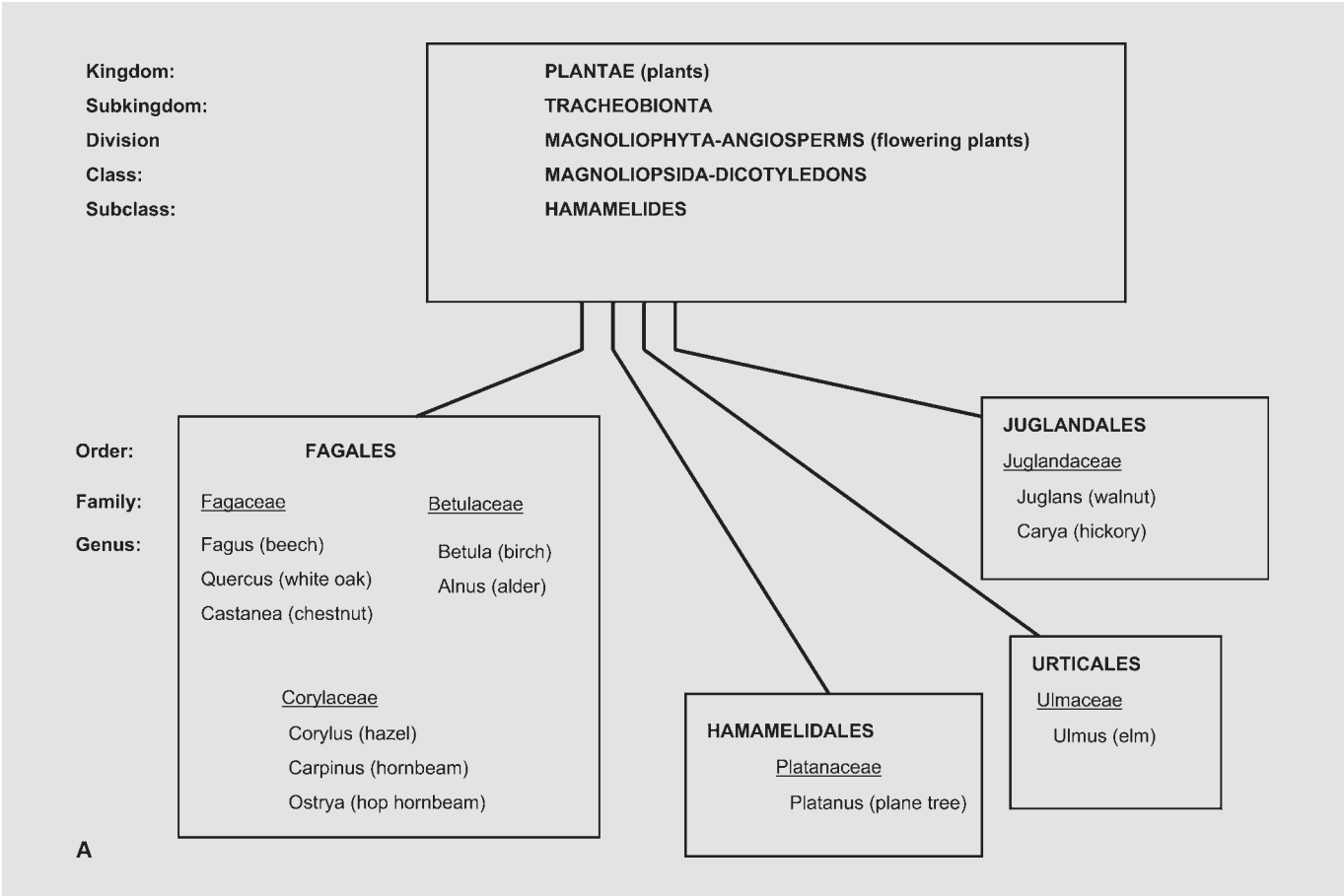


Fig. 1. A–C Taxonomy of the most relevant allergenic trees. Plants are listed following taxonomical guidelines (<http://www.itis.usda.gov>; for further information see also: Watson L. and Dallwitz M.J. (1992 onwards)). The Families of Flowering Plants: Descriptions, Illustrations, Identification, and Information Retrieval. Version: 14th December 2000 (<http://biodiversity.bio.uno.edu/delta/angio/>). Classifications are printed in bold: Kingdom, Subkingdom, Division, Class, Subclass and Order. Family names are underlined.

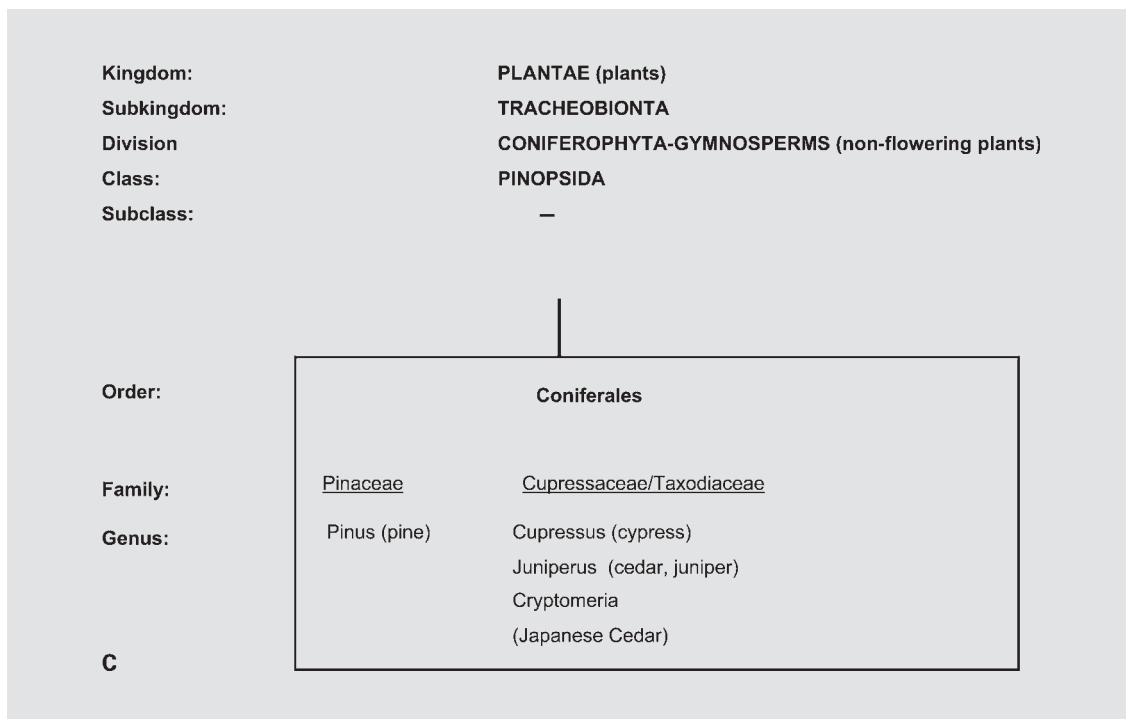
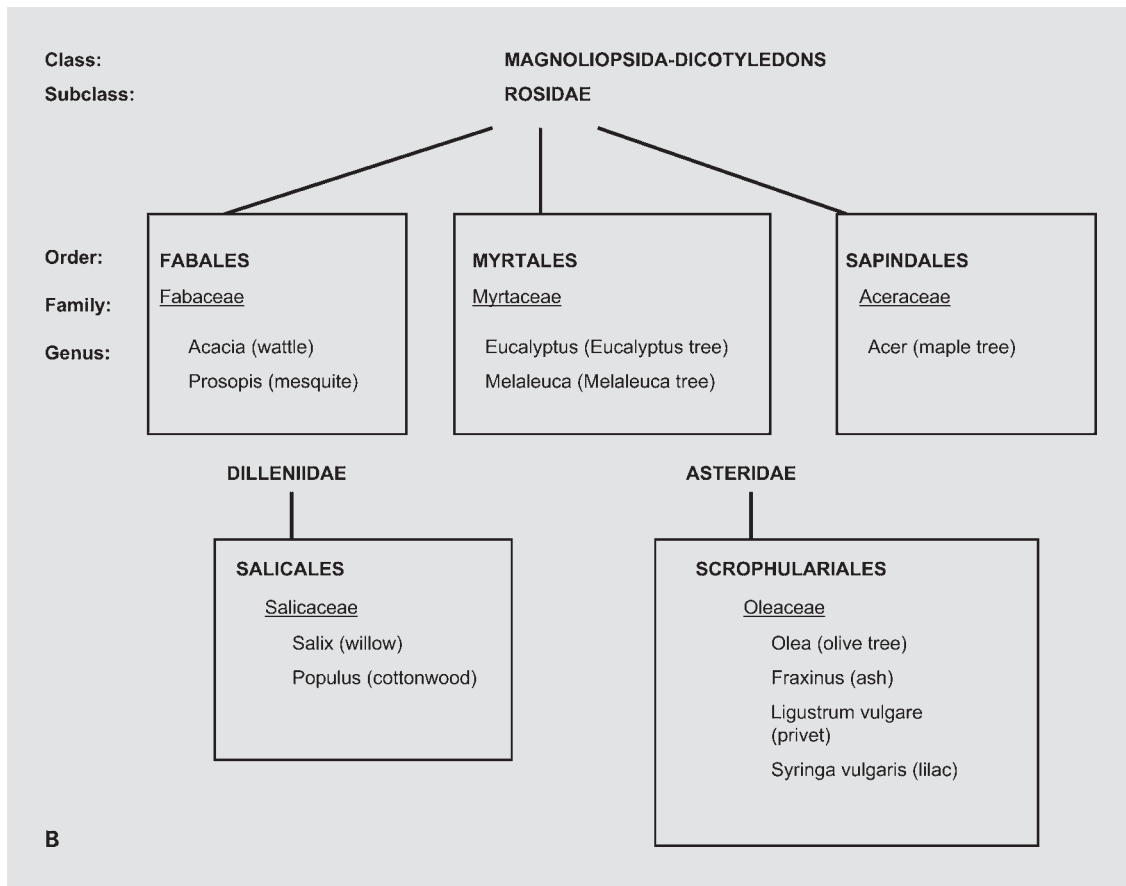


Table 1

Plant species	Allergen	kDa	Allergen biological function	Cross-reactivity		Reference, accession No.
				source	molecule	
<i>Fagales</i>						
Birch (<i>Betula verrucosa</i>)	Bet v 1	17	Pathogenesis-related protein (PRP) RNase Plant steroid carrier Nucleotide-binding protein	Pollen Trees Alder Hazel Hornbeam White oak	Aln g 1 Cor a 1 Car b 1 Que a 1	45 ^a 46, 47
				Food Fruits Apple Cherry Apricot Pear Vegetables Celery Carrot Soybean Nuts Peanut	Mal d 1 Pru av 1 Pru ar 1 Pyr c 1 Api g 1 Dau c 1 Gly m 4 Ara h 8	48–50 51–53 54 AY328088 ^c
	Bet v 2	14	Profilin	Others Spices Pollen Trees Olive tree Weeds Sunflower Food Fruits Cherry Peach Pear Vegetables Celery Tomato Soybean Spices Bell pepper Others Latex		55 56 ^a 57 58 59 60 61 62 61 63
	Bet v 3	23.7	Ca ²⁺ -binding protein	Pollen Trees Alder Prickly juniper Grasses Timothy grass	Hev b 8 Aln g 4 Jun o 4 Phl p 7	64 ^a 65
	Bet v 4	9.3	Ca ²⁺ -binding protein	Pollen Trees Olive tree	Ole e 3 Ole e 8	66, 67 ^a 68 69
	Bet v 6	33.5	Isoflavone reductase			70
	Bet v 7	18	Cyclophilin			71
	Bet v 8	66	Pectin esterase			72

^a Molecules with experimentally determined function.

