

ORIGINAL PAPER

The allergen profile of beech and oak pollen

C. Egger^{*†}, M. Focke^{*}, A. J. Bircher[‡], K. Scherer[‡], N. Mothes-Luksch^{*§}, F. Horak[¶] and R. Valenta^{*}

^{*}Christian Doppler Laboratory for Allergy Research, Division of Immunopathology, Department of Pathophysiology, Center for Physiology and Pathophysiology, Medical University of Vienna, Vienna, Austria, [†]Clinical Department of Dermatology and Venereology, Medical University of Innsbruck, Innsbruck, Austria, [‡]Allergy Unit, Department of Dermatology, University Hospital Basel, Basel, Switzerland, [§]Department of Dermatology, Medical University of Vienna, Vienna, Austria and [¶]Department of ENT, Medical University of Vienna, Vienna, Austria

Clinical and
Experimental
Allergy

Summary

Background Beech and oak pollen are potential allergen sources with a world-wide distribution. **Objective** We aimed to characterize the allergen profile of beech and oak pollen and to study cross-reactivities with birch and grass pollen allergens.

Methods Sera from tree pollen-allergic patients with evidence for beech and oak pollen sensitization from Basel, Switzerland, ($n = 23$) and sera from birch pollen-allergic patients from Vienna, Austria, ($n = 26$) were compared in immunoblot experiments for IgE reactivity to birch (*Betula pendula* syn. *verrucosa*), beech (*Fagus sylvatica*) and oak (*Quercus alba*) pollen allergens. Subsequently, beech and oak pollen allergens were characterized by IgE inhibition experiments with purified recombinant and natural allergens and with allergen-specific antibody probes. Birch-, beech- and oak pollen-specific IgE levels were determined by ELISA. **Results** Beech and oak pollen contain allergens that cross-react with the birch pollen allergens Bet v 1, Bet v 2 and Bet v 4 and with the berberine bridge enzyme-like allergen Phl p 4 from timothy grass pollen. Sera from Swiss and Austrian patients exhibited similar IgE reactivity profiles to birch, beech and oak pollen extracts. IgE levels to beech and oak pollen allergens were lower than those to birch pollen allergens.

Conclusion IgE reactivity to beech pollen is mainly due to cross-reactivity with birch pollen allergens, and a Phl p 4-like molecule represented another predominant IgE-reactive structure in oak pollen. The characterization of beech and oak pollen allergens and their cross-reactivity is important for the diagnosis and treatment of beech and oak pollen allergy.

Keywords beech pollen, birch pollen, cross-reactivity, oak pollen, pollinosis, recombinant allergen

Submitted 21 October 2007; revised 14 June 2008; accepted 8 July 2008

Correspondence:

Rudolf Valenta, Christian Doppler Laboratory for Allergy Research, Division of Immunopathology, Department of Pathophysiology, Center of Physiology and Pathophysiology, Medical University of Vienna, AKH, Währinger Gürtel 18–20, A–1090 Vienna, Austria. E-mail: rudolf.valenta@meduniwien.ac.at

Introduction

Beech and oak belong to the most common deciduous trees, with a wide distribution in Europe, North America, and certain parts of Asia and Africa [1]. They are members of a distinct botanical family, the *Fagaceae*, which can release significant amounts of pollen [2–5]. The *Fagaceae* belong to the order *Fagales*, which includes potent elicitors of spring pollinosis in Central Europe such as birch, alder, hazel and hornbeam. The presence of cross-reactive allergens in these pollen has been demonstrated in great detail and it has been shown that birch pollen allergens contain the majority of IgE epitopes present in *Fagales* pollen [6]. Nevertheless, evidence for a genuine sensitization to hazel pollen allergens has been provided [7] and the existence of a genuine oak pollen allergy has been proposed [8].

The allergens of birch pollen and of the closely related trees, alder and hazel, have been characterized in detail and have been produced as recombinant allergens resembling the allergenic activity of the natural allergens [9–14]. Previous immunotherapy studies performed with allergen extracts [15–17] and more recent studies carried out with purified recombinant derivatives of the major birch pollen allergen, Bet v 1 [18–20], suggest that immunotherapy with birch pollen extract or Bet v 1 is also sufficient to treat allergy to alder and hazel pollen.

However, the allergens in oak and beech pollen have only been partly characterized and only little information is available regarding the extent of cross-reactivity between the allergens of beech, oak and birch pollen [6, 21–23].

Basel in the North of Switzerland and Vienna, Austria, are regions where patients are exposed not only to birch pollen

but also to relevant amounts of beech and oak pollen [24]. We have therefore analysed the allergen profile of beech and oak pollen using sera from tree pollen-allergic patients from these two regions and purified recombinant allergens and allergen-specific antibody probes. In particular, we were interested in identifying beech and oak pollen allergens that cross-react with birch pollen allergens and to study the extent of cross-reactivity. The potential implications of these results on the diagnosis and immunotherapy of beech and oak pollen sensitivity are discussed.

