Biology of Tree Pollen Allergens

Nadine Mothes, MD, and Rudolf Valenta, MD

Address
Division of Immunopathology, Department of Pathophysiology, Vienna General Hospital, AKH, Medical University of Vienna, Waehringer Guertel 18-20, A-1090 Vienna, Austria.
E-mail: rudolf.valenta@meduniwien.ac.at

Current Allergy and Asthma Reports 2004, **4:**384–390
Current Science Inc. ISSN 1529-7322

More than 25% of the population suffer from type I allergy. Pollens from trees of the Fagales, Oleaceae, and Cupressaceae belong to the most potent and frequent allergen sources. During the past 15 years, the nature of the most important allergens has been identified by molecular biological techniques, and recombinant allergens equivalent to the natural allergens have been produced. These advances provide insight into the biological functions of important allergens and allow the development of novel forms of diagnosis and therapy. In this review, we focus on Fagales allergens to illustrate the impact of recombinant allergens on diagnosis and therapy. We discuss structural similarities as a molecular basis for cross-reactivities and develop diagnostic concepts by using species-specific marker allergens as well as highly cross-reactive allergens. The identification of the allergen recognition profiles of patients with recombinant allergens allows a more precise selection of patients for available forms of allergy treatment. Moreover, we describe novel recombinant allergen-based forms of specific immunotherapy.

Introduction

Pollen production is a general feature of numerous plants, but only wind-pollinated plants represent potent allergen sources [1]. This fact can be explained by the importance of respiratory allergen contact for allergic sensitization in early childhood [2,3]. The expression level of pollen allergens can be influenced by environmental factors, maturation of pollen, climate, and characteristics of certain plant species [4-8], and thus may affect overall allergenicity. The tree pollen allergens characterized to date represent low-molecular weight intracellular proteins and, sometimes, glycoproteins that are rapidly released after contact with aqueous solutions [9,10], and may be bound to respirable particles (*eg,* diesel exhaust particles). The latter fact has been related to the induction of strong Th2 immune responses to allergens due to possible adjuvant and/or immunomodulatory effects [11–13].

It is useful to classify important allergenic trees according to certain botanical orders that show a typical geographic distribution and are characterized by defined flowering periods [14]. The Fagales, including the families of the Betulaceae, Corylaceae, and Fagaceae, occur in North and Middle Europe, North-West Africa, East Asia, and from North America to the Andes, and predominantly flower in spring. The Oleaceae family reaches further south and is found in the Mediterranean area, where olive belongs to the most potent elicitor of type I allergy [14,15].

The Gymnosperms, especially the Cupressaceae/Taxodiaceae family, which grow in the Mediterranean areas, Australia, New Zealand, North America, South America, and certain parts of Asia, are responsible for allergic sensitization in these regions [16].

Allergenic Trees: From a Botanical Classification to a Classification According to Allergenic Molecules

Cross-reactivity of IgE antibodies between different allergen sources has been established as the basis for the co-occurrence of allergic symptoms to a variety of allergen sources. The elucidation of cross-reactivities started with the introduction of the radioallergosorbent (RAST) technology into the field of allergy [17]. Several RAST inhibition studies have identified cross-reactive allergens in different allergen sources [18,19]. As far as plant, and especially tree-pollen, allergy is concerned, cross-reactivity follows strongly botanical relationships between allergen sources [20]. For example, it has been demonstrated that pollens of trees belonging to the order Fagales (*eg,* Fagaceae, Corylaceae, and Betulaceae) contain cross-reactive allergens [21,22]. This observation has led to clinical immunotherapy studies demonstrating that Fagales pollen allergy can be treated equally well with a mixture of pollen extracts from different members of the Fagales or birch-pollen extract alone [23–25]. Similar observations have been made for members of the Oleaceae family, in which extensive cross-reactivities have been described among olive, ash, privet, and lilac [26].

