

Recombinant Marker Allergens: Diagnostic Gatekeepers for the Treatment of Allergy

Lili Kazemi-Shirazi^{a,b} Verena Niederberger^{b,c} Birgit Linhart^b
Jonas Lidholm^d Dietrich Kraft^b Rudolf Valenta^b

^aDivision of Gastroenterology and Hepatology, Department of Internal Medicine IV,

^bDepartment of Pathophysiology and ^cDepartment of Otorhinolaryngology, University of Vienna, Austria;

^dPharmacia Diagnostics, Uppsala, Sweden

Key Words

Allergy · IgE · Cross-reactivity · Recombinant allergen ·
Marker allergen · Immunotherapy

Abstract

During the past decade an increasing number of recombinant allergens have become available, representing a significant proportion of the epitope complexity of natural allergen extracts. Component-resolved diagnosis with recombinant allergens reveals the antibody reactivity profile of allergic patients and identifies the disease-eliciting allergen molecules. This article exemplifies how recombinant allergen molecules with high cross-reactive potential can be used as marker allergens to identify allergic patients who are cross-sensitized to a variety of allergen sources. It further demonstrates how the use of allergens with a restricted distribution in a certain group of allergen sources may allow the identification of patients who have been genuinely sensitized by a particular allergen molecule. Drawing from those examples, it is suggested how diagnostic tests based on such recombi-

nant marker allergens may be used to improve the choice and monitoring of currently available forms of specific immunotherapy.

Copyright © 2002 S. Karger AG, Basel

Introduction

Allergic individuals are characterized by their increased tendency to mount IgE antibody responses against antigens per se harmless (i.e. allergens) [1]. Diagnosis of type I allergy is hence based on the detection of allergen-specific IgE antibodies in serum or other body fluids or on positive provocation tests with the relevant allergens [2]. Already in 1880, long before the discovery of IgE antibodies, Charles Blackley performed allergy provocation tests with allergen extracts [3]. While it is possible to use allergen extract-based diagnostic tests to define allergen-containing sources they are not designed to identify the disease-eliciting allergen molecules [4]. With the introduction of molecular biology techniques to the field of allergen characterization, the molecular identities of many important allergens have been revealed and an increasing number of recombinant allergens has become available [5–7]. For many important allergen sources (e.g. tree pollen, grass pollen, mites, animal dander, moulds,

L.K.-S. and V.N. contributed equally.

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

© 2002 S. Karger AG, Basel
1018–2438/02/1274–0259\$18.50/0

Accessible online at:
www.karger.com/journals/iaa

Correspondence to: Prof. Dr. Rudolf Valenta
Molecular Immunopathology Group
Department of Pathophysiology, University of Vienna Medical School
Vienna General Hospital, Währinger Gürtel 18–20, A–1090 Vienna (Austria)
Tel. +43 1 40400 5108, Fax +43 1 40400 5130, E-Mail Rudolf.Valenta@akh-wien.ac.at

venoms), it has been possible to produce panels of recombinant allergens which may be equivalent in epitope complexity to the traditional extracts, even for diagnostic screening purposes [4, 6, 8–13]. Furthermore it has been shown that spiking of allergen extracts with recombinant allergens that are not well represented in allergen extracts can improve the sensitivity of extract-based diagnostic tests [14]. Diagnostic tests exclusively based on single recombinant allergens, allowing the precise measurement of levels of IgE antibodies to a particular allergen molecule and thus comparison of specific IgE levels with the allergenic potency of that determinant are available [4, 15]. In addition, diagnostic tests based on multiple microarrayed allergen molecules (i.e., allergen chips) are currently being developed which allow the complex reactivity profile of allergic patients to be determined using minute amount of serum in a single and fast assay [16]. Component-resolved allergy diagnosis based on recombinant allergen molecules enables analysis of the complex reactivity profile of allergic patients [4]. Using component-resolved allergy diagnosis, it is possible to precisely identify the disease-eliciting allergens for each patient and thus to group patients according to the molecular multiplicity of their sensitization, e.g. patients who are sensitized against few allergen molecules (i.e. mono- or oligosensitized) and those who are polysensitized against a great variety of immunologically unrelated allergen molecules. The latter group cannot not be precisely identified with allergen extracts because sensitization to highly cross-reactive allergens present in different allergen extracts might be interpreted as cosensitization to different allergens and thus lead to the incorrect diagnosis of polysensitization [17]. Component-resolved allergy diagnosis delivers precise information about the individual patient's sensitization profile and as a logic next step, component-resolved forms of immunotherapy are being developed [18]. For this purpose, recombinant and synthetic allergen molecules with reduced allergenic activity have been constructed which should minimize the risk of anaphylactic side effects [18–21]. Several of these molecules have been evaluated regarding safety in clinical provocation studies and have already entered clinical immunotherapy studies [22–25]. However, new forms of specific immunotherapy based on recombinant allergen molecules are not yet routinely available. It is therefore the aim of this article to illustrate how recombinant allergen-based diagnostics can assist the clinician in choosing optimal strategies among available forms of treatment (e.g. symptomatic treatment, allergen extract-based immunotherapy) and to monitor the success of the therapeutic measures applied.

Allergies to Plant Products: Pollen and Plant-Derived Foods

We illustrate the concept of using recombinant marker allergens from plants for refined allergy diagnosis for two reasons. First, allergies to plant products (e.g. pollen, vegetables, fruits, nuts and seeds) are very common and affect more than 75% of allergic patients [26]. Secondly, the allergen spectrum of grass and tree pollens and plant foods has been intensely studied, leading to the production of a large panel of well-characterized recombinant plant allergens [4, 8–10, 27]. It should, however, be mentioned that the principle of using marker allergens for diagnosis is applicable to other allergen sources as well.

Certain plant allergens representing highly conserved proteins with important biological functions are produced as highly cross-reactive allergens in a variety of botanically unrelated plants and also in different plant tissues (e.g., pollen, fruits, leaves, seeds). Other allergens are produced exclusively in certain plant tissues (e.g. pollen-specific expression) or in certain plants. On the basis of the presence of these allergens in certain plants, plant tissues and their degree of cross-reactivity, it is possible to use these allergens as markers to obtain additional important diagnostic information. Examples for such marker allergens are given below.