^b <http://www.allergenonline.com>.

^c International Union of Immunological Societies Allergen Nomenclature Sub-Committee: <http://www.allergen.org/List.htm>.

^d SDAP-Structural Database of Allergenic Proteins: <http://www.allergome.org>.

Table 1 (continued)

Plant species	Allergen	kDa	Allergen biological function	Cross-reactivity		Reference, accession No.
				source	molecule	
Alder (<i>Alnus glutinosa</i>)	Aln g 1	17	PRP		Bet v 1-related allergens	73
	Aln g 4		Ca ²⁺ -binding protein		Bet v 4-related allergens	74 ^a
Hazel (<i>Corylus avellana</i>)	Cor a 1		PRP		Bet v 1-related allergens	75
	Cor a 2	14	Profilin		Bet v 2-related allergens ^c	
	Cor a 8	9	Lipid transfer protein			76
	Cor a 10	70	Luminal-binding protein			AJ295617 ^c
Hornbeam (<i>Carpinus betulus</i>)	Car b 1	17	PRP		Bet v 1-related allergens	77
White oak (<i>Quercus alba</i>)	Que a 1	17	PRP		Bet v 1-related allergens	78
Chestnut (<i>Castanea sativa</i>)	Cas s 1	22	PRP		Bet v 1-related allergens	79
	Cas s 5		Chitinase			CAA64868 ^b
	Cas s 8	9.7	Lipid transfer protein			80
Hamamelidales						
London plane tree (<i>Platanus acerifolia</i>)	Pla a 1	18	Invertase inhibitor			81, 82
	Pla a 2	43	?			P82967 ^c

^a Molecules with experimentally determined function.^b <http://www.allergenonline.com>.^c International Union of Immunological Societies Allergen Nomenclature Sub-Committee: <http://www.allergen.org/List.htm>.^d SDAP-Structural Database of Allergenic Proteins: <http://www.allergome.org>.

allergen in *Escherichia coli* and shown to react with the majority of birch pollen allergic patients by serology, cellular and in vivo tests [110–113]. The cDNA of Bet v 1 was found to hybridize with the RNAs coding for the major allergens of alder (Aln g 1), hazel (Cor a 1), hornbeam (Car b 1) and apple (Mal d 1) by Northern blotting [114, 115]. It was thus possible to isolate the cDNAs coding for Aln g 1, Car b 1, Cor a 1 and Mal d 1 by reverse transcription and polymerase chain reaction (rT-PCR) methods using degenerate primers designed according to the N-terminal amino acid sequences of the allergens [73, 75, 77, 116]. Immunological cross-reactivity of the major allergens of trees belonging to the Fagales, fruits, vegetables and spices has been well documented [39, 40, 42, 46–55] and the corresponding allergens from carrot (Dau c 1) [51], celery (Api g 1) [52], cherry (Pru av 1) [49, 50], pear (Pyr c 1) [117] (International Union of Immunological Societies Allergen Nomenclature Sub-Committee; <http://www.allergen.org/List.htm>; Accession No. AF05730) and apricot (Pru ar 1; International Union of Immunological Societies Allergen Nomenclature Sub-Committee; <http://www.allergen.org/List.htm>; Accession No. U93165) were also obtained by rT-PCR approaches. By immunological cross-reactivity experiments it was demonstrated that the major birch pollen allergen, Bet v 1, contained most of

the epitopes present in the pollen of related trees (alder, hazel, hornbeam) and plant-derived food (fruits, vegetables) [47, 48, 118]. The latter finding indicated that Bet v 1 represented the originally sensitizing agent for the majority of patients suffering from Fagales pollen allergy and birch pollen-related plant food allergy. The three-dimensional structures of Bet v 1 and Bet v 1-related allergens [119, 49] have been determined and it is known that Bet v 1 shows high sequence homology to a group of plant proteins referred to as pathogenesis-related (PR) [120]. However, up to now there are no experimental data proving that Bet v 1 is indeed a PR protein. Several other ideas exist about the possible biological functions of the Bet v 1 allergen family. They include the hypotheses that Bet v 1 is an RNase [121], has lipid carrier functions or acts as a nucleotide-binding protein but neither of these hypotheses has been proven [122, 123].

Immunological cross-reactivity to Bet v 1 and Bet v 1-homologous proteins defines patients suffering from allergy to Fagales pollen and concomitant plant-food allergy (i.e., oral allergy syndrome) [118, 124]. Therefore, IgE reactivity to Bet v 1 can be regarded as a proof of a true and genuine sensitization to Fagales pollen and is a basis for birch pollen-related plant food allergy [118, 124–126]. Several other allergens (Bet v 2–8) have been identified in

Table 2

Plant species	Allergen	kDa	Allergen description	Cross-reactivity		Ref.
				source	molecule	
<i>Scrophulariales</i>						
Olive tree	Ole e 1	16		Pollen Trees		83
				Ash Privet Lilac	Fra e 1 Lig v 1 Syr v 1 } Bet v 2-related allergens Bet v 4-related allergens	27, 28
	Ole e 2	14.4	Profilin			84
	Ole e 3	9.2	Ca ²⁺ -binding protein			85, 86 ^a
	Ole e 4	32				87
	Ole e 5	16	Superoxide dismutase			87
	Ole e 6	5	Reductase			88
	Ole e 7	10				89
	Ole e 8	21	Ca ²⁺ -binding protein		Bet v 4-related allergens	90
	Ole e 9	46.4	Beta-1,3-glucanase			91, 92
	Ole e 10					93
Ash (<i>Fraxinus excelsior</i>)	Fra e 1	20			Ole e 1-related allergens	27, 83
Privet (<i>Ligustrum vulgare</i>)	Lig v 1	20			Ole e 1-related allergens	94
Lilac	Syr v 1	20			Ole e 1-related allergens	95
(<i>Syringa vulgaris</i>)	Syr v 3	9	Ca ²⁺ -binding protein		Ole e 1-related allergens ^d	

^a Molecules with experimentally determined function.

^b <http://www.allergenonline.com>.

^c International Union of Immunological Societies Allergen Nomenclature Sub-Committee: <http://www.allergen.org/List.htm>.

^d SDAP-Structural Database of Allergenic Proteins: <http://www.allergome.org>.

birch pollen but these molecules represent highly cross-reactive allergens which exist in a wide variety of plants (trees, grasses, weeds; table 1). IgE reactivity to the latter allergens may therefore be a result of immunological cross-reactivity to other pollen allergen sources (e.g., grasses, weeds) without underlying genuine sensitization to Fagales pollens [118, 125]. This assumption is supported by the observation that the percentage of Bet v 1-reactive patients is high in populations with high exposure to Fagales pollens, and that these patients show almost exclusive IgE reactivity to Bet v 1 but rarely react with birch pollen allergens other than Bet v 1 (e.g., Bet v 2–8) [126].

Bet v 2, the second well-described birch pollen allergen has been identified as a member of the family of profilins which are ubiquitous eukaryotic 14-kDa proteins with actin-binding properties [56, 127]. Profilins represent the most cross-reactive allergens described to date [128, 129]. Extensive cross-reactivities have been demonstrated for profilins from the pollen of botanically unrelated plants (trees, grasses, weeds), plant food (fruits, vegetables, nuts, spices), latex up to human profilin [56–63, 130, 131]. Tables 1–3 demonstrate that profilins have been identified

in a large variety of plants and show extensive cross-reactivity to Bet v 2. The high degree of cross-reactivity among profilins may be attributed to their highly conserved three-dimensional structures [132].

After Bet v 2, another family of highly cross-reactive allergens, represented by Bet v 3 and Bet v 4 was discovered in birch pollen by IgE immunoscreening of a birch pollen cDNA expression library [64, 66, 67]. Bet v 3 and Bet v 4 were the first calcium-binding pollen allergens identified according to sequence comparisons and calcium-binding assays [133]. Bet v 3, a protein with a calculated molecular weight of 23.7 kDa, contains three typical calcium-binding motifs and was found to be highly expressed in mature pollen [64]. Bet v 4, an acidic protein of 9.3 kDa belongs to a novel family of calcium-binding allergens [66, 67] which contains two calcium-binding domains and shares IgE epitopes with the structurally related two-EF-hand calcium-binding allergens present in grass pollens (Bermuda grass, Cyn d 7; timothy grass, Phl p 7) [134, 135], weed pollens (rape: Bra r 1 and Bra r 2) [136] and tree pollens (alder, Aln g 4; olive, Ole e 3) [74, 86]. All of the described EF-hand plant allergens are pri-

Table 3

Plant species	Allergen	kDa	Allergen description	Cross-reactivity		Ref.
				source	molecule	
<i>Pinales</i>						
Japanese cedar (<i>Cryptomeria japonica</i>)	Cry j 1	40.6	Pectate lyase	Pollen		96, 97
				Trees		
				Japanese cypress	Cha o 1	98
				Arizona cypress	Cup a 1	
				Mountain cedar	Jun a 1	
				Common cypress	Cup s 1	
	Cry j 2	56.6	Polymethyl-galacturonase	Pollen		99, 100
				Trees		
				Japanese cypress	Cha o 2	101
Japanese cypress	Cha o 1	40.2	Pectate lyase			102
(<i>Chamaecyparis obtusa</i>)	Cha o 2	46				103
Cypress	Cup a 1	43	Pectate lyase-like			104
(<i>Cupressus arizonica</i>)	Cup a 3	21	PRP			CAC05258 ^b
Common cypress	Cup s 1	43	Pectate lyase-like			AAF72629 ^b
(<i>Cupressus sempervirens</i>)						
Mountain cedar	Jun a 1	43	Pectate lyase-like			105, 106
(<i>Juniperus ashei</i>)	Jun a 2	55.7				107
	Jun a 3	30	PRP			108
Prickly juniper	Jun o 4	18	Ca ²⁺ -binding protein			107
(<i>Juniperus oxycedrus</i>)						
Eastern red cedar	Jun v 1	43				109
(<i>Juniperus virginiana</i>)						

^a Molecules with experimentally determined function.