Material and methods

Patients' sera

Basel (Switzerland) represents a region with birch, beech and oak pollen exposure during springtime [24]. Sera from 23 allergic patients (S1–S23) from the region around Basel, Switzerland, who had a case history of seasonal rhinoconjunctivitis with (43%) or without asthma (57%) during the tree pollen season and positive skin prick test (SPT) reactions to birch (96%) (ALK-Abelló A/S, Hørsholm, Denmark), beech (100%) and oak (100%) (Allergopharma, Reinbek, Germany) were analysed. Beech pollen-specific IgE levels were determined in 83% of these patients and were positive for each serum ranking between 0.5 and > 100 kUA/L (ImmunoCAP, Phadia AB, Uppsala, Sweden). All but two Swiss patients with IgE reactivity to oak pollen were also sensitized to grass pollen. Swiss patients were compared with sera from 26 birch pollen-allergic patients from Vienna (A1–A26). The Vienna patients had a history of seasonal birch pollen-related rhinoconjunctivitis (with or without asthma GINA grade 1–2), a positive SPT (weal diameter >3 mm) in response to birch pollen extract (Stallergenes SA, Antony, France) and serum-specific IgE levels ≥ 0.7 kUA/L as determined with the CAP System (Phadia). In addition, four Austrian sera with known IgE reactivity profiles to purified recombinant and natural birch and grass pollen allergens were used as reference sera in IgE immunoblot experiments and for IgE inhibition studies (I–IV). The sera had been tested for IgE reactivity to purified allergens by ELISA and CAP. Serum I and IV contained IgE for Bet v 1, serum II and IV reacted with Bet v 2, serum III and IV reacted with Bet v 4 as well as Phl p 7 and serum II and III reacted with Phl p 4.

Pollen protein extracts

Pollen from birch (*Betula pendula* syn. *verrucosa*), beech (*Fagus sylvatica*), oak (*Quercus alba*) and timothy grass (*Phleum pratense*) were obtained from Allergon (Välinge, Sweden). Pollen were extracted in phosphate-buffered saline (PBS) at 4 °C overnight before centrifugation at 20 000 g for 30 min [25]. Supernatants were dialysed against distilled water through Spectra/Por Membrane 1

MWCO 6–8 kDa (Spectrum, Gardena, CA, USA) and lyophilized. The quality of the extracts was analysed by 15% SDS-PAGE [26] and Coomassie blue staining [27] (Fig. 1). Total protein contents were determined according to the Bradford method [27].

Recombinant and purified natural allergens

Recombinant pollen allergens rBet v1, rBet v 2 and rPhl p 7, a cross-reactive homologous allergen of Bet v 4 [28], were obtained from BIOMAY (Vienna, Austria). The natural grass pollen allergen nPhl p 4 was purified as described previously [29].

Immunoglobulin E immunoblotting and Immunoglobulin E immunoblot inhibition experiments

For immunoblot analysis, birch, beech and oak pollen extracts were separated by 15% SDS-PAGE. Approximately 8 µg total protein/extract/cm preparative gel was loaded onto the gels. After SDS-PAGE, proteins were blotted onto 0.2 µm nitrocellulose membranes (Schleicher & Schuell, Dassel, Germany), [30] which were then blocked with buffer A (50 mM sodium phosphate, pH 7.5, 0.5% v/v Tween-20, 0.5% w/v BSA, 0.05% w/v NaN₃) and probed with patients' sera (diluted 1 : 10 in buffer A) at 4 °C overnight. Nitrocellulose strips were then washed with buffer A and incubated with ¹²⁵I-anti-huIgE (IBL, Gesellschaft für Immunchemie und Immunbiologie,

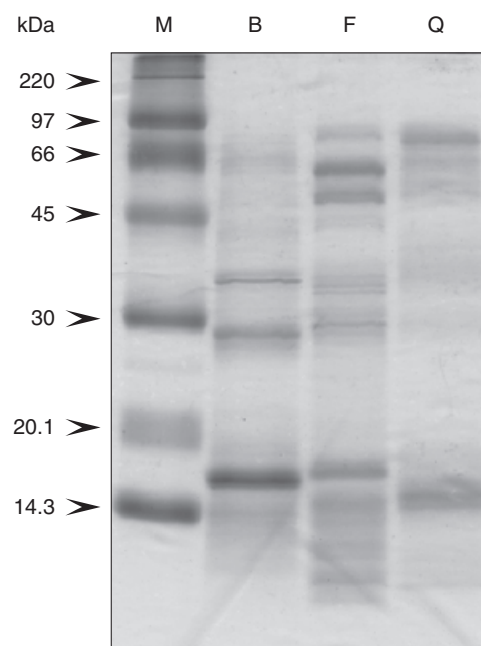


Fig. 1. Coomassie stained SDS-PAGE containing pollen protein extracts. Equal amounts of total pollen proteins were applied to each lane and separated by SDS-PAGE. M, marker; B, birch; F, beech; Q, oak. Molecular weights are displayed on the left side.

Hamburg, Germany) at room temperature overnight. After washing and air-drying, strips were exposed to Kodak X-OMAT LS Scientific Imaging films using intensifying screens (Eastman Kodak Company, Rochester, NY, USA) at -70°C . For inhibition experiments, 1:10 diluted sera were pre-adsorbed with 10 $\mu\text{g}/\text{mL}$ purified recombinant or natural allergen or 100 $\mu\text{g}/\text{mL}$ total protein of timothy grass pollen extract for 4 h at room temperature before they were reacted with the strips [31]. The molecular mass of the bands was estimated by comparison with protein markers of known molecular weight (Rainbow Marker RPN 756, Amersham, Buckinghamshire, UK).