Profilins: Predictors for Pollen and Plant Food Cross-Sensitization

Profilins were discovered as highly cross-reactive allergens and important components of the plant cytoskeleton by the isolation of a cDNA coding for a cross-reactive birch pollen allergen and its identification as profilin [28]. At that time, profilins were established as important components of the eukaryotic cytoskeleton (i.e. actin-binding proteins) and elements in signal transduction, but they were not described in plants. Because of their highly conserved biological function, profilins from various organisms share sequence homology, interact with ligands (e.g. actin) from other organisms and have preserved their three-dimensional structure [29–33]. Accordingly, there is extensive cross-reactivity of allergic patients' IgE antibodies with profilins from different plant sources but also with profilin from other organisms, including man. Profilins are well established as clinically relevant cross-reactive allergens in tree, grass and weed pollens and are also expressed at lower levels in somatic plant tissues (e.g. fruits, leaves, seeds, roots). Therefore, allergic individuals

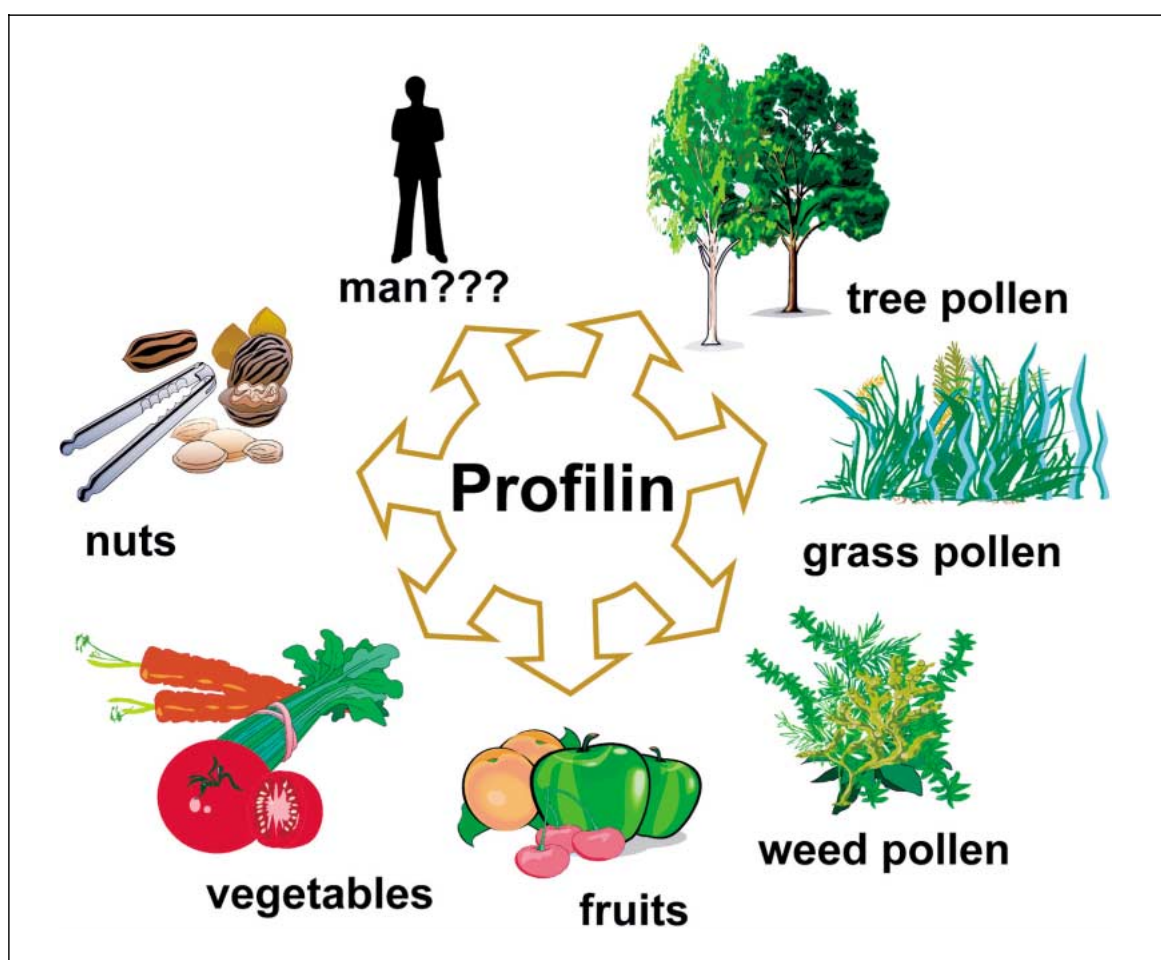


Fig. 1. Profilin, a highly cross-reactive panallergen. Profilins are structurally conserved eukaryotic proteins which have been described as allergens in trees, grasses, weed pollens, fruits, vegetables, nuts, latex and even in man. Profilin-sensitized patients either exhibit clinically relevant reactivity to several of the displayed allergen sources or are at risk of developing allergic symptoms.

producing IgE antibodies towards profilin frequently belong to a group of patients who either suffer from manifest allergic reactions to pollens from various plants and plant-derived foods or are at least at risk to mount clinically significant reactions towards a variety of profilin-containing allergen sources (fig. 1). Whether the levels of profilin-specific IgE antibodies, the amount and availability of profilin in the different allergen sources or the route and/or mode of exposure determine the extent of clinically relevant allergic reactions to profilin-containing allergen sources has not yet been fully investigated. However, the extensive cross-reactivity of profilin-specific IgE antibodies and the *in vivo* allergenic activity of profilins have been thoroughly documented [34–37]. Sensitization to

profilin can be expected in different populations at levels varying between 5 and 40% depending on exposure to various profilin-containing allergen sources [38]. The presence of profilin-specific IgE antibodies in allergic patients may be one, but not the only reason for cross-sensitization to many unrelated allergen sources. Since allergen extracts used for immunotherapy are primarily standardized regarding the presence of major allergens, but not regarding the presence of profilin, it is possible that profilin-reactive patients will benefit less from allergen-specific immunotherapy. The appearance of detectable levels of profilin-specific IgE antibodies in allergic patients during the normal course of allergic disease or during or after immunotherapy deserves attention as it