^b <http://www.allergenonline.com>.

^c International Union of Immunological Societies Allergen Nomenclature Sub-Committee: <http://www.allergen.org/List.htm>.

^d SDAP-Structural Database of Allergenic Proteins: <http://www.allergome.org>.

marily expressed in mature pollen and exhibit extensive immunological cross-reactivity [137]. IgE reactivity to the EF-hand pollen allergens therefore indicates that a patient may exhibit allergic symptoms to pollens from a wide variety of botanically unrelated plants and thus is a marker for plant pollen polysensitization [125]. Both, profilins and calcium-binding allergens known as panallergens can therefore be considered as markers of multi-pollen sensitization [36].

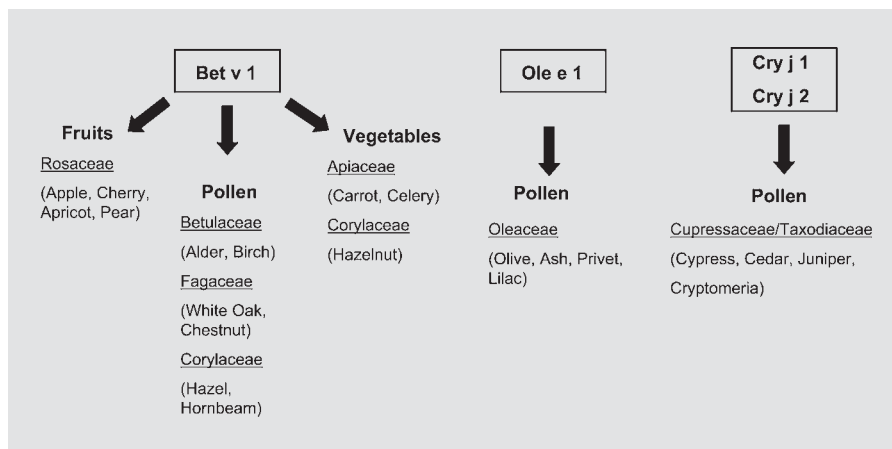
Among the other allergens described in birch pollen, Bet v 6 was found to be an isoflavone reductase [70], Bet v 7 represents a cyclophilin [71] and Bet v 8 is closely related to plant pectinesterases [72]. Pectinesterases are ubiquitously distributed in plants and are mainly known as ripening enzymes that cleave methoxylated pectins [138]. Bet v 8, therefore, may represent a cross-reactive allergen useful as a marker allergen to define a group of patients allergic to pollen, fruits, vegetables and spices [72]. The clinical importance of Bet v 6–8 has not yet been studied in detail.

In conclusion, Bet v 1 and Bet v 1-related allergens can be considered as true markers for a genuine sensitization to Fagales pollens, whereas reactivity to the other described allergens, Bet v 2–8, may also be due to cross-reactivity with other unrelated plants and/or plant products.

Marker Allergens Indicating Sensitization to Scrophulariales and Coniferales

A variety of allergens in pollens of trees belonging to the order Scrophulariales, especially to the Oleaceae family (olive, ash, privet, lilac), and the order Coniferales (cedar, cypress, pine, juniper) have been characterized by molecular biological methods and produced as recombinant proteins (tables 2, 3). Similar to Fagales, it is possible to discriminate major allergens which may serve as markers for genuine sensitizations from highly cross-reactive allergens which also occur in a variety of other unrelated plant-species. In this context, Ole e 1, the major olive pol-

Fig. 2. Definition of cross-reactivity clusters according to major marker allergens. Allergen sources which are associated with reactivity to the major birch pollen allergen, Bet v 1, the major olive pollen allergen, Ole e 1 and the major cedar allergens, Cry j 1 and Cry j 2. Family names have been underlined. Common names of the allergenic species are shown.



len allergen, may represent a marker allergen for sensitization to olive pollen and members of the Oleaceae family [139]. It cross-reacts with the major allergens of ash (Fra e 1), privet (Lig v 1) and lilac (Syr v 1) [27, 28] but shows no or only very limited cross-reactivity with homologous allergens in grasses and maize [140, 141]. Among the other olive pollen allergens several highly cross-reactive molecules such as profilin and calcium-binding allergens have been described [84–87].

In the case of allergy to pollens of trees belonging to the order Coniferales, it seems that the two major allergens of cedar, Cry j 1 and Cry j 2, [96, 97, 99–101] which fulfill the function of a pectate-lyase and polymethylgalacturonase [142, 143], respectively, can serve as marker allergens for sensitization to the Cupressaceae/Taxodiaceae families. However, only a few immunological cross-reactivity studies have been performed to support this hypothesis. Polygalacturonases have also been identified in grass pollens (i.e., group 13 allergens) but it seems that there is no relevant IgE cross-reactivity between polygalacturonases from grasses, trees and weeds (Phl p 13) [144]. So far one calcium-binding pollen allergen, Jun o 4, has been characterized in trees belonging to the order Coniferales (table 3).

Classification of Allergic Sensitization According to Reactivity with Major Marker Tree Pollen Allergens

Clusters of cross-reactive allergen sources have so far been classified according to botanical and clinical aspects [42]. These principles have also been applied to define

birch pollen-related and olive pollen-related allergies as well as allergies to Coniferales. We suggest redefining these cross-reactivity clusters by using a molecular classification based on IgE reactivity to the major allergens of birch pollen, Bet v 1, olive pollen, Ole e 1, and the Cupressaceae/Taxodiaceae allergens, Cry j 1 and Cry j 2 (fig. 2). In this classification, IgE reactivity to Bet v 1 indicates genuine sensitization to birch pollen and to allergen sources containing Bet v 1-related allergens, such as pollens of trees of the order Fagales (alder, hazel, hornbeam, oak), to certain fruits, nuts and vegetables (fig. 2). IgE reactivity to Ole e 1, may be considered as a marker for olive pollen and Scrophulariales sensitization due to cross-reactivity to ash, privet, lilac and possibly plantain (fig. 2). Cry j 1 and Cry j 2 are a pectate lyase and a polygalacturonase, respectively, from cedar with cross-reactivity to homologous proteins in pollens of other members of the Coniferales, and hence may represent marker allergens for sensitization to Coniferales pollen (fig. 2).

Besides these three main groups the order of Hamamelidales, with its representative London plane tree occurring almost everywhere in Europe, should be mentioned. The plane tree bears allergens (Pla a 1 and Pla a 2) which do not seem to match any known allergen and thus could be markers of Platanus sensitization possibly identifying a botanical family-reactive subset of patients.

It should be emphasized that the molecular definition of cross-reactivity clusters cannot rely only on sequence homologies but requires experimental studies documenting extensive cross-reactivity because it has been found that there is little or no cross-reactivity among certain allergens, although sequence homology has been signifi-

cant. For example, Ole e 1 did not show relevant cross-reactivity with Phl p 11, the homologous protein from timothy grass pollen [140].

Major, Source-Specific Marker Allergens to Determine the Disease-Eliciting Allergen Sources: Diagnostic Tests for the Selection of Patients for Allergen-Specific Immunotherapy

Allergen-specific immunotherapy is one of the few causative forms of allergy treatment. It is based on the repeated administration of the disease-eliciting allergens to patients. At present, immunotherapy is most frequently and successfully performed by subcutaneous injection of allergens which in most cases are bound to adjuvants [145, 146]. Other routes of administration such as sublingual, oral or nasal application have been evaluated in clinical trials but clinical and immunological efficacy is still a matter of debate.

The analysis of immunological effects of immunotherapy with recombinant allergens indicates that injection immunotherapy has vaccination character although several other mechanisms have been proposed [146–148].

At present, patients are selected for allergen-specific immunotherapy on the basis of clinical criteria and provocation testing with allergen extracts. Serological testing for specific IgE antibodies using allergen extracts can be used to confirm the diagnosis of an IgE-mediated allergy. However, using allergen extracts for diagnostic testing, it is impossible to discriminate between co-sensitization and cross-sensitization to different allergen sources [125]. The accurate prescription of allergen-specific immunotherapy would require that the disease-eliciting allergen source is identified so that the adequate allergens can be selected for treatment. For this purpose it has been suggested that major allergens, specific for a given allergen source, be used as diagnostic markers to confirm genuine sensitization to a given allergen source [125]. Such marker allergen tests can be used for the improved selection of patients for specific immunotherapy in addition to the well-established clinical criteria. A major feature of a marker allergen to be used as an inclusion criterion for specific immunotherapy with a certain allergen extract is its rather specific presence and abundance in the given allergen source. In this context, the major allergen of *Parietaria*, rPar j 2, has been shown to represent a marker for a genuine sensitization to *Parietaria* pollen. Par j 2 is a major allergenic component of *Parietaria* pollen but absent from other weeds (e.g., ragweed, mugwort) [149].

Likewise, the major house dust mite allergens, Der p 1 and Der p 2, may be used as marker allergens to confirm genuine sensitization to house dust mites [150]. Furthermore, the major timothy grass pollen allergens, Phl p 1 and Phl p 5, may be used as markers for genuine grass pollen sensitization and the major birch pollen allergen, Bet v 1, to confirm original sensitization to birch and *Fagales* pollen [125].

In fact, Ole e 1 and the Coniferales allergens Cry j 1 and Cry j 2 may also be useful as marker allergens to confirm original sensitization to Oleaceae family and Coniferales order, respectively (fig. 2). Diagnostic testing with such marker allergens confirms that patients are genuinely sensitized against a certain allergen source and helps to discriminate the patients with genuine sensitization to a given allergen source from those who appear wrongly positive in extract-based tests because of reactivity with highly cross-reactive allergens that also occur in other unrelated allergen sources.

The clinically important differentiation between co- and cross-sensitivity [40], later also termed co- and cross-recognition [151], can thus only be achieved by the use of purified recombinant allergens.

rBet v 1 as a Diagnostic Marker Allergen to Identify Genuinely Birch Pollen-Sensitized Patients

Bet v 1 is the major allergen in birch pollen and is also abundant in birch pollen extracts [152]. Nevertheless, patients who exhibit allergic reactions to birch pollen without IgE reactivity to Bet v 1 can be identified [125, 153]. These patients appear positive in a birch pollen extract-based diagnostic test although they were not originally sensitized against birch pollen [153]. For example, it has been found that even patients from Central Africa who were never exposed to birch pollen exhibited positive skin- and IgE reactivity to birch pollen due to profilin sensitization [153]. Birch pollen-reactive patients without Bet v 1-specific IgE are rather rare in northern parts of Europe, but their number increases towards the southern parts where other pollen allergen sources predominate, which again supports the assumption that such patients were genuinely sensitized against allergen sources other than birch pollen [125]. The latter assumption is confirmed by following experiments.

