

Original article

Ash (*Fraxinus excelsior*)-pollen allergy in central Europe: specific role of pollen panallergens and the major allergen of ash pollen, Fra e 1

Background: The role of ash (*Fraxinus excelsior*) pollen as a cause of spring pollinosis in central Europe has received little attention. It is not clear whether ash pollen is a primary cause of sensitization or whether it is implicated through cross-sensitization to other pollens.

Methods: Over a 22-month period, ash pollen was included in a screening series for inhalant allergies. Pollen data were documented from 1976 through 1999. The frequency of IgE-binding to the ash-specific allergen Fra e 1 and pollen panallergens, respectively, was compared by Western blot between mono- ($n=6$), oligo- ($n=16$), and polysensitized ($n=25$) patients.

Results: Of 5416 consecutive patients sensitized to any pollen, 920 (17.6%) had a positive skin prick test to ash. Total pollen counts varied extensively between years (229–5351) as did peak concentrations (23–837 grains/m³/24 h). Western blotting revealed Fra e 1 sensitization in 100% of monosensitized, 93% of oligosensitized, but only 44% of polysensitized patients. IgE against profilins (Fra e 2), Ca-binding proteins (Fra e 3), and carbohydrate epitopes in the three groups was found in 0/0/17%, 0/19/31%, and 32/72/60%, respectively. At least 50% of sera from patients with Fra e 1 sensitization did not bind with the protein in Western blots under reducing conditions.

Conclusions: Ash pollen should be considered a relevant factor and distinct entity in spring pollinosis. In all, only 20% of positive skin tests to ash appear to result from cross-sensitization to pollen panallergens.

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The ash tree (*Fraxinus excelsior*) is a wind-pollinated tree of the plant family Oleaceae regularly occurring in summer-green deciduous forests of the Middle European temperate zones, including northern Spain, the British Isles, France, central Europe, and southern Scandinavia. In urban areas, airborne *Fraxinus* pollen may particularly derive from ash trees cultivated in parks and gardens. While the closely related olive tree (*Olea europaea*) has long been recognized as an important source of pollinosis in southern Europe and the Middle East (1–5), respiratory allergy from ash in temperate zones is poorly documented. The potential role of ash pollinosis may have been overlooked in these regions due to the fact that the flowering of ash and birch coincide (April–May) and because of the well-documented importance of pollens of species of the order Fagales in the elicitation of spring pollinosis in this area (6). In Austria, ash pollen is not included in routine screening series for inhalant allergies, and this seems to be the case also in other countries (7, 8).

Previous studies have presented evidence of a very

similar allergen profile among Oleaceae genera, including *Olea*, *Ligustrum* (privet), *Syringa* (lilac), *Forsythia*, and *Fraxinus*. In particular, considerable amino-acid sequence identity and immunologic cross-reactivity has been found between the highly allergenic olive-pollen allergen Ole e 1 and corresponding proteins in other Oleaceae species (9–14). Cross-reactivity has also been described between *Olea* and botanically unrelated plants, such as grasses (11, 15) and birch (10). It seems reasonable to assume that at least some of this cross-reactivity is due to the presence of pollen panallergens, such as profilins and Ca-binding proteins, that have been recently described also in Oleaceae (16, 17).

The airborne ash-pollen count represents a very small part of the total pollen load in Scandinavia (6), but considerable numbers have been reported for some central European locations (8, 10, 18, 19). While there is evidence for high sensitization rates to ash from the Mediterranean-temperate borderlines (20, 21), possibly reflecting cross-reactivity in primary allergy to olive pollen, data on the prevalence of sensitivity to ash

pollen in the temperate zones is scarce. The relevance of sensitivity to ash pollen in central Europe is believed to be low (6, 18), but recent observations from Switzerland suggest that ash may be, at least locally, as important as birch in the elicitation of spring pollinosis (19). In those sensitized to ash pollen, little is known of whether reactivity is due to *Fraxinus*-specific allergens, such as Fra e 1, the homolog of Ole e 1, or is a consequence of cross-sensitization to pollen panallergens, although this knowledge would be important for specific immunotherapy. Western blot studies in olive-pollen allergens revealed a more complex binding pattern and a lower prevalence of IgE antibodies against the major olive-pollen allergen Ole e 1 in patients from France and Australia, as compared with sera from Italy (11). These data emphasize that IgE against pollen panallergens may be a major factor in positivity to ash pollen in central Europe, as has been demonstrated for other pollens (22).