Likewise, there are cross-reactivities among pollens of the Cupressaceae/Taxodiaceae [27]. With the introduction of molecular biological methods, the individual allergens in most of the above-mentioned allergen sources have been characterized down to the molecular and structural level [28,29]. Using defined recombinant allergens, it has become possible to reveal the molecular basis for cross-reactivities and to identify precisely the cross-reactive allergens' IgE as well as T-cell epitopes [28,29]. In Table 1, we provide a list of allergens that have been identified in pol-

Table 1. Tree-pollen allergens

Angiosperms					Gymnosperms
Hamamelides				Asteridae	
Fagales			**Hamamelidales**	**Scrophulariales**	**Coniferales**
Betulaceae	**Corylaceae**	**Fagaceae**	**Platanaceae**	**Oleaceae**	**Cupressaceae/Taxodiaceae**
Betula (birch)	Corylus (hazel)	Quercus (white oak)		Olea (olive tree)	Cryptomeria (Japanese cedar)
Alnus (alder)	Carpinus (hornbeam)	Castanea (chestnut)	Platanus (plane tree)	Fraxinus (ash)	Juniperus (cedar, juniper)
				Ligustrum vulgare (privet)	Cupressus (cypress)
				Syringa vulgaris (lilac)	
Bet v 1	Cor a 1	Que a 1	Pla a 1	Ole e 1	Cry j 1
Bet v 2	Cor a 2		Pla a 2	Ole e 2	Cry j 2
Bet v 3	Cor a 8	Cas s 1		Ole e 3	
Bet v 4	Cor a 10	Cas s 5		Ole e 4	Cha o 1
Bet v 6		Cas s 8		Ole e 5	Cha o 2
Bet v 7	Car b 1			Ole e 6	
Bet v 8				Ole e 7	Cup a 1
				Ole e 8	Cup a 3
Aln g 1				Ole e 9	Cup s 1
Aln g 4				Ole e 10	
				Fra e 1	Jun a 2
					Jun a 3
				Lig v 1	Jun o 4
					Jun v 1
				Syr v 1	
					Cry j 1
				Syr v 3	Cry j 2

http://www.allergenonline.com
International Union of Immunological Societies Allergen Nomenclature Sub-Committee http://www.allergenorg/list.htm
SDAP—Structural Database of Allergenic Proteins.

lens of trees of the Fagales, Hamamelidales, Scrophulariales, and Coniferales. The allergen sources are grouped according to botanical classifications, and allergens have been termed according the international allergen nomenclature. Several allergen databases (*eg*, http://www.allergenonline.com, International Union of Immunological Societies Allergen Nomenclature Sub-Committee; http://www.allergenorg/list.htm; SDAP-Structural Database of Allergenic Proteins) are available, and further information about the spectrum of tree-pollen allergens can be obtained in recently published review articles [15,30,31].

Molecular Classification of Tree Pollen Allergy: Allergy to Fagales Pollen as a Concrete Example

Trees of the Fagales order include the families of the Betulaceae, Fagaceae, and Corylaceae, which, according to RAST inhibition and immunochemical studies, contain cross-reactive allergens. Birch represents the most potent and frequent allergen source of the Fagales. Hence, the allergen spectrum of birch is well characterized [31].

Bet v 1, the major allergen of birch is a 17 kDa protein. The cDNA coding for Bet v 1 was one of the first allergen-encoding cDNAs isolated [32]. It revealed significant sequence homology to a group of proteins that are activated in plants under stressful conditions. Accordingly, these proteins were called pathogenesis-related plant proteins [32,33]. Until now, the biological function of Bet v 1 has not been revealed with certainty, although several assumptions have been made based on in vitro experiments and structural studies [34–36]. Evidence that members of the Bet v 1 family may protect plants from insects comes from a recent study that demonstrated insecticide activity of a Bet v 1–homologous protein from periwinkle, PR10 [37]. Recombinant Bet v 1 has been expressed in *Escherichia coli* as a biologically active allergen that binds the IgE of most birch-pollen allergic patients [38–40].