Detection of cross-reactive allergens using specific antibody probes

Rabbit antisera were raised against the purified recombinant tree pollen proteins rBet v 1, rBet v 2 or rAln g 4, the Bet v 4-homologous allergen from alder pollen, by immunizing New Zealand white rabbits [32, 33]. Nitrocellulose-blotted pollen extracts were exposed to the rabbit antisera (1:2000 in buffer A) at 4°C overnight. Bound rabbit antibodies were detected with a ^{125}I -labelled donkey anti-rabbit antiserum (Amersham, Buckinghamshire, UK) diluted 1:1000 in buffer A for 1 h at room temperature and subsequent autoradiography.

Determination of pollen extract-specific Immunoglobulin E antibody levels by enzyme-linked immunosorbent assay

ELISA plates (Nunc Maxisorb, Roskilde, Denmark) were coated with birch, beech or oak pollen extracts at concentrations of 25 $\mu\text{g}/\text{mL}$ total protein in carbonate buffer ($\text{pH}=9.6$) at 4°C overnight. The plates were washed five times with washing buffer (0.05% v/v Tween 20/PBS) and blocked with 2% BSA in washing buffer (w/v) for 6 h at room temperature. The plates were incubated with 100 μL /well of the sera diluted 1:10 in dilution buffer (0.5% w/v BSA, 0.05% v/v Tween 20 in PBS) at 4°C over night and washed five times with washing buffer. A peroxidase-coupled mouse monoclonal anti-human IgE-antibody (KPL, Guildford, UK) diluted 1:2500 was used to detect bound IgE antibodies. After incubation at 37°C for 1 h and 4°C for 1 h, plates were washed five times with washing buffer and the colour reaction was started with ABTS in citrate buffer (60 mM citric acid, 77 mM $\text{Na}_2\text{HPO}_4 \times \text{H}_2\text{O}$, $\text{pH}=4$, 0.03% v/v H_2O_2). The optical density (OD) was determined with an ELISA reader (Dynatech, Denkendorf, Germany). All reactions were carried out in duplicates and mean values of the ODs were calculated. Serum from a non-allergic donor was included in the analysis to create a cut-off OD that was subtracted from the OD values obtained with patients' sera. A reference serum from a tree pollen-allergic patient was analysed on each of the plates in order to calibrate results from different plates in

the form of OD equivalents and to harmonize plate-to-plate variabilities.

Statistics

Statistics were performed with Microsoft Excel 2000 and the Statistical Package for Social Sciences (SPSS) 10.0. A two-tailed Mann-Whitney *U*-test was performed to compare the ELISA results of the two populations studied. For comparisons within the populations, results were corrected for multiple testing using the Shaffer coefficients. *P*-values <0.05 were considered significant.

Results

Immunoglobulin E-reactive components in nitrocellulose-blotted birch, beech and oak pollen extracts

Sera from patients from Basel (S1-S23) (Fig. 2a) and Vienna (A1-A26) (Fig. 2b), serum from a non-allergic person (N), buffer without serum and sera from four patients with known IgE reactivity profiles (I-IV) were probed with nitrocellulose-blotted birch, beech and oak pollen extracts. Thereby, sera from Swiss and Austrian patients exhibited similar IgE reactivity profiles to birch, beech and oak pollen extracts (Figs 2a and b). A 17 kDa band in birch pollen was detected by 87% of the Swiss and 100% of the Austrian patients and by reference sera I and IV, which contained Bet v 1-specific IgE (Figs 2a and b). The 17 kDa band was also the most frequently detected beech pollen allergen (Basel: 69.6%; Vienna: 88.5%). A 14 kDa band was detected by approximately 25% of the Swiss and Austrian patients in birch and beech pollen as well as by reference serum II, which contained profilin-specific IgE (Figs 2a and b).

The IgE-reactivity profile of oak pollen was different from that of birch and beech pollen. Bands with a molecular weight of more than 35 kDa were recognized by 34.8% of the Swiss and by 50% of the Austrian patients, whereas $<50\%$ of the patients demonstrated mostly weak IgE binding to the 17 kDa band (Basel: 26.1%; Vienna: 46.2%).

The >35 kDa moieties were also frequently detected in beech pollen (Basel: 34.8%; Vienna: 46.2%). Two EF-hand calcium-binding allergens were detected by the reference serum IV in birch, beech and oak pollen and by reference serum III in beech and oak pollen. Only one Austrian patient (A13) reacted weakly with the 8 kDa component (Figs 2a and b).

Serum from the non-allergic individual and buffer without serum showed no reactivity with the blotted pollen extracts, confirming the specificity of the IgE binding observed with the patients (Figs 2a and b). Although comparable amounts of birch, beech and oak pollen proteins had been blotted onto nitrocellulose, we