We found that natural birch pollen exposure primarily increased the IgE responses to the major birch pollen allergen, Bet v 1, at 17 kDa whereas IgE reactivity to pro-

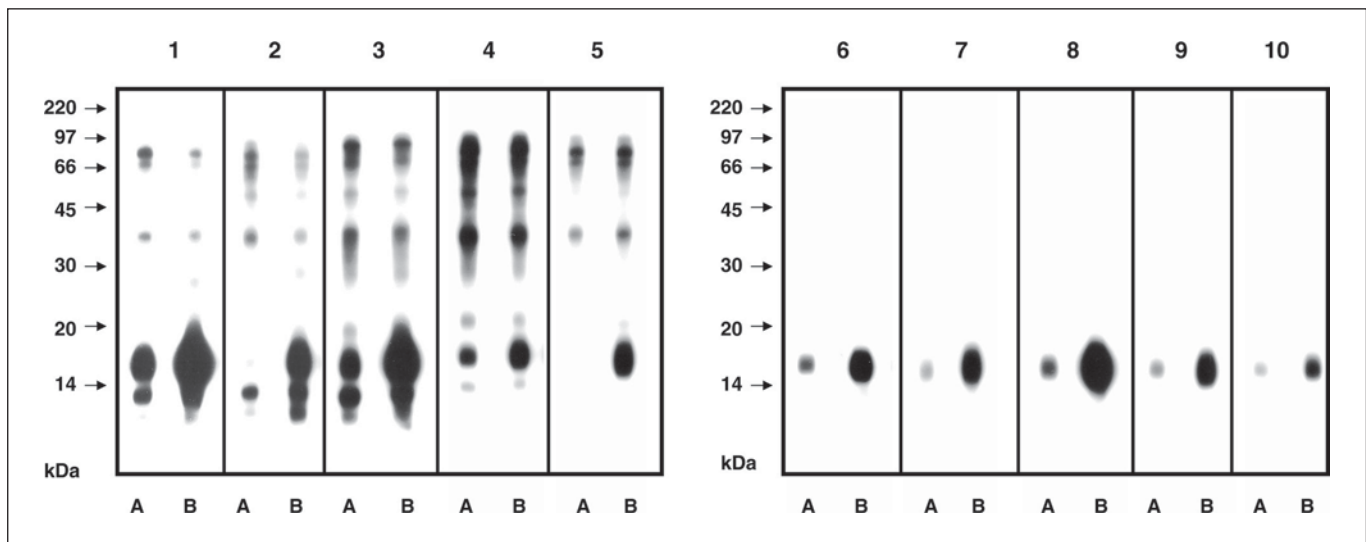


Fig. 3. Seasonal birch pollen exposure boosts IgE reactivity to the major birch pollen allergen, Bet v 1. Serum samples collected from birch pollen allergic patients (1–10) before (lanes A) and after (lanes B) birch pollen season were tested for IgE reactivity to birch pollen extract. Bound IgE antibodies were visualized by autoradiography. Molecular weights are indicated in kilodaltons (kDa) on the left margin. Seasonal birch pollen exposure strongly boosts IgE reactivity to Bet v 1 (17 kDa) but has little or no effect on IgE reactivity to other allergens in birch pollen.

filin (Bet v 2, 14 kDa) and higher molecular weight allergens (30–90 kDa) was not boosted by seasonal birch pollen exposure. Figure 3 shows the changes in the IgE responses to Bet v 1 and other birch pollen allergens in the sera of a group of birch pollen-allergic patients. Patients carrying IgE antibodies to Bet v 1 and other allergens in birch pollen (fig. 3, panels 1–5) as well as patients with exclusive IgE reactivity to Bet v 1 were studied (fig. 3, panels 6–10). In both groups seasonal exposure to birch pollen primarily increased IgE responses to Bet v 1 (fig. 3, lanes A versus B).

In order to identify the allergen source responsible for the sensitization to profilin and the high molecular weight allergens we performed IgE inhibition experiments (fig. 4). We found that IgE reactivity to the high molecular weight allergens (fig. 4, 30–90 kDa) and profilin (14 kDa) was completely inhibited by timothy grass pollen extract. This finding, together with the fact that natural birch pollen exposure did not boost IgE reactivity to profilin and the high molecular weight allergens, supports the assumption that in most cases IgE reactivity to profilin and the high molecular weight allergens in the patients analyzed was due to sensitization and cross-reactivity with grass pollen allergens but not to birch pollen.

Several other arguments support the idea that Bet v 1 can be considered as a true marker allergen for birch pol-

len and related Fagales and birch pollen-related plant food allergy. First, Bet v 1 is the major allergen in birch recognized by more than 95% of birch pollen-allergic patients [152]. Second, rBet v 1 was found to inhibit the majority of IgE reactivity to pollen allergens from trees of the order Fagales (alder, hazel, hornbeam) and plant-derived food in patients with birch pollen-related oral allergy syndrome [47, 118]. Third, it has been shown that immunotherapy with birch pollen extract was effective in treating allergies not only to birch, but also to pollens of trees of the Fagales order and to birch-related plant foods [154–157]. Fourth, it has been demonstrated that patients who reacted exclusively with Bet v 1 had a better clinical outcome when treated with birch pollen extract than patients reacting also with other allergens [158]. Figure 5 from the latter study shows the IgE reactivity profile of patients who showed clinical improvement after birch pollen immunotherapy versus patients who did not improve. Patients without clinical improvement frequently reacted with other allergens in addition to Bet v 1.

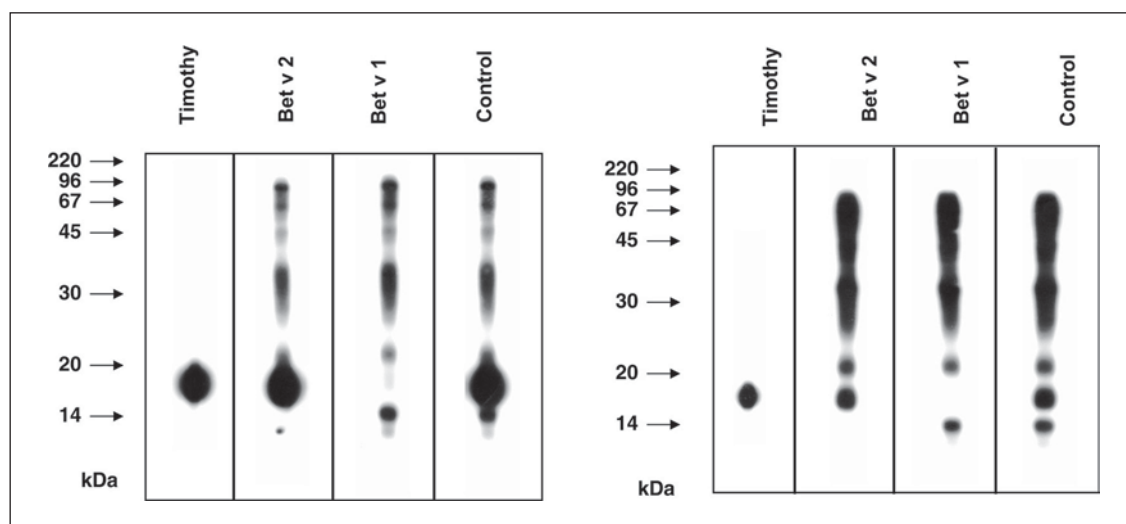
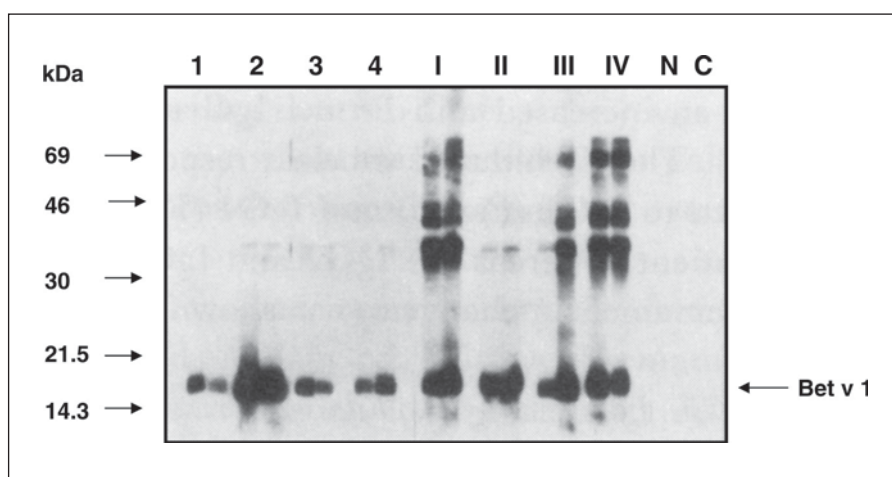


Fig. 4. Timothy grass pollen allergens inhibit IgE reactivity to birch pollen allergens other than Bet v 1. Sera from 2 birch pollen allergic patients with IgE reactivity to multiple allergens (fig. 3, sera 3 and 4) were pre-incubated with rBet v 1, rBet v 2, timothy grass pollen extract and BSA (negative control) before exposure to nitrocellulose-blotted birch pollen extract. Pre-incubation with timothy grass pollen allergens inhibits IgE reactivity to all birch pollen allergens, except to the major birch pollen allergen, Bet v 1 at 17 kDa. Molecular weights are displayed in kilodaltons (kDa) on the left margin.

Fig. 5. Sera from patients with good or no clinical response to birch pollen immunotherapy exhibit different IgE reactivity profiles. Sera were collected from birch pollen allergic patients with good (patients 1–4) or no (patients I–IV) clinical response to birch pollen-specific immunotherapy before (left) and after (right lanes) treatment. These sera, serum from a non-allergic patient (lane N) or buffer alone (lane C) were exposed to nitrocellulose-blotted birch pollen extract. Bound IgE antibodies were visualized by autoradiography and molecular weights are indicated on the left margin. The position of the major birch pollen allergen, Bet v 1 has been marked. Reprinted with permission from Birkner et al. [158].



Application of the Marker Allergen Concept for Improvement of Tree Pollen Allergen-Specific Immunotherapy

Review of the tree pollen allergens characterized so far indicates that it is possible to identify marker allergens which can be used for diagnostic testing to confirm genuine sensitization against certain allergen sources. rBet v 1 may be considered as a marker for birch pollen and re-

lated Fagales pollen as well as plant food allergy. While not yet studied, Ole e 1 may serve as a marker for Scrophulariales pollen allergy and Cry j 1 and Cry j 2 as potential markers for Coniferales allergies. Diagnostic testing with these marker allergens should enable definition of the disease-eliciting allergen sources and thus provides an additional criterion for the prescription of allergen-specific immunotherapy. Diagnostic testing with marker allergens will likely contribute to increased success of al-

lergen-specific immunotherapy and thus broaden its applications. Ultimately it will pave the road for future forms of recombinant allergen-based forms of immunotherapy which are already in clinical evaluation and may replace allergen extract-based immunotherapy in the near future [159].