Based on homologies at a nuclear acid level, cDNAs coding for major allergens of related pollens such as alder (Aln g 1), hazel (Cor a 1), hornbeam (Car b 1), and apple (Mal d 1) could be isolated by reverse transcription and polymerase chain reaction (PCR) methods [41–44]. In accordance with earlier studies, examining immunologic cross-reactivities between the major allergens of Fagales pollen, fruits, vegetables, and spices, several Bet v 1–related allergens have been identified (*eg*, in carrot, celery, cherry, and pear) [45–49] (Fig. 1). Cross-reactivity experiments using pollen of several trees of the Fagales (alder, hazel, hornbeam), fruits, vegetables, and spices revealed that most epitopes are represented by Bet v 1, the major birch-pollen allergen [40,50]. The latter studies indicate that Bet v 1 is the initial sensitizing allergen for most patients suffering from Fagales-pollen allergy and birch-pollen–related plant-food allergy (oral allergy syndrome [OAS]) [40,50] (Fig. 1). Several other population studies confirmed that Bet v 1 may be considered as a marker allergen for genuine sensitization to Fagales-pollen and birch-pollen–related food allergy [40,50,51].

Bet v 2, the second well-described birch pollen allergen, belongs to the family of profilins, a group of ubiquitous actin-binding eukaryotic proteins with a molecular weight of 14 kDa [52,53]. Profilins can be found as cross-reactive allergens not only in pollen from unrelated plants (trees, grasses, weeds) but also in other plant tissues (fruits, vegetables, nuts, spices, and latex), and even in humans .

Besides Bet v 1 and Bet v 2, there is a third group of allergens with a wide distribution in pollen of different unrelated plants. Bet v 3 and Bet v 4 were identified as EF-hand calcium-binding proteins primarily expressed in mature pollen [54–56]. They show extensive cross-reactive activity to other pollens from grasses, weeds, and trees and, thus, can serve as marker allergens for plant polysensitization [57]. Bet v 3 is a 23.7 kDa protein containing three typical calcium-binding motifs [54]. Bet v 4, a protein of 9.3 kDa, contains only two calcium-binding domains [55,56] and, thus, constitutes another family of calcium-binding allergens that share epitopes with cross-reactive allergens from grass pollen (bermuda grass: Cyn d 7; timothy grass: Phl p 7), weeds (rape: Bra r 1 and Bra r 2), and tree pollens (alder: Aln g 4; olive: Ole e 3) [57].

Several other birch-pollen allergens have been characterized. One of those, Bet v 6, was identified as an isoflavone reductase [58]; Bet v 7 is a cyclophilin [59]; and Bet v 8 is a plant pectin esterase [60]. The clinical relevance of Bet v 6–8 has not yet been investigated in detail. In summary, it can be stated that Bet v 1 is the major allergen of birch. It contains not only most of the epitopes present in birch pollen, but, owing to cross-reactivity, also those of pollens from trees of the Fagales order and related plant foods. Accordingly, Bet v 1 has been suggested as a diagnostic marker allergen to identify patients with a genuine birch-pollen sensitization [61]. However, highly cross-reactive allergens, such as Bet v 2 and Bet v 4, may be considered as marker allergens that are cross-reactive with numerous unrelated plants/plant products [61].

Recombinant Birch Pollen Allergens for Novel Forms of Allergy Diagnosis

In 1991 the first study using recombinant birch pollen allergens for serological diagnosis of birch pollen allergy was published [38]. Since then, the common allergens from most allergen sources have been identified by molecular cloning techniques and produced as recombinant allergens, which can now be used for component-resolved diagnosis (CRD) of allergy [62–64]. The advantage of CRD versus allergen-extract–based diagnosis is that CRD allows the identification of the disease-eliciting allergens for each patient and, thus, establish a detailed IgE reactivity profile. By contrast, extract-based diagnosis only tells us that a patient reacts to unspecified components in the given