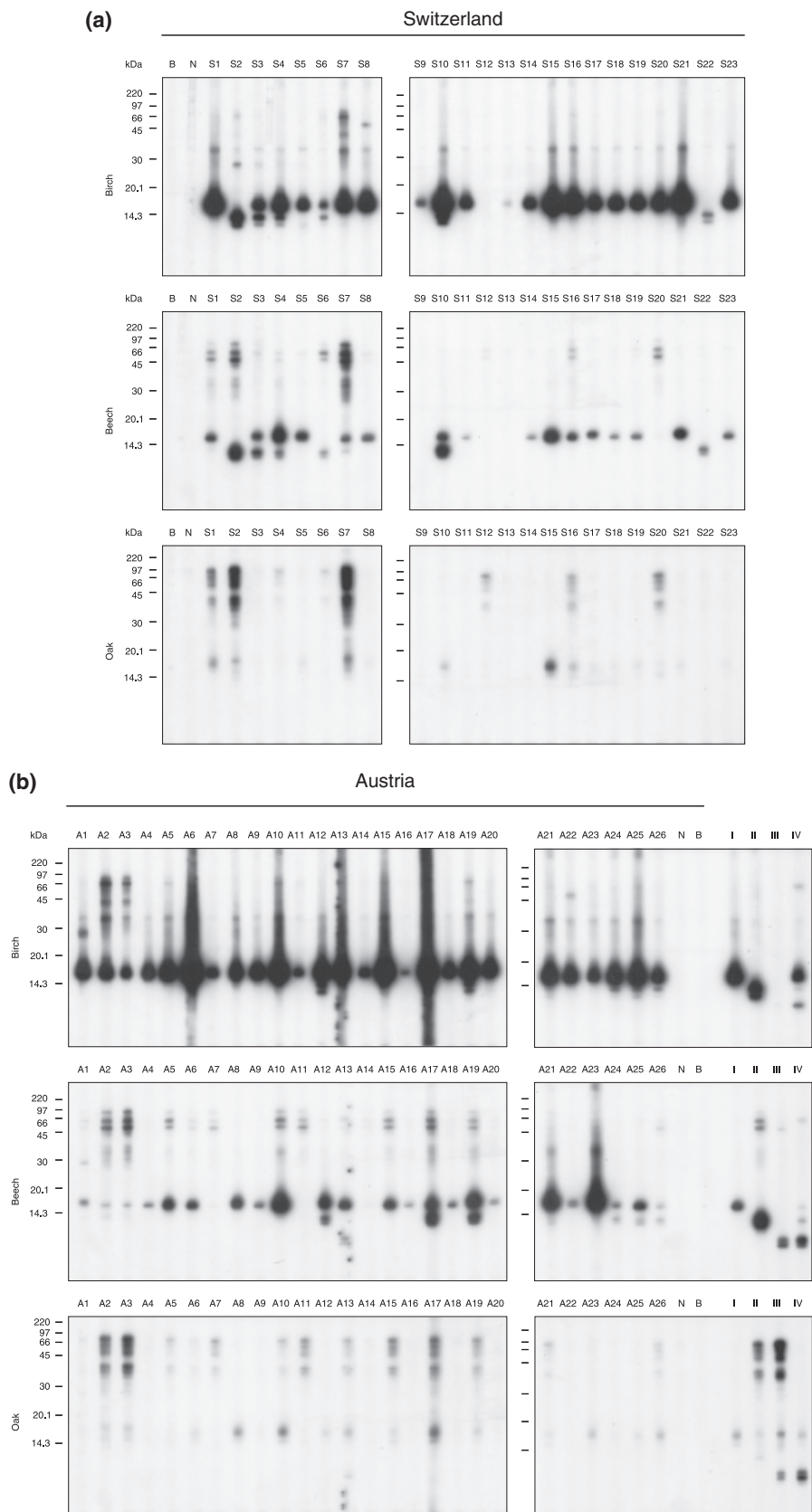


Fig. 2. IgE reactivity profile to nitrocellulose-blotted extracts from birch, beech and oak pollen. Sera of 23 patients from Basel, Switzerland, (S1–S23) with seasonal rhinoconjunctivitis in spring (a), 26 patients from Vienna, Austria, (A1–A26) allergic to birch pollen and four reference patients (I–IV) (b) were exposed to blotted pollen extracts. B, buffer; N, non-allergic donor serum. Molecular weights are indicated on the left side.

found that IgE reactivities were most intense to birch pollen, followed by beech pollen and oak pollen extracts (Figs 2a and b).

Specific immunoglobulin E levels are the highest for birch pollen in both patient groups

ELISAs were performed to compare specific IgE levels to birch, beech and oak pollen extracts. The results are displayed in Fig. 3 for each patient's serum. Specific IgE levels were highest to birch pollen extract, followed by beech pollen extract and oak pollen extract (Fig. 3). Median OD values were 0.481 for birch, 0.063 for beech and 0.047 for oak pollen extracts in patients from Basel and 0.668, 0.081 and 0.021 in patients from Vienna,