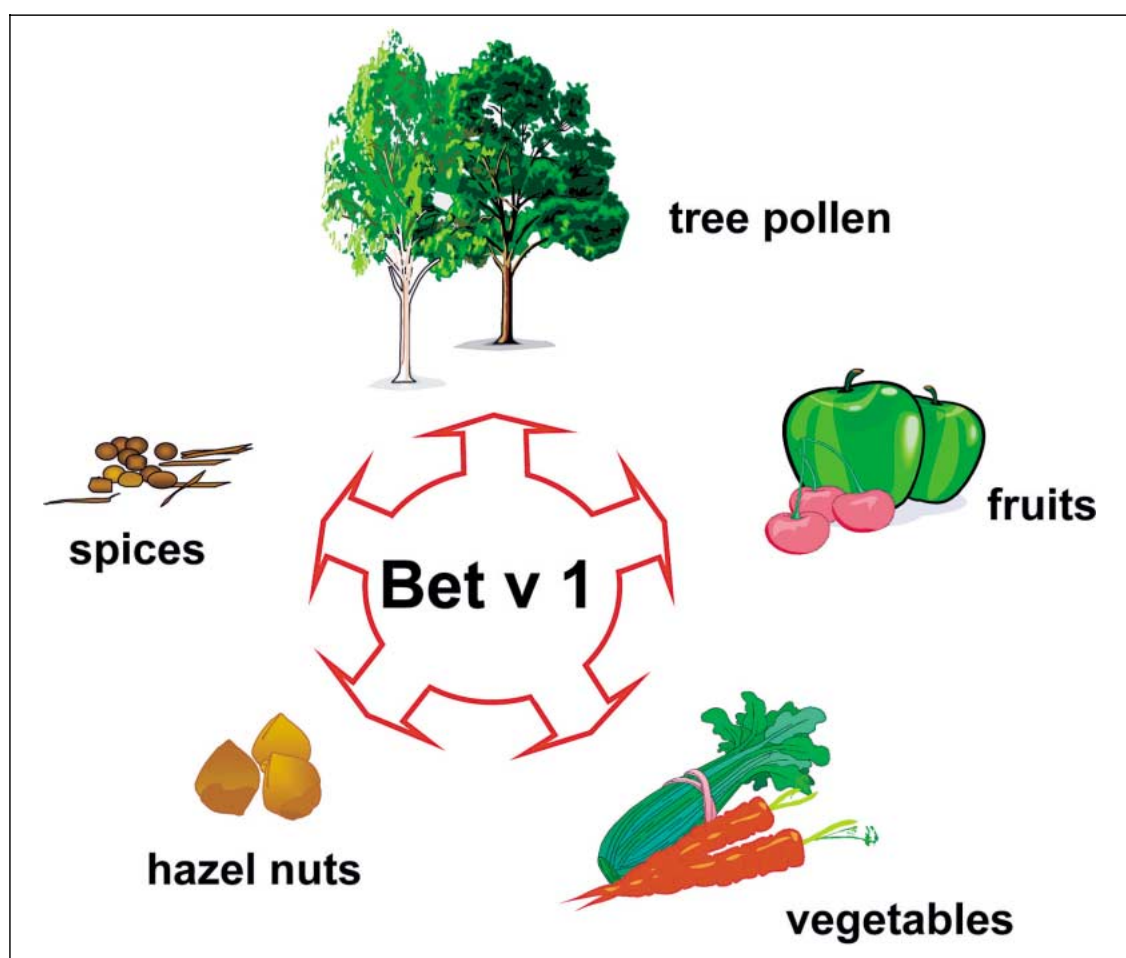


Fig. 2. Bet v 1, the major birch pollen allergen, cross-reacts with allergens in *Fagales* pollens and plant-derived foods. The presence of allergens cross-reacting with the major birch pollen allergen, Bet v 1, has been demonstrated for pollens of trees belonging to the order *Fagales* (birch, alder, hazel, hornbeam, oak), fruits, vegetables, certain nuts and spices. Patients showing Bet v 1-specific IgE either suffer from allergies to the displayed allergen sources or are at risk of progressing to allergic symptoms.

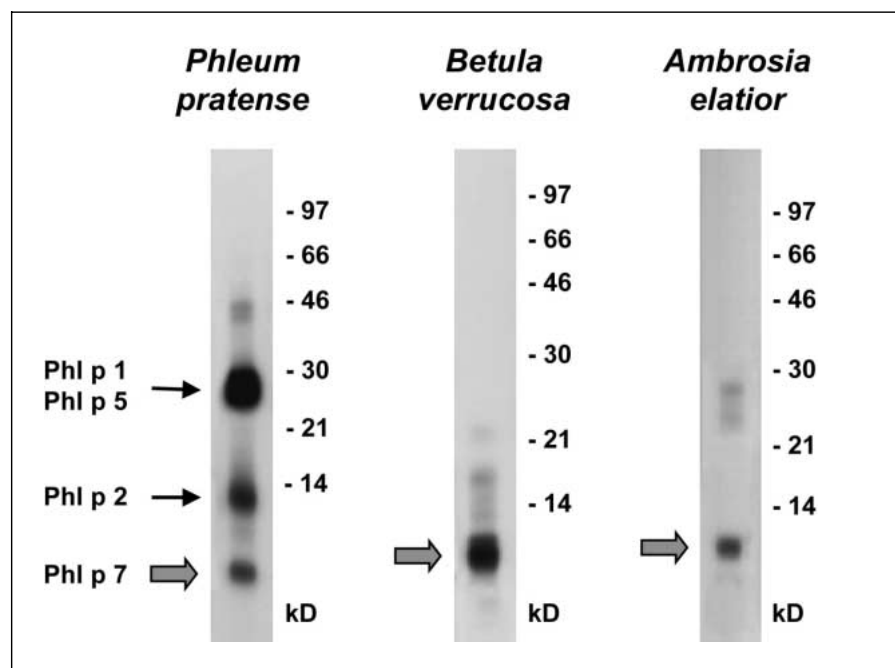
may indicate that the patient is at risk of broadening his or her sensitivity towards several allergen sources [39].

Bet v 1, the Major Birch Pollen Allergen: Predictor for *Fagales* Pollen and Plant Food Sensitization

Bet v 1, a 17-kD protein, is the major allergen of birch pollen [40]. More than 95% of birch pollen-allergic patients show IgE reactivity to Bet v 1 and more than 60% are exclusively sensitized to Bet v 1 in birch pollen [41]. Bet v 1-specific IgE antibodies cross-react with Bet v 1-related allergens in pollens of trees belonging to the order

Fagales, in particular with *Aln g 1* (alder), *Cor a 1* (hazel) and *Car b 1* (hornbeam) but pollens from other trees contain Bet v 1-related proteins (oak, chestnut) as well [8, 42, 43]. Consequently, patients suffering from birch pollen allergy can exhibit allergic symptoms also upon exposure to pollens from trees belonging to the order *Fagales* due to IgE-mediated cross-reactivity to Bet v 1-homologous allergens in these pollens. Furthermore, it has been demonstrated that various plant foods (fruits, vegetables and spices) contain Bet v 1-related allergens (e.g. carrot: *Dau c 1*; celery: *Api g 1*; apple: *Mal d 1*; cherry: *Pru av 1*; hazelnut, but not peanut or walnuts) [44–49]. Likewise, birch pollen allergy can be associated with IgE-mediated hypersensitivity to spices due to cross-reactivity with Bet