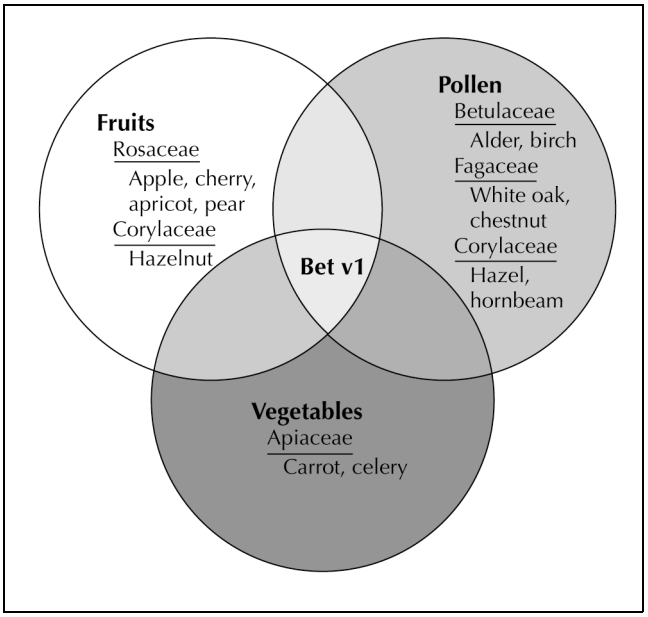

Figure 1. Redefinition of cross-reactivities among various allergen sources based on cross-reactivity to the major birch-pollen allergen, Bet v 1. Family names are underlined, and common names are listed.

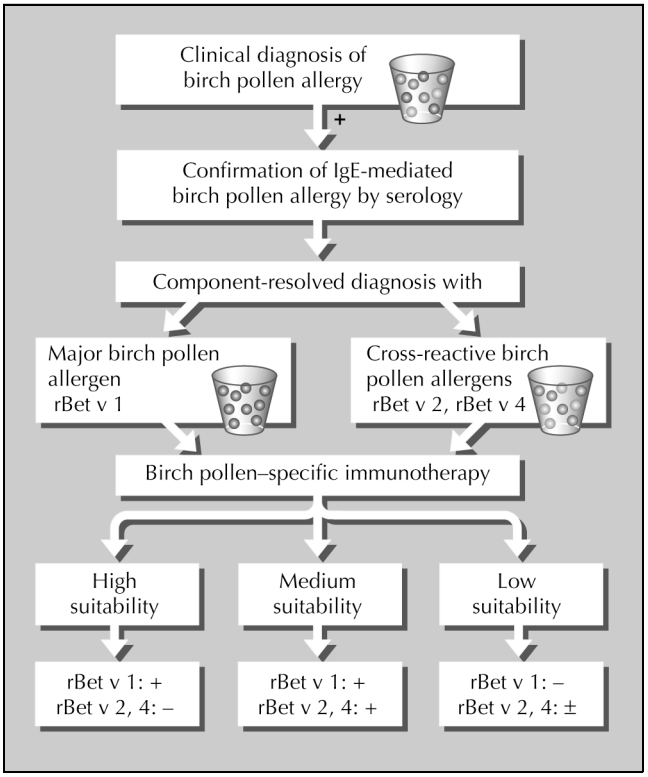

Figure 2. A suggested decision tree for the selection of patients for birch-pollen–specific immunotherapy.

extract and, thus, only provides a determination of the allergen source.