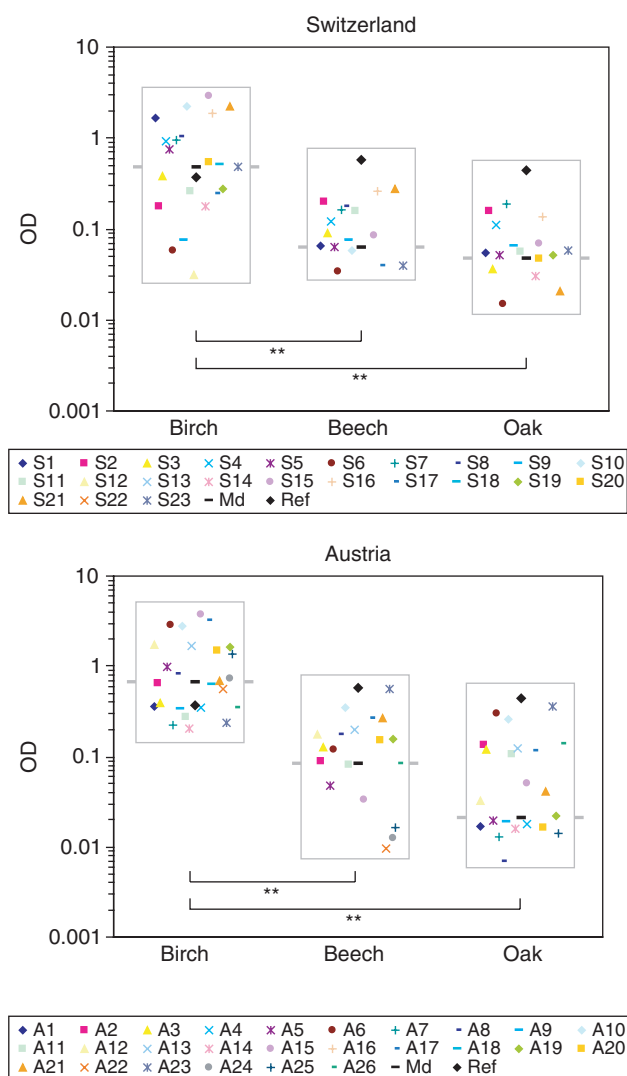


Fig. 3. Levels of specific IgE to birch, beech and oak pollen extracts as determined by ELISA in patients from Vienna (A1–A26) and Basel (S1–S23). Significant differences ($P < 0.001$) between median optical density (OD) values (Md) are indicated **Ref=patient IV. OD values corresponding to bound IgE are displayed on the y-axis.

respectively. Thus, birch pollen-specific IgE levels were significantly higher than beech and oak pollen-specific IgE levels in the Austrian and Swiss population ($P < 0.001$). Differences between the Swiss and Austrian population were not statistically significant ($P = 0.186$ for birch, $P = 0.648$ for beech, and $P = 0.762$ for oak).

Identification of Bet v 1-, Bet v 2- and Bet v 4-like allergens in beech and oak pollen with specific antibody probes

Rabbit antisera raised against purified recombinant allergens rBet v 1, rBet v 2 and rAln g 4, the Bet v 4-homologous allergen from alder pollen, were used to detect cross-reactive allergens in beech and oak pollen (Fig. 4). Rabbit anti-Bet v 1 antibodies reacted strongly with birch pollen-derived Bet v 1 and a Bet v 1-cross-reactive beech pollen allergen at 17 kDa whereas only weak binding to a 17 kDa moiety in oak pollen was observed (Fig. 4). Profilins and 2 EF-hand calcium-binding allergens were detected at 14 and 8 kDa, respectively, in birch, beech and oak pollen (Fig. 4).

Demonstration of Bet v 1-, Bet v 2-, Bet v 4- and Phl p 4-like allergens in beech and oak pollen by immunoglobulin E inhibition studies

Four sera (I–IV) with known IgE reactivity profiles were used to identify cross-reactive allergens by IgE inhibition experiments (Fig. 5). Pre-adsorption of serum I and IV with rBet v 1 inhibited IgE reactivity to a 17 kDa band in birch and beech pollen and to a weakly IgE-reactive band in oak pollen (Fig. 5). IgE reactivity to birch, beech and oak pollen profilin was inhibited by pre-incubation of serum II and IV with rBet v 2. rPhl p 7 inhibited IgE binding to Bet v 4 (birch pollen) and Bet v 4-related allergens in beech and oak after pre-adsorption of serum III and IV (Fig. 5). Interestingly, we could identify a Phl p 4-related allergen of approximately 60 kDa and several smaller bands of 40 and 17 kDa in beech and oak but not in birch pollen using sera II and III and purified Phl p 4 and timothy grass pollen extract in the IgE inhibition studies (Fig. 5, lanes nPhl p 4 and timothy).

Discussion

Besides pollen of trees belonging to the family *Betulaceae* and *Corylaceae* (e.g. birch, alder, hazel) and *Oleaceae* (e.g. ash), beech and oak pollen are widely distributed potential allergen sources for patients suffering from spring pollinosis [1]. In the present study, we analysed the allergen profile of beech and oak pollen using sera from two regions with relevant birch, beech and oak pollen exposure. All patients had a positive history of allergic rhinoconjunctivitis during tree pollen season and there were no