Acknowledgements

This study was supported by grant Y078GEN of the Austrian Science Fund, by the CeMM project of the Austrian Academy of Sciences and by a research grant from Pharmacia Diagnostics, Uppsala, Sweden.

References

- Gregory PH: The Microbiology of the Atmosphere. Aylesbury, Hill, 1973.
- Accorsi CA, Bandini-Mazzanti M, Romano B, et al: Allergenic pollen: Morphology and microscopic photographs; in D'Amato G, Spiekma FThM, Bonini S (eds): Allergenic Pollen and Pollenosis in Europe. Oxford, Blackwell, 1991, pp 24–44.
- D'Amato G, Spiekma FThM, Liccardi G, et al: Pollen-related allergy in Europe. Allergy 1998;53:567–578.
- D'Amato G, Bonini S (eds): Pollenosis 2000, Global Approach. Naples, JGC Editions, 2001.
- Lewis WH, Vinay P, Zenger VE: Airborne and Allergenic Pollen of North America. Baltimore, Johns Hopkins University Press, 1983.
- Charpin J, Surinych R: Atlas of European Allergenic Pollens. Paris, Sandoz, Editions, 1974.
- Durham SR, Gould HJ, Hamid QA: Local IgE production in nasal allergy. Int Arch Allergy Immunol 1997;113:128–130.
- Niederberger V, Niggemann B, Kraft D, Spitzauer S, Valenta R: Evolution of IgM, IgE and IgG(1–4) antibody responses in early childhood monitored with recombinant allergen components: Implications for class switch mechanisms. Eur J Immunol 2002;32:576–584.
- Florido JF, Delgado PG, de San Pedro BS, Quiralte J, de Saavedra JM, Peralta V, Valenzuela LR: High levels of *Olea europaea* pollen and relation with clinical findings. Int Arch Allergy Immunol 1999;119:133–137.
- Horak F, Jäger S: Die Erreger des Heufiebers. Medizinisch-botanische Dokumentation der Pollenallergie in Mitteleuropa. München, Urban & Schwarzenberg, 1979.
- Mittermann I, Swoboda I, Pierson E, Eller N, Kraft D, Valenta R, Heberle-Bors E: Molecular cloning and characterization of profilin from tobacco (*Nicotina tabacum*): Increased profilin expression during pollen maturation. Plant Mol Biol 1995;27:137–146.
- Ahlholm JU, Helander ML, Savolainen J: Genetic and environmental factors affecting the allergenicity of birch (*Betula pubescens* ssp. *Czerepanovii* [Orl.] *Hamet-ahiti*) pollen. Clin Exp Allergy 1998;28:1384–1388.
- Kondo Y, Ipsen H, Lowenstein H, Karpas A, Hsieh LS: Comparison of concentrations of Cry j 1 and Cry j 2 in diploid and triploid Japanese cedar (*Cryptomeria japonica*) pollen extracts. Allergy 1997;52:455–459.
- Helander ML, Savolainen J, Ahlholm J: Effects of air pollution and other environmental factors on birch pollen allergens. Allergy 1997;52:1207–1214.
- Diaz-Sanchez D, Tsien A, Fleming J, Saxon A: Combined diesel exhaust particulate and ragweed allergen challenge markedly enhances human in vivo nasal ragweed-specific IgE and skews cytokine production to a T helper cell 2 type pattern. J Immunol 1997;158:2406–2413.
- Suphioglu C, Singh MB, Taylor P, Bellomo R, Holmes P, Puy R, Knox RB: Mechanisms of grass-pollen-induced asthma. Lancet 1992;339:569–572.
- Vrtala S, Grote M, Duchene M, van Ree R, Kraft D, Scheiner O, Valenta R: Properties of tree and grass pollen allergens: Reinvestigation of the linkage between solubility and allergenicity. Int Arch Allergy Immunol 1993;102:160–169.
- Schappi GF, Monn C, Wüthrich B, Wanner HU: Direct determination in ambient aerosols: Methodological aspects. Int Arch Allergy Immunol 1996;110:364–370.
- Grote M, Valenta R, Reichelt R: Abortive pollen germination: A mechanism of allergen release in birch, alder, and hazel revealed by immunogold electron microscopy. J Allergy Clin Immunol 2003;111:1017–1023.
- Dvorin DJ, Lee JJ, Belecanech GA, Goldstein MF, Dunskey EH: A comparative, volumetric survey of airborne pollen in Philadelphia, Pennsylvania (1991–1997) and Cherry Hill, New Jersey (1995–1997). Ann Allergy Asthma Immunol 2001;87:394–404.
- Kosisky SE, Carpenter GB: Predominant tree aeroallergens of the Washington, DC area: A six year survey (1989–1994). Ann Allergy Asthma Immunol 1997;78:381–392.
- Wüthrich B, Schindler C, Leuenberger P, Ackermann-Lieblich U: Prevalence of atopy and pollinosis in the adult population of Switzerland (SAPALDIA study). Swiss Study on Air Pollution and Lung Diseases in Adults. Int Arch Allergy Immunol 1995;106:149–156.
- Bousquet J, Cour P, Guerin B, Michel FB: Allergy in the Mediterranean area. I. Pollen counts and pollinosis of Montpellier. Clin Allergy 1984;14:249–258.
- D'Amato G, Mullins J, Noland N, Spiekma FT, Wächter R: City spore concentrations in the European Economic Community (EEC). VII. Oleaceae (*Fraxinus*, *Ligustrum*, *Olea*). Clin Allergy 1988;18:541–547.
- Kerneman SM, McCullough J, Green J, Ownby DR: Evidence of cross-reactivity between olive, ash, privet, and Russian olive tree pollen allergens. Ann Allergy 1992;69:493–496.
- Liccardi G, D'Amato M, D'Amato G: *Oleaceae* pollinosis: A review. Int Arch Allergy Immunol 1996;111:210–217.
- Niederberger V, Purohit A, Oster JP, Spitzauer S, Valenta R, Pauli G: The allergen profile of ash (*Fraxinus excelsior*) pollen: Cross-reactivity with allergens from various plant species. Clin Exp Allergy 2002;32:933–941.
- Hemmer W, Focke M, Wantke F, Götz M, Jarisch R, Jäger S: Ash (*Fraxinus excelsior*) pollen allergy in Central Europe: Specific role of pollen pan allergens and the major allergen of ash pollen, Fra e 1. Allergy 2000;55:923–930.
- Lin RY, Clauss AE, Bennett ES: Hypersensitivity to common tree pollens in New York City patients. Allergy Asthma Proc 2002;23:253–258.
- Weber RW: American elm (*Ulmus americana*) is a native tree that has had a wide range from the entire Eastern States through the Central Plains. Ann Allergy Asthma Immunol 2001;86:A7.
- Decco ML, Wendland BI, O'Connell EJ: Volumetric assessment of airborne pollen and spore levels in Rochester, Minnesota, 1992 through 1995. Mayo Clin Proc 1998;73:225–229.
- Panzani R, Zerbini R, Ariano R: Allergenic significance of *Cupressaceae* pollen in some parts of the Mediterranean area; in D'Amato G, Spiekma FThM, Bonini S (eds): Allergenic Pollen and Pollenosis in Europe. Oxford, Blackwell, 1991, pp 81–84.
- Freeman GL: Pine pollen allergy in northern Arizona. Ann Allergy 1993;70:491–494.
- De Luca A, Graziani E, Anticoli S, Simeoni S, Terzano C, Mannino F: Respiratory allergy to *Cupressus sempervirens* in Rome. Allergol Immunopathol (Madr) 1997;25:229–232.
- Rawat A, Singh A, Singh AB, Gaur SN, Kumar L, Roy I, Ravindran P: Clinical and immunologic evaluation of *Cedrus deodara* pollen: A new allergen from India. Allergy 2000;55:620–626.
- Mari A: Multiple pollen sensitization: A molecular approach to the diagnosis. Int Arch Allergy Immunol 2001;125:57–65.
- Ito H, Nishimura J, Suzuki M, Mamiya S, Sato K, Takagi I, Baba S: Specific IgE to Japanese cypress (*Chamaecyparis obtusa*) in patients with nasal allergy. Ann Allergy Asthma Immunol 1995;74:299–303.