The advantage of using recombinant allergens for diagnosis can be illustrated with a concrete example. Birch pollen allergy is frequently treated with allergen-specific immunotherapy. The clinician has to make a careful decision as to which patient may be suitable for this treatment, because immunotherapy represents a sometimes risky, costly, and time-consuming treatment. From population studies, we know that clinically relevant sensitization to birch pollen can be found in patients who were not originally sensitized to birch. For example, it has been reported that allergic patients from Central Africa showed positive skin reactivity and serology to birch pollen although they were not exposed to birch [65•]. It turned out that these patients were sensitized to cross-reactive allergens from other plants. Such patients can be identified in populations all over the world, to a varying degree [51]. They are rather rare in countries with high exposure to birch pollen but are more frequently observed in countries with mixed vegetation (e.g., Middle and southern parts of Europe) [51]. Population studies performed with recombinant allergens can distinguish patients who are genuinely sensitized to birch pollen by their IgE reactivity to the major birch pollen allergen Bet v 1. In contrast, patients who exhibit positive skin tests to birch pollen extracts, but who have not been exposed to birch, have IgE to cross-reactive allergens, such as Bet v 2. Thus, the use of rBet v 1 for diagnostic testing to identify patients with genuine birch pollen sensitization and to confirm the diagnosis of birch pollen allergy before initiating immunotherapy with birch pollen extract is recommended. Figure 2 illustrates the possible diagnostic procedure for the confirmation of genuine birch pollen allergy. It is recommended to treat only those patients with birch pollen immunotherapy who react to the major allergen of birch, Bet v 1. There are two reasons for this suggestion. First, genuine sensitization to birch without reactivity to the major allergen is very unlikely. Second, most birch pollen extracts used for immunotherapy contain large amounts of Bet v 1 but are not standardized for other birch pollen allergens.

Recombinant Birch Pollen Allergens for the Monitoring of Therapy and for Immunotherapy

The introduction of the recombinant allergens in the diagnosis of type I allergy not only facilitates the decision whether a patient is suitable for immunotherapy but allows the measurement of IgE and IgG responses in response to allergen-specific immunotherapy [66,67•,68•]. Studies using recombinant allergens for the monitoring of antibody profiles and levels during the course of immunotherapy have reemphasized the importance of blocking antibodies for successful immunotherapy [66,67•]. On the other hand, it has turned out that immunotherapy with crude allergen extracts may induce de novo IgE sensitizations to new allergens or extract components [66,68•,69]. The latter studies emphasize a need for patient-tailored forms of treatment, which, in fact, can only be achieved with defined recombinant allergens.

Due to the fact that recombinant allergens can be produced that contain most of epitopes present in complex

allergen sources, there is hope that recombinant allergens will replace traditional allergen extracts for immunotherapy [70•,71•]. Furthermore, it is possible to apply recombinant DNA technology and synthetic peptide chemistry to produce new generations of allergy vaccines with reduced allergenic activity [70•,71•]. Such candidate vaccines have already been tested for safety in patients and for efficacy in animal models. Currently, several immunotherapy trials are being conducted with recombinant allergen based vaccines. It may, therefore, be anticipated that the practice of immunotherapy will dramatically change in the near future [72].

Conclusions

Advances made in the field of molecular allergen characterization have provided the basis for the transition from a botanical to a molecular classification of tree pollens. An overview over the most important tree pollen allergens is given, and the importance and usefulness of recombinant allergens for allergy diagnosis and treatment is exemplified for birch pollen and related allergies. The introduction of species-specific marker allergens and cross-reactive allergens allows the precise identification of a patient's sensitization profile as well as the monitoring of extract-based immunotherapy. Moreover, new allergy vaccines based on recombinant allergens have already entered clinical trials and will likely replace conventional, extract-based forms of allergen-specific treatment.