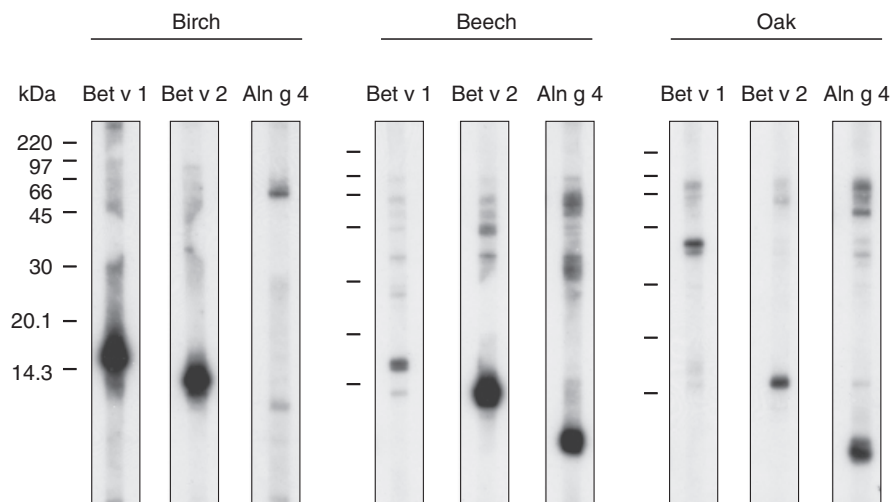


Fig. 4. Detection of birch-, beech- and oak pollen allergens with specific antibody probes. Nitrocellulose-blotted pollen extracts were probed with rabbit antisera raised against purified rBet v 1, rBet v 2 or rAln g 4. Molecular weights are displayed on the left side.

striking differences regarding the IgE reactivity profiles recognized in beech and oak pollen between the two patients groups.

In beech pollen, we identified allergens cross-reactive with the major birch pollen allergen Bet v 1, Bet v 2 and Bet v 4 by using purified recombinant pollen allergens and specific antibody probes. In accordance with the IUIS allergen nomenclature (<http://www.allergome.org>), we suggest that these allergens are designated Fag s 1, Fag s 2 and Fag s 4. Our findings that IgE reactivity to Fag s 1, Fag s 2 and Fag s 4 was inhibited with Bet v 1, Bet v 2 and the Bet v 4-homologous allergen Phl p 7 [28, 34] and that IgE levels to beech pollen were lower than to birch pollen in each but one of the tested patients suggests that reactivity to beech pollen is primarily due to cross-reactivity with birch pollen allergens.

The IgE reactivity profile to oak pollen allergens was different from that to birch and beech pollen allergens. Although we could identify a Bet v 1-, Bet v 2- and Bet v 4-related allergen in oak pollen for which we suggest the designations Que a 1, Que a 2 and Que a 4, these allergens exhibited lower IgE reactivity than the corresponding allergens in birch and beech and were recognized by fewer patients than in birch and beech pollen. The relatively weak IgE reactivity of Que a 1 may be explained by the fact that the oak pollen extract contained little Que a 1 (Fig. 1), but it is also in good agreement with a recent study performed with purified Que a 1 showing that the N-terminus of Que a 1 has only moderate sequence homology with that of Bet v 1 [35]. In birch and beech pollen, IgE reactivity to Bet v 1 and Fag s 1 predominated (Bet v 1: 87% of Swiss and 100% of Austrian patients) whereas fewer patients (Basel: 26.1%; Vienna: 46.2%) showed weak IgE reactivity to Que a 1. These results are similar to the results obtained in an

earlier study that did not identify the nature of the IgE-reactive components but described their molecular weight [36]. The most prominent IgE-reactive components in oak pollen appeared to be a high-molecular-weight allergen of 60 kDa and several low-molecular-weight IgE-reactive components of 40 and 17 kDa that we found by IgE inhibition experiments to be cross-reactive with the timothy grass pollen allergen Phl p 4, a recently described berberine bridge enzyme-related protein [37]. Because we used natural glycosylated Phl p 4 in the IgE inhibition experiments, it is possible that the cross-reactivity is due to the carbohydrate moieties on Phl p 4, Phl p 4-derived peptides or both types of epitopes [29]. In summary, it seems that oak pollen is a much less important allergen source than birch pollen.

The dissection of allergen profiles and their cross-reactivities are of clinical importance because they can contribute to improved allergy diagnosis and immunotherapy. Our findings provide evidence that allergy to beech pollen is mainly due to cross-reactivity with birch pollen allergens. Thus, patients who exhibit signs of beech pollen allergy and react with the major birch pollen allergen Bet v 1 in diagnostic tests may be adequately treated with birch pollen or Bet v 1-based immunotherapy protocols.

Furthermore, oak and also beech pollen allergy may be due to cross-sensitization to grass pollen allergens. Patients with negative test results to Bet v 1 should therefore be further tested for reactivity with cross-reactive pollen allergens (e.g. Bet v 2, Bet v 4, Phl p 4) and diagnostic markers (e.g. Phl p 1, Phl p 5) that are suited to confirm grass pollen allergy. In case of a positive test result with the latter, these patients may be treated with grass pollen allergens.

In conclusion, beech and oak pollen are allergen sources of little importance in Austria and Switzerland