Fig. 3. IgE reactivity to two-EF-hand calcium-binding allergens present in pollens of botanically unrelated grasses, trees and weeds. A patient with IgE antibodies against the calcium-binding allergen from timothy grass, Phl p 7, exhibits IgE cross-reactivity with related allergens in birch and ragweed pollens.



v 1-related allergens [50, 51]. Allergic patients showing Bet v 1-specific IgE-reactivity are therefore not only sensitized to birch pollen and pollens of trees belonging to the *Fagales* order but frequently also suffer from allergies to plant-derived foods (fig. 2). Allergen-specific immunotherapy of Bet v 1-reactive patients with birch pollen extract is indicated according to the guidelines for specific immunotherapy because most birch pollen extracts contain sufficient amounts of Bet v 1 and are mostly standardized regarding their Bet v 1 contents. Since Bet v 1 cross-reacts with the major *Fagales* pollen allergens and related plant food allergens immunotherapy with birch pollen extract may improve allergy to *Fagales* pollens and the concomitant oral allergy syndrome [52, 53]. Since Bet v 1 is the most important and abundant allergen in birch pollen and most birch pollen-specific IgE is directed against rBet v 1 [8], it is likely that patients who produce IgE antibodies to birch pollen extract but lack Bet v 1-specific IgE were sensitized against cross-reactive allergens from other sources (e.g. profilins or EF-hand allergens from grasses). Such patients may appear positive in a birch pollen extract-based test due to cross-reactivity with other homologous proteins represented in birch pollen but are probably not genuinely sensitized to birch (fig. 3, 4). For this reason, and because birch pollen extracts used for immunotherapy are primarily standardized regarding their Bet v 1 contents, it is suggested not to treat such

patients with birch pollen-specific immunotherapy. It has also been reported that birch pollen-allergic patients can develop IgE antibodies against cross-reactive birch pollen allergens other than Bet v 1 (e.g. birch profilin: Bet v 2; two-EF-hand allergens from birch: Bet v 4) in the course of immunotherapy [39], possibly as a result of the treatment. Such patients deserve attention in order to clarify whether such induction of IgE antibodies against cross-reactive allergens can lead to clinically relevant sensitization to other allergen sources (e.g., grass, weed pollens).

Two-EF-Hand Calcium-Binding Pollen Allergens: Predictors for Pollen Polysensitization

Calcium-binding proteins containing two binding sites for calcium, termed EF-hands, have been identified in pollens of numerous plants (trees, grasses, weeds) [reviewed in ref. 54, 55]. These allergens are remarkable because they are produced preferentially in pollen but not in other plant tissues. Moreover, two-EF-hand allergens from various pollens (alder: Aln g 4; birch: Bet v 4; olive: Ole e 3; ash: Fra e 3; Bermuda grass: Cyn d 7; timothy grass: Phl p 7; rape: Bra n 1, 2, Bra r 1, 2) contain cross-reactive IgE epitopes [56–64]. Patients producing IgE antibodies against two-EF-hand pollen allergens therefore

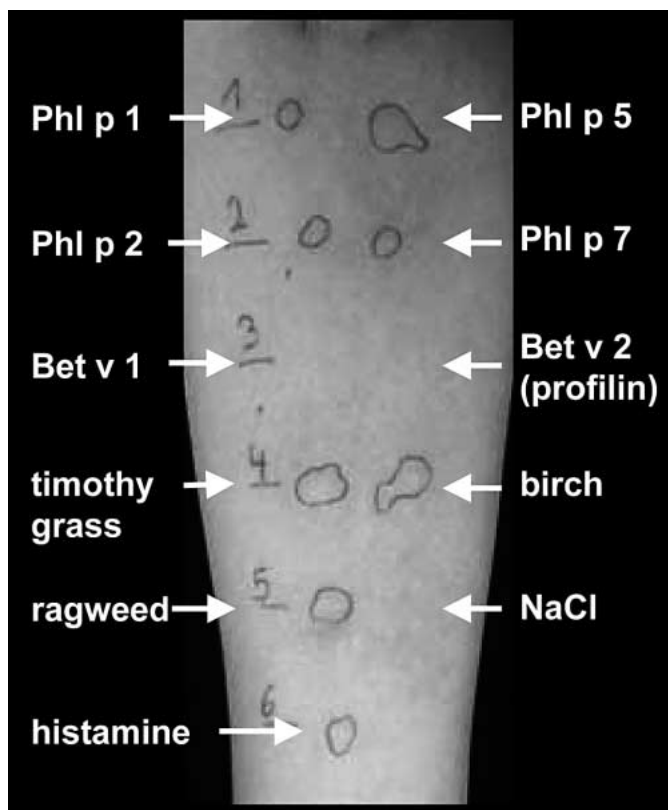


Fig. 4. Clinically relevant sensitization to cross-reactive calcium-binding allergens. The patient lacks IgE antibodies and skin reactivity to Bet v 1 and Bet v 2 and birch pollen is therefore unlikely as a primary sensitizer. IgE reactivity to Phl p 1, Phl p 2 and Phl p 5 demonstrates sensitization to grass pollen and in addition there are IgE antibodies to the calcium-binding allergen Phl p 7. The immediate type skin reaction to birch pollen extract is apparently caused by IgE cross-reactivity between Phl p 7 and the homologous protein in birch pollen, Bet v 4.

belong to a group of patients who are either already allergic or are at risk of developing allergic symptoms upon contact with pollens from a broad spectrum of plants [65, 66]. Two-EF-hand calcium-binding allergens may therefore be considered as markers for broad pollen sensitization, but are not implicated in allergy to plant-derived foods because they have as yet not been detected in somatic plant tissues. Figures 3 and 4 show *in vitro* and *in vivo* test results for a 27-year-old male patient with allergic symptoms during the flowering period of trees, grasses and weeds (spring to autumn). Skin prick testing and serology with allergen extracts yielded positive test results for numerous tree pollens including birch, alder, hazel, olive, grass pollens (timothy grass, rye grass, Bermuda grass) and weed pollens (ragweed, mugwort, parietaria).