References and Recommended Reading

Papers of particular interest, published recently, have been highlighted as:

- • Of importance
- •• Of major importance

1. D'Amato G, Bonini S (eds): *Pollenosis 2000: Global Approach.* Naples, Italy: JGC Editions; 2001.
2. Kulig M, Klettke U, Wahn V, *et al.*: **Development of seasonal allergic rhinitis during the first 7 years of life.** *J Allergy Clin Immunol* 2000, **106:**832–839.
3. Niederberger V, Niggemann B, Kraft D, *et al.*: **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.
4. Mittermann I, Swoboda I, Pierson E, *et al.*: **Molecular cloning and characterization of profilin from tobacco (Nicotina tabacum): increased profilin expression during pollen maturation.** *Plant Mol Biol* 1995, **27:**137–146.
5. Ahlholm JU, Helander ML, Savolainen J: **Genetic and environmental factors affecting the allergenicity of birch (Betula pubescens ssp. Czerepanovii [Orl.] Hamet-ahti) pollen.** *Clin Exp Allergy* 1998, **28:**1384–1388.
6. Kondo Y, Ipsen H, Lowenstein H, *et al.*: **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.
7. Helander ML, Savolainen J, Ahlholm J: **Effects of air pollution and other environmental factors on birch pollen allergens.** *Allergy* 1997, **52:**1207–1214.
8. Tashpulatov AS, Clement P, Akimcheva SA, *et al.*: **A model system to study the environment-dependent expression of the Bet v 1a gene encoding the major birch pollen allergen.** *Int Arch Allergy Immunol* 2004, **134:**1–9.
9. Grote M, Vrtala S, Valenta R: **Monitoring of two allergens, Bet v 1 and profilin, in dry and rehydrated birch pollen by immunogold electron microscopy and immunoblotting.** *J Histochem Cytochem* 1993, **41:**745–750.
10. 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.
11. 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.
12. Knox RB, Suphioglu C, Taylor P, *et al.*: **Major grass pollen allergen Lol p 1 binds to diesel exhaust particles: implications for asthma and air pollution.** *Clin Exp Allergy* 1997, **27:**246–251.
13. Traidl-Hoffmann C, Kasche A, Menzel A, *et al.*: **Impact of pollen on human health: more than allergen carriers?** *Int Arch Allergy Immunol* 2003, **131:**1–13.
14. Mothes N, Westritschnig K, Valenta R: **Tree pollen allergens.** In *Allergens and Allergen Immunotherapy for Allergic Diseases,* edn 3. Edited by Lockey RF, Bukantz SC, Bousquet J. New York: Marcel Dekker; 2004:165–184.
15. Rodriguez R, Villalba M, Monsalve RI, Batanero E: **The spectrum of olive pollen allergens.** *Int Arch Allergy Immunol* 2001, **125:**185–195.
16. Schwietz LA, Goetz DW, Whisman BA, Reid MJ: **Cross-reactivity among conifer pollens.** *Ann Allergy Asthma Immunol* 2000, **84:**87–93.
17. Wide L, Bennich H, Johnsson SG: **Diagnosis of allergy by an in-vitro test for allergen antibodies.** *Lancet* 1967, **2:**1105–1107.
18. Vik H, Florvaag E, Apold J, *et al.*: **Comparative studies on tree pollen allergens. IV. Evaluation of two commercially available allergen extracts of alder (Alnus incana) and birch (Betula verrucosa) pollen.** *Int Arch Allergy Appl Immunol* 1982, **68:**371–376.
19. 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.
20. Yman L: *Botanical Relations and Immunological Cross-reactions in Pollen Allergy.* Uppsala, Sweden: Pharmacia Diagnostics AB; 1978.
21. Moller C, Dreborg S: **Cross-reactivity between deciduous trees during immunotherapy. I. In vivo results.** *Clin Allergy* 1986, **16:**135–143.
22. Hemmens VJ, Baldo BA, Elsayed S, Bass D: **Allergic response to birch and alder pollen allergens influenced by geographical location of allergic subjects.** *Int Arch Allergy Appl Immunol* 1988, **87:**321–328.
23. Petersen BN, Janniche H, Munch EP, *et al.*: **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.
24. Wihl JA, Ipsen H, Petersen BN, *et al.*: **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.
25. Ipsen H, Schwartz B, Wihl JA, *et al.*: **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.
26. D'Amato G, Mullins J, Nolard N, *et al.*: **City spore concentrations in the European Economic Community (EEC). VII. Oleaceae (Fraxinus, Ligustrum, Olea).** *Clin Allergy* 1988, **18:**541–547.