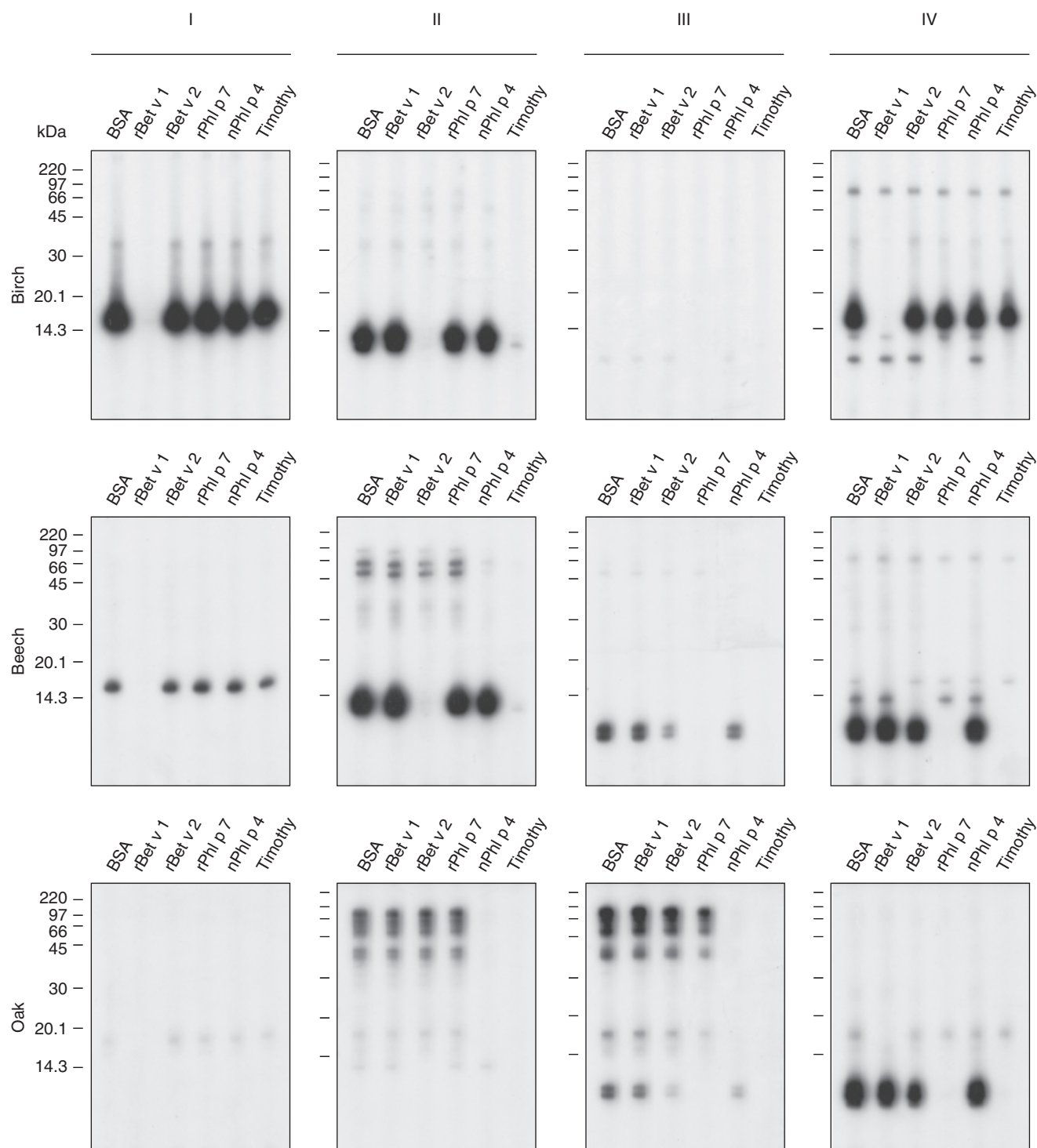


Fig. 5. Identification of allergens in birch-, beech- and oak pollen by IgE inhibition experiments. Sera from four pollen allergic patients (I–IV) were pre-adsorbed with purified allergens (rBet v 1, rBet v 2, rPhl p 7, nPhl p 4), BSA or timothy grass pollen extract as described on the top of the figure and then exposed to nitrocellulose-blotted birch-, beech- or oak pollen extracts. Bound IgE antibodies were visualized by autoradiography. Molecular weights are displayed on the left side.

and symptoms of beech and oak pollen allergy may be treated with birch- and/or grass pollen-specific immunotherapy according to the molecular sensitization profiles.

Acknowledgements

This study was supported in part by grant L214-B13 of the Austrian Science Fund and by a research grant from the

Christian Doppler Association, Austria and Biomay, Vienna.