- 38 van Dalen G, Voorhorst R: Allergen community in pollen from certain tree species. *Ann Allergy* 1981;46:276–278.
- 39 Eriksson NE, Wihl JA, Arrendal H, Strandhede SO: Tree pollen allergy. III. Cross reactions based on results from skin prick tests and the RAST in hay fever patients. A multi-centre study. *Allergy* 1987;42:205–214.
- 40 Pauli G: Evolution in the understanding of cross-reactivities of respiratory allergens: The role of recombinant allergens. *Int Arch Allergy Immunol* 2000;123:183–195.
- 41 Schwietz LA, Goetz DW, Whisman BA, Reid MJ: Cross-reactivity among conifer pollens. *Ann Allergy Asthma Immunol* 2000;84:87–93.
- 42 Weber RW: Patterns of pollen cross-allergenicity. *J Allergy Clin Immunol* 2003;112:229–239.
- 43 Kosman E, Eshel A, Waisel Y: The ‘Travelling Salesman Problem’: A new approach for identification of differences among pollen allergens. *Int Arch Allergy Immunol* 1997;112:371–377.
- 44 Mari A, Di Felice G, Afferni C, Barletta B, Tinghino R, Sallusto F, Pini C: Assessment of skin prick test and serum specific IgE detection in the diagnosis of Cupressaceae pollinosis. *J Allergy Clin Immunol* 1996;98:21–31.
- 45 Breiteneder H, Pettenburger K, Bito A, Valenta R, Kraft D, Rumpold H, Scheiner O, Breitenbach M: The gene coding for the major birch pollen allergen, Bet v 1, is highly homologous to a pea disease resistance response gene. *EMBO J* 1989;8:1935–1938.
- 46 Rohac M, Birkner T, Reimtzter I, Bohle B, Steiner R, Breitenbach M, Kraft D, Scheiner O, Gabl F, Rumpold H: The immunological relationship of epitopes on major tree pollen allergens. *Mol Immunol* 1991;28:897–906.
- 47 Niederberger V, Pauli G, Gronlund H, Froschl R, Rumpold H, Kraft D, Valenta R, Spitzauer S: Recombinant birch pollen allergens (rBet v 1 and rBet v 2) contain most of the IgE epitopes present in birch, alder, hornbeam, hazel and oak pollen: A quantitative IgE inhibition study with sera from different populations. *J Allergy Clin Immunol* 1998;102:579–591.
- 48 Ebner C, Hirschwehr R, Bauer L, Breiteneder H, Valenta R, Ebner H, Kraft D, Scheiner O: Identification of allergens in fruits and vegetables: IgE cross-reactivities with the important birch pollen allergens Bet v 1 and Bet v 2 (birch profilin). *J Allergy Clin Immunol* 1995;95:962–969.
- 49 Neudecker P, Schweimer K, Nerkamp J, Scheurer S, Vieths S, Sticht H, Rosch P: Allergic cross-reactivity made visible: Solution structure of the major cherry allergen Pru av 1. *J Biol Chem* 2001;276:22756–22763.
- 50 Scheurer S, Son DY, Boehm M, Karamloo F, Franke S, Hoffmann A, Haustein D, Vieths S: Cross-reactivity and epitope analysis of Pru av 1, the major cherry allergen. *Mol Immunol* 1999;36:155–167.
- 51 Hoffmann-Sommergruber K, O’Riordain G, Ahorn H, Ebner C, Laimer Da Camara Machado M, Pühringer H, Scheiner O, Breiteneder H: Molecular characterization of Dau c 1, the Bet v 1 homologous protein from carrot and its cross-reactivity with Bet v 1 and Api g 1. *Clin Exp Allergy* 1999;29:840–847.
- 52 Breiteneder H, Hoffmann-Sommergruber K, O’Riordain G, Susani M, Ahorn H, Ebner C, Kraft D, Scheiner O: Molecular characterization of Api g 1, the major allergen of celery (*Apium graveolens*), and its immunological and structural relationships to a group of 17-kDa tree pollen allergens. *Eur J Biochem* 1995;233:484–489.
- 53 Neudecker P, Lehmann K, Nerkamp J, Haase T, Wangorsch A, Fotisch K, Hoffmann S, Rosch P, Vieths S, Scheurer S: Mutational epitope analysis of Pru av 1 and Api g 1, the major allergens of cherry (*Prunus avium*) and celery (*Apium graveolens*): Correlating IgE reactivity with three-dimensional structure. *Biochem J* 2003;376:97–107.
- 54 Mittag D, Vieths S, Vogel L, Becker WM, Rihs HP, Helbling A, Wüthrich B, Ballmer-Weber BK: Soybean allergy in patients allergic to birch pollen: Clinical investigation and molecular characterization of allergens. *J Allergy Clin Immunol* 2004;113:148–154.
- 55 Jensen-Jarolim E, Leitner A, Hirschwehr R, Kraft D, Wüthrich B, Scheiner O, Graf J, Ebner C: Characterization of allergens in *Apiaceae* spices: Anise, fennel, coriander cumin. *Clin Exp Allergy* 1997;27:1299–1306.
- 56 Valenta R, Duchene M, Pettenburger K, Sillaber C, Valent P, Bettelheim P, Breitenbach M, Rumpold H, Kraft D, Scheiner O: Identification of profilin as a novel pollen allergen: IgE autoreactivity in sensitized individuals. *Science* 1991;253:557–560.
- 57 Ledesma A, Rodriguez R, Villalba M: Olive-pollen profilin. Molecular and immunologic properties. *Allergy* 1998;53:520–526.
- 58 Asturias JA, Arilla MC, Gomez-Bayon N, Aguirre M, Martinez A, Palacios R, Martinez J: Cloning and immunological characterization of the allergen Hel a 2 (profilin) from sunflower pollen. *Mol Immunol* 1998;35:469–478.
- 59 Rodriguez-Perez R, Fernandez-Rivas M, Gonzalez-Mancebo E, Sanchez-Monge R, Diaz-Perales A, Salcedo G: Peach profilin: Cloning, heterologous expression and cross-reactivity with Bet v 2. *Allergy* 2003;58:635–640.
- 60 Scheurer S, Wangorsch A, Haustein D, Vieths S: Cloning of the minor allergen Api g 4 profilin from celery (*Apium graveolens*) and its cross-reactivity with birch pollen profilin Bet v 2. *Clin Exp Allergy* 2000;30:962–971.
- 61 Willeroeder M, Fuchs H, Ballmer-Weber BK, Focke M, Susani M, Thalhammer J, Ferreira F, Wüthrich B, Scheiner O, Breiteneder H, Hoffmann-Sommergruber K: Cloning and molecular and immunological characterisation of two new food allergens, Cap a 2 and Lyc e 1, profilins from bell pepper (*Capsicum annuum*) and tomato (*Lycopersicon esculentum*). *Int Arch Allergy Immunol* 2003;131:245–255.
- 62 Rihs HP, Chen Z, Rueff F, Petersen A, Rozynek P, Heimann H, Baur X: IgE binding of the recombinant allergen soybean profilin (rGly m 3) is mediated by conformational epitopes. *J Allergy Clin Immunol* 1999;104:1293–1301.
- 63 Ganglberger E, Radauer C, Wagner S, Roridain G, Beezhold DH, Brehler R, Niggemann B, Scheiner O, Jensen-Jarolim E, Breiteneder H: Hev b 8, the *Hevea brasiliensis* latex profilin, is a cross-reactive allergen of latex, plant foods and pollen. *Int Arch Allergy Immunol* 2001;125:216–227.
- 64 Seiberler S, Scheiner O, Kraft D, Lonsdale D, Valenta R: Characterization of a birch pollen allergen, Bet v III, representing a novel class of Ca²⁺ binding proteins: Specific expression in mature pollen and dependence of patients’ IgE binding on protein-bound Ca²⁺. *EMBO J* 1994;13:3481–3486.
- 65 Tinghino R, Twardosz A, Barletta B, Puggioni EM, Iacovacci P, Buteroni C, Afferni C, Mari A, Hayek B, Di Felice G, Focke M, Westritschnig K, Valenta R, Pini C: Molecular, structural, and immunologic relationships between different families of recombinant calcium-binding pollen allergens. *J Allergy Clin Immunol* 2002;109:314–320.
- 66 Twardosz A, Hayek B, Seiberler S, Vangelista L, Elfmann L, Grönlund H, Kraft D, Valenta R: Molecular characterization, expression in *Escherichia coli* and epitope analysis of a two EF-hand calcium-binding birch pollen allergen, Bet v 4. *Biochem Biophys Res Commun* 1997;239:197–204.
- 67 Engel E, Richter K, Obermeyer G, Briza P, Kungl AJ, Simon B, Auer M, Ebner C, Rheinberger HJ, Breitenbach M, Ferreira F: Immunological and biological properties of Bet v 4, a novel birch pollen allergen with two EF-hand calcium-binding domains. *J Biol Chem* 1997;272:8630–8637.
- 68 Ledesma A, Villalba M, Batanero E, Rodriguez R: Molecular cloning and expression of active Ole e 3, a major allergen from olive-tree pollen and member of a novel family of Ca²⁺-binding proteins (polcalcins) involved in allergy. *Eur J Biochem* 1998;258:454–459.
- 69 Ledesma A, Villalba M, Vivanco F, Rodriguez R: Olive pollen allergen Ole e 8: Identification in mature pollen and presence of Ole e 8-like proteins in different pollens. *Allergy* 2002;57:40–43.
- 70 Karamloo F, Wangorsch A, Kasahara H, Davin LB, Haustein D, Lewis NG, Vieths S: Phenylcoumaran benzylic ether and isoflavonoid reductases are a new class of cross-reactive allergens in birch pollen, fruits and vegetables. *Eur J Biochem* 2001;268:5310–5320.
- 71 Cadot P, Diaz JF, Proost P, Van Damme J, Engelborghs Y, Stevens EA, Ceuppens JL: Purification and characterization of an 18-kD allergen of birch (*Betula verrucosa*) pollen: Identification as a cyclophilin. *J Allergy Clin Immunol* 2000;105:286–291.