27. Yoo TJ, Spitz E, McGerity JL: **Conifer pollen allergy: studies of immunogenicity and cross antigenicity of conifer pollens in rabbit and man.** *Ann Allergy* 1975, **34:**87–93.
28. Valenta R, Kraft D: **Recombinant allergen molecules: tools to study effector cell activation.** *Immunol Rev* 2001, **179:**119–127.
29. Valenta R, Kraft D: **From allergen structure to new forms of allergen-specific immunotherapy.** *Curr Opin Immunol* 2002, **14:**718–727.
30. Di Felice G, Barletta B, Tinghino R, Pini C: **Cupressaceae pollinosis: identification, purification and cloning of relevant allergens.** *Int Arch Allergy Immunol* 2001, **125:**129.
31. Mothes N, Horak F, Valenta R: **The transition from a botanical to a molecular classification in tree pollen allergy: Implications for diagnosis and therapy.** *Int Arch Allergy Immunol* 2004, In press.
32. Breiteneder H, Pettenburger K, Bito A, *et al.*: **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.
33. Hoffmann-Sommergruber K: **Plant allergens and pathogenesis-related proteins: What do they have in common?** *Int Arch Allergy Immunol* 2000, **122:**155–166.
34. Bufe A, Spangfort MD, Kahlert H, *et al.*: **The major birch pollen allergen, Bet v 1, shows ribonuclease activity.** *Planta* 1996, **199:**413–415.
35. Markovic-Housley Z, Degano M, Lamba D, *et al.*: **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.
36. Mogensen JE, Wimmer R, Larsen JN, *et al.*: **The major birch allergen, Bet v 1, shows affinity for a broad spectrum of physiological ligands.** *J Biol Chem* 2002, **277:**23684–23592.
37. Elbaz M, Bremer J, Thibout E, *et al.*: **A periwinkle PR-10 protein, CrPR-10a, has insecticide activity.** *Planta* 2004, In press.
38. Valenta R, Duchene M, Vrtala S, *et al.*: **Recombinant allergens for immunoblot diagnosis of tree pollen allergy.** *J Allergy Clin Immunol* 1991, **88:**889–894.
39. Menz G, Dolecek C, Schonheit-Kenn U, *et al.*: **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.
40. Niederberger V, Pauli G, Gronlund H, *et al.*: **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.
41. Valenta R, Breiteneder H, Pettenburger, *et al.*: **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.
42. Breiteneder H, Ferreira F, Reikerstorfer A, *et al.*: **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.
43. Larsen JN, Stroman P, Ipsen H: **PCR based cloning and sequencing of isogenes encoding the tree pollen major allergen Car b I from Carpinus betulus, hornbeam.** *Mol Immunol* 1992, **29:**703–711.
44. Vanek-Krebitz M, Hoffmann-Sommergruber K, Laimer da Camara Machado M, *et al.*: **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.
45. Ebner C, Hirschwehr R, Bauer L, *et al.*: **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.
46. Jensen-Jarolim E, Leitner A, Hirschwehr R, *et al.*: **Characterization of allergens in Apiaceae spices: anise, fennel, coriander cumin.** *Clin Exp Allergy* 1997, **27:**1299–1306.
47. Hoffmann-Sommergruber K, O'Riordain G, Ahorn H, *et al.*: **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.
48. Scheurer S, Son DY, Boehm M, *et al.*: **Cross-reactivity and epitope analysis of Pru av 1, the major cherry allergen.** *Mol Immunol* 1999, **36:**155–167.
49. Karamloo F, Scheurer S, Wangorsch A, *et al.*: **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.
50. Kazemi-Shirazi L, Pauli G, Purohit A, *et al.*: **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.
51. Moverare R, Westritschnig K, Svensson M, *et al.*: **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.
52. Valenta R, Duchene M, Pettenburger K, *et al.*: **Identification of profilin as a novel pollen allergen: IgE autoreactivity in sensitized individuals.** *Science* 1991, **253:**557–560.
53. Valenta R, Ferreira F, Grote M, *et al.*: **Identification of profilin as an actin-binding protein in higher plants.** *J Biol Chem* 1993, **268:**22777–22781.
54. Seiberler S, Scheiner O, Kraft D, *et al.*: **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.
55. Twardosz A, Hayek B, Seiberler S, *et al.*: **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.
56. Engel E, Richter K, Obermeyer G, *et al.*: **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.
57. Valenta R, Hayek B, Seiberler S, *et al.*: **Calcium-binding allergens: from plants to man.** *Int Arch Allergy Immunol* 1998, **117:**160–166.
58. Karamloo F, Wangorsch A, Kasahara H, *et al.*: **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.
59. Cadot P, Diaz JF, Proost P, *et al.*: **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.
60. Mahler V, Fischer S, Heiss S, *et al.*: **cDNA cloning and characterization of a cross-reactive birch pollen allergen: identification as a pectin esterase.** *Int Arch Allergy Immunol* 2001, **124:**64–66.
61. Kazemi-Shirazi L, Niederberger V, Linhart B, *et al.*: **Recombinant marker allergens: Diagnostic gate keepers for therapy of allergy.** *Int Arch Allergy Immunol* 2002, **127:**259–268.
62. Valenta R, Lidholm J, Niederberger V, *et al.*: **The recombinant allergen-based concept of component-resolved diagnostics and immunotherapy (CRD and CRIT).** *Clin Exp Allergy* 1999, **29:**896–904.
63. Hiller R, Laffer S, Harwanegg C, *et al.*: **Microarrayed allergen molecules: diagnostic gatekeepers for allergy treatment.** *FASEB J* 2002, **16:**414–416.
64. Harwanegg C, Laffer S, Hiller R, *et al.*: **Microarrayed recombinant allergens for diagnosis of allergy.** *Clin Exp Allergy* 2003, **33:**7–13.