Immunoblot results demonstrated that reactivity to birch pollen and ragweed pollen were mainly due to a two-EF-hand allergen from birch and ragweed which cross-reacts with the Phl p 7 allergen from timothy grass pollen (fig. 3). Skin testing with recombinant allergens from timothy grass and birch showed that this patient was sensitized to the two-EF-hand allergen from timothy grass, Phl p 7, in addition to major timothy grass pollen allergens (Phl p 1, Phl p 2, Phl p 5), but did not react with the major birch pollen allergen, Bet v 1, or birch profilin, Bet v 2 (fig. 4). Although only about 5–10% of pollen-allergic patients are cross-sensitized against two-EF-hand pollen allergens, these patients should be identified because of their multiple pollen sensitivity. Indication for immunotherapy in such patients needs careful consideration. The patient shown in figures 3 and 4 will probably not benefit from immunotherapy with birch pollen or ragweed pollen extract because of his unusual exclusive sensitization to the two-EF-hand allergen and the lack of IgE against the major allergens in birch (Bet v 1) and ragweed (Amb a 1). Whether immunotherapy with grass pollen extract can be expected to be effective is difficult to predict because currently available pollen allergen extracts used for immunotherapy are not standardized regarding their two-EF-hand allergen contents.

Similar as for profilin, it has been reported that patients who had received birch pollen-specific immunotherapy developed detectable antibody levels against two-EF-hand allergens in birch (i.e. Bet v 4). It may therefore be important to use cross-reactive allergens such as profilins and two-EF-hand allergens to monitor pollen-allergic patients during the natural course of their disease, and especially during and after immunotherapy with pollen extracts to detect the development of IgE reactivities against such structures [39, 67]. Although not yet proven, it cannot be excluded that induction of such cross-reactive IgE antibodies may result in a broadening of clinically relevant sensitivity.

Grass Pollen-Specific Marker Allergens

Grass pollen allergens belong to the most frequent and potent elicitors of allergic symptoms. Due to the worldwide occurrence of grasses and their abundant pollen production, it can be estimated that more than 40% of allergic patients are sensitized against grasses [68]. Pollen from the different grass species, corn and other monocotyledonous plants contain a variety of different allergen molecules with cross-reactive potential. For example, profilins and two EF-hand allergens have been described in

grasses and shown to cross-react with homologous allergens from botanically unrelated plants (e.g. trees, weeds) [61–63, 69]. On the other hand, it is well established that certain grass pollen allergens (groups 1, 2, 5, 6) are exclusively present in pollen of grasses [70]. The latter four allergens are of particular interest because they can be used as diagnostic markers for a genuine grass pollen sensitization. Group 1 grass pollen allergens with a molecular weight of approximately 30 kD are the most frequently recognized grass pollen allergens [71]. They are expressed in all grasses, corn and in many other monocotyledonous plants [72]. Approximately 90% of grass pollen-allergic patients show IgE antibody reactivity to group 1 allergens [72, 73]. Group 2 grass pollen allergens, with a molecular weight of 10–12 kD, are recognized by IgE antibodies of approximately 60% of grass pollen-allergic individuals [73, 74]. In contrast to group 1 allergens, IgE-reactive group 2 allergens are not expressed in pollens of all grass species; for example, they were not detected in *Cynodon dactylon*, *Zea mays* and *Phragmites australis* [10].

Group 5 allergens are important and major grass pollen allergens with high allergenic activity in IgE-binding capacity [75–79]. Approximately 80% of grass pollen-allergic patients react with group 5 allergens which occur as proteins with a molecular weight of approximately 30 kD in a variety of grass species (e.g. timothy grass (*Phleum pratense*), rye (*Secale cereale*), Kentucky bluegrass (*Poa pratense*) and rye grass (*Lolium perenne*). However, they could not be detected in *Z. mays* and *C. dactylon* [10].

Group 6 allergens are proteins with a molecular weight of approximately 10 kD, which are recognized by 60–70% of grass pollen-allergic patients [80–82] and have been detected in only a few grass species (e.g. timothy grass, Kentucky bluegrass) [82]. Due to the specific expression of group 1, 2, 5, and 6 allergens in grass pollens and their absence in other plants or plant products, these allergens can be used as marker allergens for the diagnosis of grass pollen allergy. Diagnostic tests based on recombinant Phl p 1, rPhl p 2, rPhl p 5 and rPhl p 6 will thus specifically detect patients with a genuine sensitization to grasses. rPhl p 2, rPhl p 5 and rPhl p 6 should be useful tools to further define the potentially sensitizing grass for a given patient. For example, patients showing rPhl p 1-specific IgE but lacking IgE to rPhl p 2, rPhl p 5, rPhl p 6 are likely sensitized to certain grass or monocot pollens (e.g. *C. dactylon*, *Z. mays*) [10]. On the other hand, patients with rPhl p 6-specific IgE are likely sensitized preferentially towards timothy grass or Kentucky bluegrass [82]. Patients lacking IgE antibodies against any of the four recombinant marker allergens may appear positive in

grass pollen extract-based diagnostic tests because of IgE binding to other grass pollen proteins with cross-reactive potential, but may have been sensitized towards allergen sources other than grass pollen.

Because of the latter arguments and the fact that grass pollen extracts used for immunotherapy are mainly standardized regarding their contents of group 1, 2, and 5 allergens, we would recommend caution against immunotherapy with grass pollen extracts in patients lacking IgE antibodies against any of the four marker allergens (i.e. rPhl p 1, rPhl p 2, rPhl p 5, rPhl p 6).

Summary, Conclusions and Suggestions

Well-defined recombinant allergens may be used as diagnostic marker allergens to characterize allergic patients and to select optimal therapy strategies. IgE reactivity to highly cross-reactive allergens allows to identify patients with a broad sensitization or who are at risk of developing allergic symptoms to many allergen sources. On the other hand, allergens with a very restricted occurrence in certain allergen sources can be used to identify patients with genuine sensitization to those sources.

Pollen Profilins (e.g. Birch: Bet v 2; Timothy Grass: Phl p 12)

Profilins occur as highly cross-reactive allergens in a variety of botanically unrelated plants and plant products. Patients producing specific IgE antibodies are either sensitized or at risk of developing allergic reactions to various plant pollens and plant-derived foods. Patients with profilin-based polysensitization may be difficult to treat with current extract-based forms of immunotherapy. The appearance of profilin-specific IgE reactivity in the course of natural allergic disease or during immunotherapy warrants monitoring of the patients for signs of broadening sensitivity towards multiple allergen sources.