References

- 1 Mothes N, Horak F, Valenta R. Transition from a botanical to a molecular classification in tree pollen allergy: implications for diagnosis and therapy. *Int Arch Allergy Immunol* 2004; 135:357–73.
- 2 Helbling A, Leuschner RM, Wuthrich B. Pollinosis. IV. Welche Pollen sollten in der Praxis getestet werden? Ergebnisse der Bestimmungen von allergie-erzeugenden Pollen in der Luft von Zuerich in den Jahren 1981–1984 unter Berücksichtigung von Schwellenkonzentrationen. *Schweiz Med Wochenschr* 1985; 115:1150–9.
- 3 Subiza J, Jerez M, Jimenez JA et al. Allergenic pollen pollinosis in Madrid. *J Allergy Clin Immunol* 1995; 96:15–23.
- 4 Kosisky SE, Carpenter GB. Predominant tree aeroallergens of the Washington, DC area: a six year survey (1989–1994). *Ann Allergy Asthma Immunol* 1997; 78:381–92.
- 5 Lin RY, Clauss AE, Bennett ES. Hypersensitivity to common tree pollens in New York City patients. *Allergy Asthma Proc* 2002; 23:253–8.
- 6 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; 102:579–91.
- 7 Mari A, Wallner M, Ferreira F. Fagales pollen sensitization in a birch-free area: a respiratory cohort survey using Fagales pollen extracts and birch recombinant allergens (rBet v 1, rBet v 2, rBet v 4). *Clin Exp Allergy* 2003; 33:1419–28.
- 8 Kropp R, Laumen R. Seltener Pollenallergien. *Allergologie* 1999; 22:611–6.
- 9 Breiteneder H, Pettenburger K, Bito A et al. The gene coding for the major birch pollen allergen Betv1, is highly homologous to a pea disease resistance response gene. *EMBO J* 1989; 8:1935–8.
- 10 Valenta R, Duchene M, Vrtala S et al. Recombinant allergens for immunoblot diagnosis of tree-pollen allergy. *J Allergy Clin Immunol* 1991; 88:889–94.
- 11 Breiteneder H, Ferreira F, Reikerstorfer A et al. Complementary DNA cloning and expression in *Escherichia coli* of Aln g I, the major allergen in pollen of alder (*Alnus glutinosa*). *J Allergy Clin Immunol* 1992; 90:909–17.
- 12 Breiteneder H, Ferreira F, Hoffmann-Sommergruber K et al. Four recombinant isoforms of Cor a I, the major allergen of hazel pollen, show different IgE-binding properties. *Eur J Biochem* 1993; 212:355–62.
- 13 Valenta R, Sperr WR, Ferreira F et al. Induction of specific histamine release from basophils with purified natural and recombinant birch pollen allergens. *J Allergy Clin Immunol* 1993; 91:88–97.
- 14 Pauli G, Oster JP, Deviller P et al. 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; 97:1100–9.
- 15 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; 43:353–62.
- 16 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; 43:363–9.
- 17 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; 43:370–7.
- 18 Niederberger V, Horak F, Vrtala S et al. Vaccination with genetically engineered allergens prevents progression of allergic disease. *Proc Natl Acad Sci USA* 2004; 101 (Suppl. 2):14677–82.
- 19 Klimek L, Bachert C, Doemer C, Meyer H, Narkus A. Specific immunotherapy with recombinant birch pollen allergen rBet v 1-FV is clinically efficacious. *Allergy Clin Immunol Int* 2005; (Suppl. 1):15.
- 20 Pauli G, Larsen TH, Rak S et al. Efficacy of recombinant birch pollen vaccine for the treatment of birch allergic rhinoconjunctivitis. *J Allergy Clin Immunol* (in press).
- 21 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; 28:1279–88.
- 22 Calvo AP, Lorenzo O, Nicolas C, Rodriguez D. Expression and characteristics of a cDNA encoding a pathogenesis-related protein (accession No. AJ130889) from *Fagus sylvatica* seeds (PGR 99-090). *Plant Physiol* 1999; 120:933–5.
- 23 Ferreira F, Hawranek T, Gruber P, Wopfner N, Mari A. Allergic cross-reactivity: from gene to the clinic. *Allergy* 2004; 59:243–67.
- 24 Frei T, Oertmann C, Bergmann KC. Vergleich von Pollenflugdaten und pollenassoziertem oralem Allergie-Syndrom. *Allergologie* 1998; 21:98–104.
- 25 Vrtala S, Grote M, Duchene M et al. Properties of tree and grass pollen allergens: reinvestigation of the linkage between solubility and allergenicity. *Int Arch Allergy Immunol* 1993; 102:160–9.
- 26 Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1970; 227:680–5.
- 27 Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 1976; 72:248–54.
- 28 Niederberger V, Hayek B, Vrtala S et al. 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; 13:843–56.
- 29 Stumvoll S, Lidholm J, Thunberg R et al. Purification, structural and immunological characterization of a timothy grass (*Phleum pratense*) pollen allergen, Phl p 4, with cross-reactive potential. *Biol Chem* 2002; 383:1383–96.
- 30 Towbin H, Staehelin T, Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc Natl Acad Sci USA* 1979; 76:4350–4.

- 31 Valenta R, Duchene M, Ebner C *et al.* Profilins constitute a novel family of functional plant pan-allergens. *J Exp Med* 1992; **175**:377–85.
- 32 Vrtala S, Akdis CA, Budak F *et al.* T cell epitope-containing hypoallergenic recombinant fragments of the major birch pollen allergen, Bet v 1, induce blocking antibodies. *J Immunol* 2000; **165**:6653–9.
- 33 Hayek B, Vangelista L, Pastore A *et al.* 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**: 7031–9.
- 34 Tinghino R, Twardosz A, Barletta B *et al.* Molecular, structural, and immunologic relationships between different families of recombinant calcium-binding pollen allergens. *J Allergy Clin Immunol* 2002; **109**:314–20.
- 35 Movérare R, Everberg H, Carlsson R *et al.* Purification and characterization of the major oak pollen allergen Que a 1 for component-resolved diagnostics using ImmunoCAP®. *Int Arch Allergy Immunol* 2008; **146**:203–11.
- 36 Loria RC, Wilson P, Wedner HJ. Identification of potential allergens in white oak (*Quercus alba*) pollen by immunoblotting. *J Allergy Clin Immunol* 1989; **84**:9–18.
- 37 Marknell DeWitt A, Andersson K, Peltre G, Lidholm J. Cloning, expression and immunological characterization of full-length timothy grass pollen allergen Phl p 4, a berberine bridge enzyme-like protein with homology to celery allergen Api g 5. *Clin Exp Allergy* 2006; **36**:77–86.