This study investigates the frequency and the patterns of sensitization to ash pollen in patients from eastern Austria, where birch, grass, and mugwort pollen are the most important causes of pollinosis. Western blot experiments in selected patients were used to establish the role of ash as an active sensitizer by evaluating the occurrence of specific IgE antibodies against the ash-specific allergen Fra e 1 and IgE antibodies against pollen panallergens such as profilins, Ca-binding proteins, and carbohydrate epitopes.

Material and methods

Patients

A total of 12812 patients with suspected inhalant allergy were tested within a 22-month period between 1997 and 1999 with a screening series for inhalant allergies comprising pollens from ash (*F. excelsior*), Fagales species (birch, hazel, alder), and Gramineae species (rye, mixed grasses), as well as several weed pollens (mugwort, ragweed, plantain, nettle, oilseed rape, and plane tree; all ALK, Denmark). Forty-seven patients with a positive skin prick test reaction to ash (wheal diameter >3 mm) and with specific IgE antibodies to ash pollen (Pharmacia CAP; positive cutoff 0.7 kU/l) were selected for further investigation by Western blot. Of the 47 patients, six were mono-sensitized to ash pollen, 16 were classified as oligosensitized (reactivity also with pollens from one or two other pollen families, e.g., ash plus Fagales plus grasses), and 25 were classified as polysensitized (reactivity to three or more other pollen families). Seven patients in the latter group were also allergic to house-dust mite and/or animal dander. Rhinitis and rhinoconjunctivitis were the predominant symptoms in

39 patients (83%), 17 patients (36%) suffered from bronchial asthma, and four patients (9%) suffered from recurrent bronchitis or recurrent cough.

Allergen extracts

Proteins were extracted from *F. excelsior* and *O. europaea* pollen (Kabi-Pharmacia-Allergon AB, Ängelholm, Sweden) overnight at 4°C in phosphate-buffered saline, pH 7.4 (1 g pollen/10 ml). The supernatants were recovered after centrifugation (12000 rpm for 30 min at 4°C), dialyzed against three changes of distilled water at 4°C for 3 days, filtered through a 0.22 µm membrane (Millipore Intertech, MA, USA), lyophilized, and stored at -20°C.

SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting

Extracted pollen proteins were separated by SDS-PAGE using 13.5% homogeneous gels (10.5 × 8.0 × 0.1 cm) with 5% stacking gels at 25 mA and constant voltage with two lanes of standardized molecular weight marker proteins (Rainbow Marker, Amersham, UK). An amount of 120 µg protein was loaded onto a single gel representing approximately 6 µg protein per lane. Transfer to nitrocellulose (Schleicher & Schüll, Germany) was performed according to Towbin et al. (23). SDS-PAGE was first carried out under reducing conditions with protein treatment by 5% mercaptoethanol and boiling (24). Patients giving negative immunoblot results with this method were reinvestigated with SDS-PAGE using nonreduced/nondenatured proteins (i.e., no boiling and mercaptoethanol treatment).

IgE blotting was done in a buffer system made up from 50 mmol/l sodium phosphate, 0.5% (v/v) Tween 20, 0.5% (w/v) bovine serum albumin (BSA), and 0.05% (w/v) sodium azide, giving a final pH of 7.5. After transfer, sheets were cut into strips of 3 mm and blocked with BSA in buffer for 30 min. Each strip was incubated overnight with 1 ml serum (diluted 1:10) at 4°C under continuous shaking, washed in buffer for 30 min, and incubated overnight with ¹²⁵I-labeled rabbit antihuman IgE (Pharmacia, Sweden) diluted to achieve 200000 cpm per strip. Strips were washed again, dried at room temperature, and exposed to a high-performance autoradiography film (Hyperfilm MP, Amersham, UK) at -70°C for ≥3 days.