The Major Birch Pollen Allergen, Bet v 1

Bet v 1 is the major allergen in birch pollen and cross-reacts with the major pollen allergens from *Fagales* trees and a variety of plant food allergens. Patients reacting with Bet v 1 may be suited for immunotherapy with birch pollen extract whereas a lack of specific reactivity to Bet v 1 suggests that birch pollen-specific immunotherapy may not be the most appropriate treatment. There is evidence that successful birch pollen-specific immunotherapy may have beneficial effects on allergy to *Fagales* pollen allergy and perhaps on certain forms of plant food allergy.

Two-EF-Hand Calcium-Binding Pollen Allergens (e.g. Phl p 7, Bet v 4)

These occur as highly cross-reactive allergens in pollens from a variety of plant species but have not been detected in plant-derived foods. Patients carrying IgE antibodies specific for two-EF-hand pollen allergens frequently suffer from multiple pollen allergies and may be difficult to treat with specific immunotherapy based on pollen extract.

Grass Pollen-Specific Marker Allergens (e.g. Phl p 1, Phl p 2, Phl p 5, Phl p 6)

These allergens may serve as highly specific markers for sensitization to grass pollen. Accordingly, patients

with reactivity to these allergens should be suited for grass pollen-specific immunotherapy whereas patients lacking IgE to any of the four molecules should be investigated for identification of a more likely sensitizer. Since Phl p 2, Phl p 5 and Phl p 6 are not present in all grass species, they may be instrumental in the selection of appropriate grass pollen extract for immunotherapy.

Acknowledgments

This study was supported by grant Y078GEN from the Austrian Science Fund, by the ICP program of the Austrian Federal Ministry for Education, Science and Culture and by Pharmacia Diagnostics, Uppsala, Sweden.

References

- 1 Wills-Karp M, Santeliz J, Karp CL: The germless theory of allergic disease: Revisiting the hygiene hypothesis. *Nat Rev Immunol* 2001;1: 69–75.
- 2 Kay AB: *Allergy and Allergic Diseases*. Oxford, Blackwell Science, 1997.
- 3 Blackley CH: *Hay Fever: Its Causes, Treatment and Effective Prevention*. London, Baillière, 1880.
- 4 Valenta R, Lidholm J, Niederberger V, Hayek B, Kraft D, Grönlund H: The recombinant allergen-based concept of component-resolved diagnosis and immunotherapy (CRD and CRIT). *Clin Exp Allergy* 1999;29:896–904.
- 5 Valenta R, Kraft D: Recombinant allergens for diagnosis and therapy of allergic diseases. *Curr Opin Immunol* 1995;7:751–756.
- 6 Thomas WR, Smith W: Towards defining the full spectrum of important house dust mite allergens. *Clin Exp Allergy* 1999;29:1583–1587.
- 7 Chapman MD, Smith AM, Vailes LD, Arruda LK, Dhanaraj V, Pomes A: Recombinant allergens for diagnosis and therapy of allergic disease. *J Allergy Clin Immunol* 2000;106:409–418.
- 8 Niederberger V, Pauli G, Grönlund H, Fröschl R, Rumpold H, Kraft D, Valenta R, Spitzauer S: Recombinant birch pollen allergens (rBet v 1, rBet v 2) contain most of the IgE epitopes present in birch, alder, hornbeam, hazel, and oak pollen. A quantitative IgE inhibition study using sera from different populations. *J Allergy Clin Immunol* 1998;102:579–591.
- 9 Rodriguez R, Villalba M, Monsalve RI, Batañero E: The spectrum of olive pollen allergens. *Int Arch Allergy Immunol* 2001;125:185–195.
- 10 Niederberger V, Laffer S, Fröschl R, Kraft D, Rumpold H, Kapiotis S, Valenta R, Spitzauer S: IgE antibodies to recombinant pollen allergens (Phl p 1, Phl p 2, Phl p 5, and Bet v 2) account for a high percentage of grass pollen-specific IgE. *J Allergy Clin Immunol* 1998;101: 258–264.
- 11 Spitzauer S: Allergy to mammalian proteins: At the borderline between foreign and self? *Int Arch Allergy Immunol* 1999;120:259–269.
- 12 Cramer R: Recombinant *Aspergillus fumigatus* allergens: From the nucleotide sequences to clinical applications. *Int Arch Allergy Immunol* 1998;115:99–114.
- 13 King TP, Spangfort MD: Structure and biology of stinging insect venom allergens. *Int Arch Allergy Immunol* 2000;123:99–106.
- 14 Lundberg M, Chen Z, Rihs HP, Wrangsjö K: Recombinant spiked allergen extract. *Allergy* 2001;56:794–795.
- 15 Niederberger V, Stübner P, Spitzauer S, Kraft D, Valenta R, Ehrenberger K, Horak F: Skin test results but not serology reflect immediate type respiratory sensitivity: A study performed with recombinant allergen molecules. *J Invest Dermatol* 2001;117:848–851.
- 16 Hiller R, Laffer S, Harwanegg C, Huber M, Schmidt WM, Twardosz A, Barletta B, Becker WM, Blaser K, Breiteneder H, Chapman M, Cramer R, Duchene M, Ferreira F, Fiebig H, Hoffman-Sommergruber K, King TP, Kleber-Janke T, Kurup VP, Leher SB, Lidholm J, Müller U, Pini C, Reese G, Scheiner O, Scheynius A, Shen HD, Spitzauer S, Suck R, Swoboda I, Thomas W, Tinghino R, van Hage-Hamsten M, Virtanen T, Kraft D, Müller MW, Valenta R: Microarrayed allergen molecules: Diagnostic gatekeepers for allergy therapy. *FASEB* 2002;16:414–416.
- 17 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.
- 18 Valenta R, Kraft D: Recombinant allergens; in Hansel TJ, Barnes PJ (eds): *New Drugs for Allergy, Asthma and COPD*. Prog Resp Res. Basel, Karger, 2001, pp 195–200.
- 19 Valenta R, Vrtala S, Focke-Tejkl M, Bugajska-Schretter A, Ball T, Twardosz A, Spitzauer S, Grönlund H, Kraft D: Genetically engineered and synthetic allergen derivatives: Candidates for vaccination against type I allergy. *Biol Chem* 1999;380:815–824.
- 20 Singh MB, de Weerd N, Bhalla PL: Genetically engineered plant allergens with reduced anaphylactic activity. *Int Arch Allergy Immunol* 1999;119:75–85.
- 21 Valenta R, Vrtala S, Focke-Tejkl M, Twardosz A, Swoboda I, Bugajska-Schretter A, Spitzauer A, Kraft D: Synthetic and genetically engineered allergen derivatives for specific immunotherapy of type I allergy; in Zweiman B, Schwartz LB (eds): *Inflammatory Mechanisms in Allergic Diseases*. Clin Allergy Immunol. New York, Marcel Dekker, 2001, vol 16, pp 495–517.
- 22 Van Hage-Hamsten M, Kronqvist M, Zetterström O, Johansson E, Niederberger V, Vrtala S, Grönlund H, Grönneberg R, Valenta R: Skin test evaluation of genetically engineered hypoallergenic derivatives of the major birch pollen allergen, Bet v 1. Results obtained with a mix of two recombinant Bet v 1 fragments and rBet v 1 trimer in a Swedish population before the birch pollen season. *J Allergy Clin Immunol* 1999;104:969–977.
- 23 Pauli G, Purohit A, Oster JP, de Blay F, Vrtala S, Niederberger V, Kraft D, Valenta R: Clinical evaluation of genetically engineered hypoallergenic rBet v 1 derivatives by skin prick and intradermal testing: Results obtained in a French population. *Clin Exp Allergy* 2000;30: 1076–1084.
- 24 Nopp A, Haldén G, Lundahl J, Johansson E, Vrtala S, Valenta R, Grönneberg R, van Hage-Hamsten M: Genetically engineered hypoallergenic derivatives of the major birch pollen allergen, Bet v 1, induce less eosinophilic activity in skin chamber fluids collected from birch pollen allergic patients than rBet v 1 wild type. *J Allergy Clin Immunol* 2000;106:101–109.