- 72 Mahler V, Fischer S, Heiss S, Duchene M, Kraft D, Valenta R: cDNA cloning and characterization of a cross-reactive birch pollen allergen: Identification as a pectin esterase. *Int Arch Allergy Immunol* 2001;124:64–66.
- 73 Breiteneder H, Ferreira F, Reikerstorfer A, Duchene M, Valenta R, Hoffmann-Sommergruber K, Ebner C, Breitenbach M, Kraft D, Scheiner O: cDNA cloning and expression in *Escherichia coli* of Aln g I, the major allergen in pollen of alder (*Alnus glutinosa*). *J Allergy Clin Immunol* 1992;90:909–917.
- 74 Hayek B, Vangelista L, Pastore A, Sperr WR, Valent P, Vrtala S, Niederberger V, Twardosz A, Kraft D, Valenta R: Molecular and immunological characterization of a highly cross-reactive two EF-hand calcium-binding alder pollen allergen, Aln g 4. Structural basis for calcium-modulated IgE recognition. *J Immunol* 1998;161:7031–7039.
- 75 Breiteneder H, Ferreira F, Hoffmann-Sommergruber K, Ebner C, Breitenbach M, Kraft D, Scheiner O: Four recombinant isoforms of Cor a I, the major allergen of hazel pollen, show different IgE-binding properties. *Eur J Biochem* 1993;212:355–362.
- 76 Schocker F, Luttkopf D, Scheurer S, Petersen A, Cistero-Bahima A, Enrique E, San Miguel-Moncín M, Akkerdaas J, van Ree R, Vieths S, Becker WM: Recombinant lipid transfer protein Cor a 8 from hazelnut: A new tool for in vitro diagnosis of potentially severe hazelnut allergy. *J Allergy Clin Immunol* 2004;113:141–147.
- 77 Larson JN, Stroman P, Ipsen H: PCR based cloning and sequencing of isogenes encoding the tree pollen major allergen Car b I from *Carpinus betulus*, hornbeam. *Mol Immunol* 1992;29:703–711.
- 78 Ipsen H, Hansen OC: The NH₂-terminal amino acid sequence of the immunochemically partial identical major allergens of alder (*Alnus glutinosa*) Aln g I, birch (*Betula verrucosa*) Bet v I, hornbeam (*Carpinus betulus*) Car b I and oak (*Quercus alba*) Que a I pollens. *Mol Immunol* 1991;28:1279–1288.
- 79 Kos T, Hoffmann-Sommergruber K, Ferreira F, Hirschwehr R, Ahorn H, Horak F, Jäger S, Sperr WR, Kraft D, Scheiner O: Purification, characterization and N-terminal amino acid sequence of a new major allergen from European chestnut pollen, Cas s I. *Biochem Biophys Res Commun* 1993;196:1086–1092.
- 80 Díaz-Perales A, Lombardero M, Sánchez-Monge R, García-Sellés FJ, Pernas M, Fernández-Rivas M, Barber D, Salcedo G: Lipid-transfer proteins as potential plant panallergens: Cross-reactivity among proteins of *Artemisia* pollen, *Castanea* nut and *Rosaceae* fruits, with different IgE-binding capacities. *Clin Exp Allergy* 2000;30:1403–1410.
- 81 Asturias JA, Ibarrola I, Bartolome B, Ojeda I, Malet A, Martínez A: Purification and characterization of Pla a 1, a major allergen from *Platanus acerifolia* pollen. *Allergy* 2002;57:221–227.
- 82 Asturias JA, Ibarrola I, Eraso E, Arilla MC, Martínez A: The major *Platanus acerifolia* pollen allergen Pla a 1 has sequence homology to invertase inhibitors. *Clin Exp Allergy* 2003;33:978–985.
- 83 Villalba M, Batanero E, Monsalve RI, Delapena MAG, Lahoz C, Rodríguez R: Cloning and expression of Ole e 1, the major allergen from olive tree pollen. Polymorphism analysis and tissue specificity. *J Biol Chem* 1994;269:15217–15222.
- 84 Asturias JA, Arilla MC, Gomez-Bayon N, Martínez J, Martínez A, Palacios R: Cloning and expression of the panallergen profilin and the major allergen (Ole e 1) from olive tree pollen. *J Allergy Clin Immunol* 1997;100:365–372.
- 85 Batanero E, Villalba M, Ledesma A, Puente XSM, Rodríguez R: Ole e 3, an olive-tree allergen, belongs to a widespread family of pollen proteins. *Eur J Biochem* 1996;241:772–778.
- 86 Ledesma A, Villalba M, Rodríguez R: Molecular cloning and expression of active Ole e 3, a major allergen from olive-tree pollen and member of a novel family of calcium-binding proteins (polcalcins) involved in allergy. *Eur J Biochem* 1998;258:454–459.
- 87 Boluda L, Alonso C, Fernandez-Caldas E: Characterization of two new allergens of *Olea europaea*, Ole e 4 and Ole e 5. *Allergy* 1997(suppl):52–81.
- 88 Batanero E, Ledesma A, Villalba M, Rodríguez R: Purification, amino acid sequence and immunological characterization of Ole e 6, a cysteine-enriched allergen from olive tree pollen. *FEBS Lett* 1997;410:293–296.
- 89 Tejera ML, Villalba M, Batanero E, Rodríguez R: Identification, isolation, and characterization of Ole e 7, a new allergen of olive tree pollen. *J Allergy Clin Immunol* 1999;104:797–802.
- 90 Ledesma A, Villalba M, Rodríguez R: Cloning, expression and characterization of a novel four EF-hand Ca(2+)-binding protein from olive pollen with allergenic activity. *FEBS Lett* 2000;466:192–196.
- 91 Huelas S, Villalba M, Rodríguez R: Ole e 9, a major olive pollen allergen is a 1,3-beta-glucanase. Isolation, characterization, amino acid sequence, and tissue specificity. *J Biol Chem* 2001;276:27959–27966.
- 92 Palomares O, Villalba M, Rodríguez R: The C-terminal segment of the 1,3-beta-glucanase Ole e 9 from olive (*Olea europaea*) pollen is an independent domain with allergenic activity: Expression in *Pichia pastoris* and characterization. *Biochem J* 2003;369:593–601.
- 93 Barral P, Batanero E, Palomares O, Quiralte J, Villalba M, Rodríguez R: A major allergen from pollen defines a novel family of plant proteins and shows intra- and interspecies cross-reactivity. *J Immunol* 2004;172:3644–3651.
- 94 Batanero E, Gonzalez de la Pena MA, Villalba M, Monsalve RI, Martín-Esteban M, Rodríguez R: Isolation, cDNA cloning and expression of Lig v 1, the major allergen from privet pollen. *Clin Exp Allergy* 1996;26:1401–1410.
- 95 Batanero E, Villalba M, Lopez-Otin C, Rodríguez R: Isolation and characterization of an olive allergen-like protein from lilac pollen. *Eur J Biochem* 1994;221:187–193.
- 96 Griffith IJ, Lussier A, Garman R, Koury R, Yeung H, Pollock J: The cDNA cloning of Cry j I, the major allergen of *Cryptomeria japonica* (Japanese cedar) (abstract). *J Allergy Clin Immunol* 1993;91:339.
- 97 Sone T, Komiyama N, Shimizu K, Kusakabe T, Morikubo K, Kino K: Cloning and sequencing of cDNA coding for Cry j I, a major allergen of Japanese cedar pollen. *Biochem Biophys Res Commun* 1994;199:619–628.
- 98 Aceituno E, Del Pozo V, Minguez A, Arrieta I, Cortegano I, Cardaba B, Gallardo S, Rojo M, Palomino P, Lahoz C: Molecular cloning of major allergen from *Cupressus arizonica* pollen: Cup a 1. *Clin Exp Allergy* 2000;30:1750–1758.
- 99 Sakaguchi M, Inouye S, Taniai M, Ando S, Usui M, Matuhashi T: Identification of the second major allergen of Japanese cedar pollen. *Allergy* 1990;45:309–312.
- 100 Komiyama N, Sone T, Shimizu K, Morikubo K, Kino K: cDNA cloning and expression of Cry j II the second major allergen of Japanese cedar pollen. *Biochem Biophys Res Commun* 1994;201:1021–1028.
- 101 Yasueda H, Saito A, Sakaguchi M, Ide T, Saito S, Taniguchi Y, Akiyama K, Inouye S: Identification and characterization of a group 2 conifer pollen allergen from *Chamaecyparis obtusa*, a homologue of Cry j 2 from *Cryptomeria japonica*. *Clin Exp Allergy* 2000;30:546–550.
- 102 Suzuki M, Komiyama N, Itoh M, Itoh H, Sone T, Kino K, Takagi I, Ohta N: Purification, characterization and molecular cloning of Cha o 1, a major allergen of *Chamaecyparis obtusa* (Japanese cypress) Pollen. *Mol Immunol* 1996;33:451–460.
- 103 Mori T, Yokoyama M, Komiyama N, Okano M, Kino K: Purification, identification and cDNA cloning of Cha o 2, the second major allergen of Japanese cypress pollen. *Biochem Biophys Res Commun* 1999;263:166–171.
- 104 Mistrello G, Roncarolo D, Zanoni D, Zanotta S, Amato S, Falagiani P, Ariano R: Allergenic relevance of *Cupressus arizonica* pollen extract and biological characterization of the allergoid. *Int Arch Allergy Immunol* 2002;129:296–304.
- 105 Liu D, Midor-Horiuti T, White MA, Brooks EG, Goldblum RM, Czerwinski EW: Crystallization and preliminary X-ray diffraction analysis of Jun a 1, the major allergen isolated from pollen of the mountain cedar *Juniperus ashei*. *Acta Crystallogr D Biol Crystallogr* 2003;59:1052–1054.
- 106 Di Felice G, Barletta B, Tinghino R, Pini C: Cupressaceae pollinosis: Identification, purification and cloning of relevant allergens. *Int Arch Allergy Immunol* 2001;125:280–289.