65.• Westritschnig K, Sibanda E, Thomas W, *et al.*: **Analysis of the sensitization profile towards allergens in central Africa.** *Clin Exp Allergy* 2003, **33:**22–27.

This paper reveals unexpected sensitization profiles in a Central African population of allergic patients.

66. Ball T, Sperr WR, Valent P, *et al.*: **Induction of antibody responses to new B cell epitopes indicates vaccination character of allergen immunotherapy.** *Eur J Immunol* 1999, **29:**2026–2036.

67.• Mothes N, Heinzkill M, Drachenberg KJ, *et al.*: **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:**1–11.

Using recombinant allergens for monitoring of immunotherapy, the importance of blocking antibodies has been reevaluated.

68.• Moverare R, Elfman L, Vesterinen E, *et al.*: **Development of new IgE specificities to allergenic components in birch pollen extract during specific immunotherapy studied with immunoblotting and Pharmacia CAP System.** *Allergy* 2002, **57:**423–430.

Using recombinant allergens, the authors discover that extract-based immunotherapy can induce de novo IgE sensitization in patients.

69. van Hage-Hamsten M, Valenta R: **Specific immunotherapy: the induction of new IgE-specificities?** *Allergy* 2002, **57:**375–358.

70.• Valenta R: **The future of antigen-specific immunotherapy of allergy.** *Nat Rev Immunol* 2002, **2:**446–453.

The mechanisms of immunotherapy and future therapy strategies are discussed.

71.• Valenta R, Ball T, Focke M, *et al.*: **Immunotherapy of allergic disease.** *Adv Immunol* 2004, **82:**105–153.

The mechanisms of immunotherapy and future therapy strategies are discussed.

72. Westritschnig K, Valenta R: **Can we genetically engineer safer and more effective immunotherapy reagents?** *Curr Opin Allergy Immunol* 2003, **3:**495–500.