- 25 Arquint O, Helbing A, Crameri R, Ferreira F, Breitenbach M, Pichler WJ: Reduced in vivo allergenicity of Bet v 1d isoform, a natural component of birch pollen. *J Allergy Clin Immunol* 1999;104:1239–1243.
- 26 Wüthrich B, Schindler C, Leuenberger P, Ackermann-Lieblich U: Prevalence of atopy and pollinosis in the adult population of Switzerland (SALPADIA study). Swiss study on air pollution and lung diseases in adults. *Int Arch Allergy Immunol* 1995;106:149–156.
- 27 Breiteneder H, Ebner C: Molecular and biochemical classification of plant-derived food allergens. *J Allergy Clin Immunol* 2000;106:27–36.
- 28 Valenta R, Duchêne 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.
- 29 Valenta R, Duchêne 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.
- 30 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.
- 31 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.
- 32 Valenta R, Ferreira F, Grote M, Swoboda I, Vrtala S, Duchêne M, Deviller P, Meagher RB, McKinney E, Heberle-Bors E, Kraft D, Scheiner O: Identification of profilin as an actin-binding protein in higher plants. *J Biol Chem* 1993;268:22777–22781.
- 33 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.
- 34 Van Ree R, Fernandez-Rivas M, Cuevas M, van Wijngaarden M, Aalberse RC: Pollen-related allergy to peach and apple: An important role for profilin. *J Allergy Clin Immunol* 1995;95:726–734.
- 35 Ferreira F, Mayer P, Sperr WR, Valent P, Seiberler S, Ebner C, Liehl E, Scheiner O, Kraft D, Valenta R: Induction of IgE antibodies with predefined specificity in rhesus monkeys with recombinant birch pollen allergens, Bet v 1 and Bet v 2. *J Allergy Clin Immunol* 1996;97:95–103.
- 36 Pauli G, Oster J, Deviller P, Heiss S, Bessot J, Susani M, Ferreira F, Kraft D, Valenta R: Skin testing with recombinant allergens rBet v 1 and rBet v 2: Diagnostic value for birch pollen and associated allergies. *J Allergy Clin Immunol* 1996;97:1100–1109.
- 37 Heiss S, Mahler V, Steiner R, Spitzauer S, Schweiger C, Kraft D, Valenta R: Component-resolved diagnosis (CRD) of type I allergy with recombinant grass and tree pollen allergens by skin testing. *J Invest Dermatol* 1999;113:830–837.
- 38 Elfman L, Svensson M, Lidholm J, Pauli G, Valenta R: Different profiles in specific IgE to rBet v 1 and rBet v 2 in patients allergic to birch pollen from six countries. *Int Arch Allergy Immunol* 1997;113:249–251.
- 39 Movérare R, Elfman L, Vesterinen E, Metso T, Haahtela T: Development of new IgE reactivities to allergenic components in pollen extracts during specific immunotherapy studied with immunoblotting and the Pharmacia CAP system. *Allergy*, in press.
- 40 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.
- 41 Valenta R, Duchêne 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.
- 42 Rohac M, Birkner T, Reimitzer I, Bohle B, Steiner R, Breitner M, Kraft D, Scheiner O, Gabel F, Rumpold H: The immunological relationship of epitopes on major tree pollen allergens. *Mol Immunol* 1991;28:897–906.
- 43 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 1. *Biochem Biophys Res Commun* 1993;196:1086–1092.
- 44 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.
- 45 Hirschwehr R, Valenta R, Ebner C, Ferreira F, Sperr WR, Valent P, Rohac M, Rumpold H, Scheiner O, Kraft D: Identification of common allergenic structures in hazel pollen and hazelnuts: A possible explanation for sensitivity to hazelnuts in patients allergic to tree pollen. *J Allergy Clin Immunol* 1992;90:927–936.
- 46 Hoffman-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.
- 47 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.
- 48 Neudecker P, Schweimer K, Nerkamp J, Scheurer S, Vieths S, Sticht H, Rösch P: Allergic cross-reactivity made visible: Solution structure of the major cherry allergen Pru av 1. *Biol Chem* 2001;276:22756–22763.
- 49 Vanek-Krebitz M, Hoffmann-Sommergruber K, Laimer Da Camara Machado M, Susani 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.
- 50 Leitner A, Jensen-Jarolim E, Grimm R, Wüthrich B, Ebner C, Scheiner O, Kraft D, Ebner C: Allergens in pepper and paprika. Immunologic investigation of the celery-birch-mugwort-spice syndrome. *Allergy* 1998;53:36–41.
- 51 Jensen-Jarolim E, Santner B, Leitner A, Grimm R, Scheiner O, Ebner C, Breiteneder H: Bell peppers (*Capsicum annuum*) express allergens (profilin, pathogenesis-related protein P23 and Bet v 1) depending on the horticultural strain. *Int Arch Allergy Immunol* 1998;116:103–109.
- 52 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.
- 53 Asero R: Effects of birch pollen-specific immunotherapy on apple allergy in birch pollen-hypersensitive patients. *Clin Exp Allergy* 1998;28:1368–1373.
- 54 Valenta R, Hayek B, Seiberler S, Bugajska-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.
- 55 Valenta R, Twardosz A, Swoboda I, Hayek B, Spitzauer S, Kraft D: Calcium-binding proteins in type I allergy: Elicitors and vaccines; in Pochet R (ed): *Calcium: The Molecular Basis of Calcium Action in Biology and Medicine*. Dordrecht, Kluwer Academic Publisher, 2000, pp 365–377.
- 56 Hayek B, Vangelista L, Pastore A, Sperr WR, Valent P, Vrtala S, Niederberger V, Twardosz A, Kraft D, Valenta R: Molecular and immunologic 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.