- 107 Yokoyama M, Miyahara M, Shimizu K, Kino K, Tsunoo H: Purification, identification, and cDNA cloning of Jun a 2, the second major allergen of mountain cedar pollen. *Biochem Biophys Res Commun* 2000;275:195–202.
- 108 Midoro-Horiuti T, Goldblum RM, Kurosky A, Wood TG, Brooks EG: Variable expression of pathogenesis-related protein allergen in mountain cedar (*Juniperus ashei*) pollen. *J Immunol* 2000;164:2188–2192.
- 109 Midoro-Horiuti T, Goldblum RM, Brooks EG: Identification of mutations in the genes for the pollen allergens of eastern red cedar (*Juniperus virginiana*). *Clin Exp Allergy* 2001;31:771–778.
- 110 Valenta R, Duchene M, Vrtala S, Birkner T, Ebner C, Hirschwehr R, Breitenbach M, Rumpold H, Scheiner O, Kraft D: Recombinant allergens for immunoblot diagnosis of tree pollen allergy. *J Allergy Clin Immunol* 1991;88:889–894.
- 111 Valenta R, Sperr WR, Ferreira F, Valent P, Sillaber C, Tejkl M, Duchene M, Ebner C, Lechner K, Kraft D: Induction of specific histamine release from basophils with purified natural and recombinant birch pollen allergens. *J Allergy Clin Immunol* 1993;91:88–97.
- 112 Menz G, Dolecek C, Schonheit-Kenn U, Ferreira F, Moser M, Schneider T, Suter M, Boltz-Nitulescu G, Ebner C, Kraft D, Valenta R: Serological and skin test diagnosis of birch pollen allergy with recombinant Bet v 1, the major birch pollen allergen. *Clin Exp Allergy* 1996;26:50–60.
- 113 Pauli G, Oster JP, Deviller P, Heiss S, Bessot JC, Susani M, Ferreira F, Kraft D, Valenta R: Skin testing with recombinant allergens rBet v 1 and birch profilin, rBet v 2: diagnostic value for birch pollen and associated allergies. *J Allergy Clin Immunol* 1996;97:1100–1109.
- 114 Valenta R, Breiteneder H, Pettenburger K, Breitenbach M, Rumpold H, Kraft D, Scheiner O: Homology of the major birch-pollen allergen, Bet v 1, with the major pollen allergens of alder, hazel, and hornbeam at the nucleic acid level as determined by cross-hybridization. *J Allergy Clin Immunol* 1991;87:977–982.
- 115 Ebner C, Birkner T, Valenta R, Rumpold H, Breitenbach M, Scheiner O, Kraft D: Common epitopes of birch pollen and apples: Studies by western and northern blot. *J Allergy Clin Immunol* 1991;88:588–594.
- 116 Vanek-Krebitz M, Hoffmann-Sommergruber K, Laimer da Camara Machado M, Ebner C, Kraft D, Scheiner O, Breiteneder H: Cloning and sequencing of Mal d 1, the major allergen from apple (*Malus domestica*), and its immunological relationship to Bet v 1, the major birch pollen allergen. *Biochem Biophys Res Commun* 1995;214:538–551.
- 117 Karamloo F, Scheurer S, Wangorsch A, May S, Hausteiner D, Vieths S: Pyr c 1, the major allergen from pear (*Pyrus communis*), is a new member of the Bet v 1 allergen family. *J Chromatogr B Biomed Sci Appl* 2001;756:281–293.
- 118 Kazemi-Shirazi L, Pauli G, Purohit A, Spitzauer S, Fröschl R, Hoffmann-Sommergruber K, Breiteneder H, Scheiner O, Kraft D, Valenta R: Quantitative IgE inhibition experiments with purified recombinant allergens indicate pollen-derived allergens as the sensitizing agents responsible for many forms of plant food allergy. *J Allergy Clin Immunol* 2000;105:116–125.
- 119 Gajhede M, Osmark P, Poulsen FM, Ipsen H, Larsen JN, Joost van Neerven RJ, Schou C, Lowenstein H, Spangfort MD: X-ray and NMR structure of Bet v 1, the origin of birch pollen allergy. *Nat Struct Biol* 1996;3:1040–1045.
- 120 Hoffmann-Sommergruber K: Plant allergens and pathogenesis-related proteins. What do they have in common? *Int Arch Allergy Immunol* 2000;122:155–166.
- 121 Bufer A, Spangfort MD, Kahlert H, Schlaak M, Becker WM: The major birch pollen allergen, Bet v 1, shows ribonuclease activity. *Planta* 1996;199:413–415.
- 122 Markovic-Housley Z, Degano M, Lamba D, von Roepenack-Lahaye E, Clemens S, Susani M, Ferreira F, Scheiner O, Breiteneder H: Crystal structure of a hypoallergenic isoform of the major birch pollen allergen Bet v 1 and its likely biological function as a plant steroid carrier. *J Mol Biol* 2003;325:123–133.
- 123 Mogensen JE, Wimmer R, Larsen JN, Spangfort MD, Otzen DE: The major birch allergen, Bet v 1, shows affinity for a broad spectrum of physiological ligands. *J Biol Chem* 2002;277:23684–23692.
- 124 Valenta R, Kraft D: Type I allergic reactions to plant-derived food: A consequence of primary sensitization to pollen allergens. *J Allergy Clin Immunol* 1996;97:893–895.
- 125 Kazemi-Shirazi L, Niederberger V, Linhart B, Lidholm J, Kraft D, Valenta R: Recombinant marker allergens: Diagnostic gate keepers for therapy of allergy. *Int Arch Allergy Immunol* 2002;127:259–268.
- 126 Moverare R, Westritschnig K, Svensson M, Hayek B, Bende M, Pauli G, Sorva R, Haahela T, Valenta R, Elfman L: Different IgE reactivity profiles in birch pollen-sensitive patients from six European populations revealed by recombinant allergens: An imprint of local sensitization. *Int Arch Allergy Immunol* 2002;128:325–335.
- 127 Valenta R, Ferreira F, Grote M, Swoboda I, Vrtala S, Duchene M, Deviller P, Meagher RB, McKinney E, Heberle-Bors E, et al: Identification of profilin as an actin-binding protein in higher plants. *J Biol Chem* 1993;268:22777–22781.
- 128 Valenta R, Duchene M, Ebner C, Valent P, Sillaber C, Deviller P, Ferreira F, Tejkl M, Edelmann H, Kraft D, Scheiner O: Profilins constitute a novel family of functional plant pan-allergens. *J Exp Med* 1992;175:377–385.
- 129 van Ree R, Voitenko V, van Leeuwen WA, Aalberse RC: Profilin is a cross-reactive allergen in pollen and vegetable foods. *Int Arch Allergy Immunol* 1992;98:97–104.
- 130 Vallier P, DeChamp C, Valenta R, Vial O, Deviller P: Purification and characterization of an allergen from celery immunochemically related to an allergen present in several other plant species. Identification as a profilin. *Clin Exp Allergy* 1992;22:774–782.
- 131 Valenta R, Duchene M, Breitenbach M, Pettenburger K, Koler L, Rumpold H, Scheiner O, Kraft D: A low molecular weight allergen of white birch (*Betula verrucosa*) is highly homologous to human profilin. *Int Arch Allergy Immunol* 1991;94:368–370.
- 132 Fedorov AA, Ball T, Mahoney NM, Valenta R, Almo SC: The molecular basis for allergen cross-reactivity: Crystal structure and IgE-epitope mapping of birch pollen profilin. *Structure* 1997;5:33–45.
- 133 Seiberler S, Scheiner O, Kraft D, Valenta R: Homology of two cDNAs coding for birch pollen allergens with calmodulin: Protein-bound Ca²⁺ affects the IgE-binding capacity. *Int Arch Allergy Immunol* 1992;99:380–381.
- 134 Niederberger V, Hayek B, Vrtala S, Laffer S, Twardosz A, Vangelista L, Sperr WR, Valent P, Rumpold H, Kraft D, Ehrenberger K, Valenta R, Spitzauer S: Calcium-dependent immunoglobulin E recognition of the apo- and calcium-bound form of a cross-reactive two EF-hand timothy grass pollen allergen, Phl p 7. *FASEB J* 1999;13:843–856.
- 135 Smith PM, Xu H, Swoboda I, Singh MB: Identification of a Ca²⁺ binding protein as a new Bermuda grass pollen allergen Cyn d 7: IgE cross-reactivity with oilseed rape pollen allergen Bra r 1. *Int Arch Allergy Immunol* 1997;114:265–271.
- 136 Okada T, Swoboda I, Bhalla PL, Toriyama K, Singh MB: Engineering of hypoallergenic mutants of the *Brassica* pollen allergen, Bra r 1, for immunotherapy. *FEBS Lett* 1998;434:255–260.
- 137 Valenta R, Hayek B, Seiberler S, Bugajski-Schretter A, Niederberger V, Twardosz A, Natter S, Vangelista L, Pastore A, Spitzauer S, Kraft D: Calcium-binding allergens: From plants to man. *Int Arch Allergy Immunol* 1998;117:160–166.
- 138 Hamilton AJ, Fray RG, Grierson D: Sense and antisense inactivation of fruit ripening genes in tomato. *Curr Top Microbiol Immunol* 1995;197:77–89.
- 139 Rodriguez R, Villalba M, Monsalve RI, Batanero E: The spectrum of olive pollen allergens. *Int Arch Allergy Immunol* 2001;125:185–195.

- 140 Marknell DeWitt A, Niederberger V, Lehtonen P, Spitzauer S, Sperr WR, Valent P, Valenta R, Lidholm J: Molecular and immunological characterization of a novel timothy grass (*Phleum pratense*) pollen allergen, Phl p 11. *Clin Exp Allergy* 2002;32:1329–1340.
- 141 Heiss S, Flicker S, Hamilton DA, Kraft D, Mascarenhas JP, Valenta R: Expression of Zm13, a pollen specific maize protein, in *Escherichia coli* reveals IgE-binding capacity and allergenic potential. *FEBS Lett* 1996;381: 217–221.
- 142 Taniguchi Y, Ono A, Sawatani M, Nanba M, Kohno K, Usui M, Kurimoto M, Matuhasi T: Cry j 1, a major allergen of Japanese cedar pollen, has pectate lyase enzyme activity. *Allergy* 1995;50:90–93.
- 143 Ohtsuki T, Taniguchi Y, Kohno K, Fukuda S, Usui M, Kurimoto M: Cry j 2, a major allergen of Japanese cedar pollen, shows polymethylgalacturonase activity. *Allergy* 1995; 50:483–488.
- 144 Petersen A, Suck R, Hagen S, Cromwell O, Fiebig H, Becker WM: Group 13 grass allergens: Structural variability between different grass species and analysis of proteolytic stability. *J Allergy Clin Immunol* 2001;107: 856–862.
- 145 Bousquet J, Lockey R, Malling HJ: Allergen immunotherapy: Therapeutic vaccines for allergic diseases. A WHO position paper. *J Allergy Clin Immunol* 1998;102:558–562.
- 146 Valenta R, Ball T, Focke M, Linhart B, Mothes N, Niederberger V, Spitzauer S, Swo-boda I, Vrtala S, Westritschnig K, Kraft D: Immunotherapy of allergic disease. *Adv Immunol* 2003;82:105–154.
- 147 Ball T, Sperr WR, Valent P, Lidholm J, Spitzauer S, Ebner C, Kraft D, Valenta R: Induction of antibody responses to new B cell epitopes indicates vaccination character of allergen immunotherapy. *Eur J Immunol* 1999; 29:2026–2036.
- 148 Mothes N, Heinzkill M, Drachenberg KJ, Sperr WR, Krauth MT, Majlesi H, Semper P, Valent P, Niederberger V, Kraft D, Valenta R: Allergen-specific immunotherapy with a monophosphoryl lipid A-adjuvanted vaccine: Reduced seasonally boosted IgE production and inhibition of basophil histamine release by therapy-induced blocking antibodies. *Clin Exp Allergy* 2003;33:1198–1208.
- 149 Stumvoll S, Westritschnig K, Lidholm J, Spitzauer S, Colombo P, Duro G, Kraft D, Geraci D, Valenta R: Identification of cross-reactive and genuine *Parietaria judaica* pollen allergens. *J Allergy Clin Immunol* 2003; 111:974–979.
- 150 Pittner G, Vrtala S, Thomas WR, Weghofer M, Kundi M, Horak F, Kraft D, Valenta R: Component-resolved diagnosis of house dust mite allergy with purified natural and recombinant mite allergens. *Clin Exp Allergy* 2004; 34:597–603.
- 151 Ferreira F, Hawranek T, Gruber P, Wopfner N, Mari A: Allergic cross-reactivity: From gene to the clinic. *Allergy* 2004;59:243–267.
- 152 Jarolim E, Rumpold H, Endler AT, Ebner H, Breitenbach M, Scheiner O, Kraft D: IgE and IgG antibodies of patients with allergy to birch pollen as tools to define the allergen profile of *Betula verrucosa*. *Allergy* 1989;44:385–395.
- 153 Westritschnig K, Sibanda E, Thomas W, Auer H, Aspöck H, Pittner G, Vrtala S, Spitzauer S, Kraft D, Valenta R: Analysis of the sensitization profile towards allergens in central Africa. *Clin Exp Allergy* 2003;33:22–27.
- 154 Petersen BN, Janniche H, Munch EP, Wihl JA, Bowadt H, Ipsen H, Lowenstein H: Immunotherapy with partially purified and standardized tree pollen extracts. I. Clinical results from a three-year double-blind study of patients treated with pollen extracts either of birch or combinations of alder, birch and hazel. *Allergy* 1988;43:353–362.
- 155 Wihl JA, Ipsen H, Petersen BN, Munch EP, Janniche H, Lowenstein H: Immunotherapy with partially purified and standardized tree pollen extracts. II. Results of skin prick tests and nasal provocation tests from a three-year double-blind study of patients treated with pollen extracts either of birch or combinations of alder, birch and hazel. *Allergy* 1988; 43:363–369.
- 156 Ipsen H, Schwartz B, Wihl JA, Petersen BN, Munch EP, Janniche H, Lowenstein H: Immunotherapy with partially purified and standardized tree pollen extracts. III. Specific IgE response to the major allergens of alder, birch and hazel pollen during immunotherapy. *Allergy* 1988;43:370–377.
- 157 Asero R: How long does the effect of birch pollen injection SIT on apple allergy last? *Allergy* 2003;58:435–438.
- 158 Birkner T, Rumpold H, Jarolim E, Ebner C, Breitenbach M, Skvaril F, Scheiner O, Kraft D: Evaluation of immunotherapy-induced changes in specific IgE, IgG and IgG subclasses in birch pollen allergic patients by means of immunoblotting. *Allergy* 1990;45:418–426.
- 159 Niederberger V, Horak F, Vrtala S, Spitzauer S, Krauth MT, Valent P, Reisinger J, Pelzmann M, Hayek B, Kronqvist M, Gafvelin G, Grönlund H, Purohit A, Suck R, Fiebig H, Cromwell O, Pauli G, van Hage-Hamsten M, Valenta R: Vaccination with genetically engineered allergens prevents progression of allergic disease. *Proc Natl Acad Sci USA* 2004; 101(suppl 2):14677–14682.