The allergen profile of beech and oak pollen


*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 Venerology, 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

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

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 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

\[ 100 \text{ kUA/L} \]

(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

\[ \geq 0.7 \text{ kUA/L} \]

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 4 as 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 20000 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 v 1, 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

\[ ^{125}\text{I}-\text{anti-} \text{IgE} \]

(IBL, Gesellschaft für Immunchemie und Immunbiologie,

\[ \text{20.1 kDa M B F Q} \]

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.

© 2008 The Authors

Journal compilation © 2008 Blackwell Publishing Ltd, *Clinical and Experimental Allergy*, 38 : 1688–1696
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 125I-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 μg/mL total protein in carbonate buffer (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 Na2HPO4 • H2O, pH = 4, 0.03% v/v H2O2). 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, 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
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. 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.
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


12 Breitenberger H, Ferreira F, Hofmann-Sommergruber K et al. Four recombinant isoforms of Cor a 1, the major allergen of hazel pollen, show different IgE-binding properties. *Eur J Biochem* 1993; 212:355–62.