- 57 Twardosz A, Hayek B, Seiberler S, Vangelista L, Elfman 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.
- 58 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; 272:28630–28637.
- 59 Ledesma A, Villalba M, 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 calcium-binding proteins (polcalcins) involved in allergy. *Eur J Biochem* 1998;258:454–459.
- 60 Hemmer W, Focke M, Wantke F, Götz M, Jarisch R, Jäger S: Ash (*Fraxinus excelsior*) pollen allergy in Central Europe: Specific role of pollen panallergens and the major allergen of ash pollen, Fra e 1. *Allergy* 2000;55:923–930.
- 61 Suphioglu C, Ferreira F, Knox RB: Molecular cloning and immunological characterization of Cyn d 7, a novel calcium-binding allergen from Bermuda grass pollen. *FEBS Lett* 1997;402: 167–172.
- 62 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; 114:75–85.
- 63 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.
- 64 Toriyama K, Okada T, Watanabe M, Ide T, Ashida T, Xu H, Singh MB: A cDNA clone encoding and IgE binding protein from *Brassica anthus* has significant sequence similarity to Ca²⁺-binding proteins. *Plant Mol Biol* 1995;29: 1157–1165.
- 65 Mari A: Multiple pollen sensitization: A molecular approach to the diagnosis. *Int Arch Allergy Immunol* 2001;125:57–65.
- 66 Tinghino R, Twardosz A, Barletta B, Puggioni EMR, Iacovacci P, Buteroni C, Afferni C, Mari A, Hayek B, Di Felici 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.
- 67 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.
- 68 Esch RE: Grass pollen allergens. *Clin Allergy Immunol* 1999;12:103–120.
- 69 Valenta R, Ball T, Vrtala S, Duchêne M, Kraft D, Scheiner O: cDNA cloning and expression of timothy grass (*Phleum pratense*) pollen profilin in *Escherichia coli*: Comparison with birch pollen profilin. *Biochem Biophys Res Commun* 1994;199:106–118.
- 70 Suphioglu C: What are the important allergens in grass pollen that are linked to human allergic diseases? *Clin Exp Allergy* 2000;30:1335–1341.
- 71 Johnson P, Marsh DG: Isoallergens from rye grass pollen. *Nature* 1965;206:935–937.
- 72 Laffer S, Valenta R, Vrtala S, Susani M, van Ree R, Kraft D, Scheiner O, Duchêne M: cDNA cloning of the major allergen Phl p I from timothy grass (*Phleum pratense*); recombinant Phl p I inhibits IgE-binding to group I allergens from eight different grass species. *J Allergy Clin Immunol* 1994;94:689–698.
- 73 Laffer S, Spitzauer S, Susani M, Pairleitner H, Schweiger C, Grönlund H, Menz G, Pauli G, Ishii T, Nolte H, Ebner C, Sehon AH, Kraft D, Eichler HG, Valenta R: Comparison of recombinant timothy grass pollen allergens with natural extract for diagnosis of grass pollen allergy in different populations. *J Allergy Clin Immunol* 1996;98:652–658.
- 74 Ansari AA, Shenbagamurthi P, Marsh DG: Complete primary structure of a *Lolium perenne* (perennial rye grass) pollen allergen, Lol p II. *J Biol Chem* 1989;265:11181–11185.
- 75 Ong EK, Griffith IJ, Knox RB, Singh MB: Cloning of a cDNA encoding a group V (group IX) allergen isoform from rye grass pollen that demonstrates specific antigenic immunoreactivity. *Gene* 1993;134:235–240.
- 76 Silvanovich A, Astwood J, Zhang L, Olsen E, Kisil F, Sehon A, Mohapatra S, Hill R: Nucleotide sequence analysis of three cDNAs coding for Poa p IX isoallergens of Kentucky bluegrass pollen. *J Biol Chem* 1991;266:1204–1210.
- 77 Vrtala S, Sperr WR, Reimitzer I, van Ree R, Laffer S, Müller WD, Valent P, Lechner K, Rumpold H, Kraft D, Scheiner O, Valenta R: cDNA cloning of a major allergen from timothy grass (*Phleum pratense*) pollen: Characterization of the recombinant Phl p V allergen. *J Immunol* 1993;151:4773–4781.
- 78 Bufer A, Becker WM, Schramm G, Petersen A, Mamat U, Schlaak M: Major allergen Phl p Va (timothy grass) bears at least two different IgE-reactive epitopes. *J Allergy Clin Immunol* 1994;94:173–181.
- 79 Flicker S, Vrtala S, Steinberger P, Vangelista L, Bufer A, Petersen A, Ghannadan M, Sperr WR, Valent P, Norderhaug L, Bohle B, Stockinger H, Suphioglu C, Ong EK, Kraft D, Valenta R: A human monoclonal IgE antibody defines a highly allergenic fragment of the major timothy grass pollen allergen, Phl p 5: Molecular, immunological and structural characterization of the epitope-containing domain. *J Immunol* 2000;165:3849–3859.
- 80 Petersen A, Bufer A, Schlaak M, Becker M: Characterization of the allergen group VI in timothy grass pollen (Phl p 6). Immunological and biochemical studies. *Int Arch Allergy Immunol* 1995;108:49–54.
- 81 Petersen A, Bufer A, Schramm G, Schlaak M, Becker WM: Characterization of the allergen group VI in timothy grass pollen (Phl p 6). II. cDNA cloning of Phl p 6 and structural comparison to grass group V. *Int Arch Allergy Immunol* 1995;108:55–59.
- 82 Vrtala S, Fischer S, Grote M, Vangelista L, Pastore A, Sperr WR, Valent P, Reichelt R, Kraft D, Valenta R: Molecular, immunological, and structural characterization of Phl p 6, a major allergen and P-particle-associated protein from timothy grass (*Phleum pratense*) pollen. *J Immunol* 1999;163:5489–5496.