Identification of cross-reactive panallergens in ash-pollen extracts

Ash-pollen profilin was identified with the help of a polyclonal rabbit antiserum directed against purified celery root profilin (RP1) (25). The bound antiprolin antibodies were detected with ¹²⁵I-labeled antirabbit IgG (Amersham, UK). In addition, serum from a patient with known sensitization to birch-pollen profilin was tested on ash and olive.

Identification of Ca-binding proteins was done with the help of two selected patients' sera with proven IgE-reactivity with Ca-binding proteins from oilseed rape (Bra n 1) and alder pollen (Aln g 4) (22).

High-molecular-weight (HMW) glycoproteins assumed to be cross-reactive via sugar moieties (CCDs) were located with two selected sera containing IgE antibodies against periodate-sensitive carbohydrate epitopes on oilseed-rape and grass-pollen glycoproteins (22).

RAST inhibition with bromelain-BSA neoglycoprotein

A neoglycoprotein construct prepared from isolated N-linked xylose/fucose-containing bromelain glycans covalently coupled to BSA was employed to investigate in RAST-inhibition assays the role of carbohydrate epitopes in IgE-binding to ash pollen. Preparation of the neoglycoprotein was described in detail by Wilson et al. (26). In brief, commercially available bromelain (Sigma) was digested with pepsin in 5% formic acid to obtain small M0XF glycopeptides, which were subsequently cross-linked to BSA. According to amino-sugar analysis, the resulting neoglycoprotein had about 3–4 mol glycopeptide per mol protein.

RAST inhibition was carried out in 10 patients binding to Fra e 1 in the immunoblot, and in one of the CCD-specific reference sera. An amount of 25 µl of serum was incubated with 25 µg/ml bromelain-BSA and unreduced crude ash-pollen extract, respectively, at a final protein concentration of 75 µg/ml. PBS was used as negative control. All experiments were performed with the Pharmacia CAP system.

Pollen counts

Daily *Fraxinus* pollen load in Vienna was measured by the Aerobiologic Section of the ENT Department, University of Vienna, by means of a Hirst type volumetric pollen and spore trap (Burkhard Manufacturing, Rickmansworth, UK). Microscopic analysis was done with a Zeiss compound light microscope, objective ×40 Planapochromat oil immersion n.A. 1.0, reading five

horizontal sweeps at 250 µm per slide, equivalent to the deposit of 1 m³ of air per day. Pollen counts are expressed as daily mean per cubic meter of air.

Results

Frequency and patterns of sensitization to ash pollen

Exactly 5416 (42.3%) of the 12812 tested patients reacted positively to at least one pollen allergen. The relative proportion of single pollen species is shown in Table 1. Sensitization to ash pollen was diagnosed on average in 17.6% of pollen-allergic patients (1–8/98: 14.1%, 1–8/99: 23.6%, $n=920$), with a peak lasting from April to June in each year. The highest incidence ever observed was 35.6% in April 1999 (birch, April 1999: 65.9%).

Of 920 positive prick test reactions to ash pollen, 87 (9.5%) occurred in monosensitized, 438 (47.6%) in oligosensitized, and 395 (42.9%) in polysensitized individuals. Within the oligosensitive group, the combination ash plus Fagales, ash plus grasses, and ash plus mugwort was found in, respectively, 26.7%, 13.9%, and 2.2%. Taking into account the overall incidence of sensitization to Fagales, grasses, and mugwort in the studied population, ash-pollen sensitivity was significantly more often associated with sensitization to Fagales pollens than with grasses ($P<0.0001$, chi-square-test) and mugwort ($P<0.001$).

IgE immunoblot reactivity with reduced ash-pollen allergens

Of the 47 sera, 33 were positive in immunoblots when

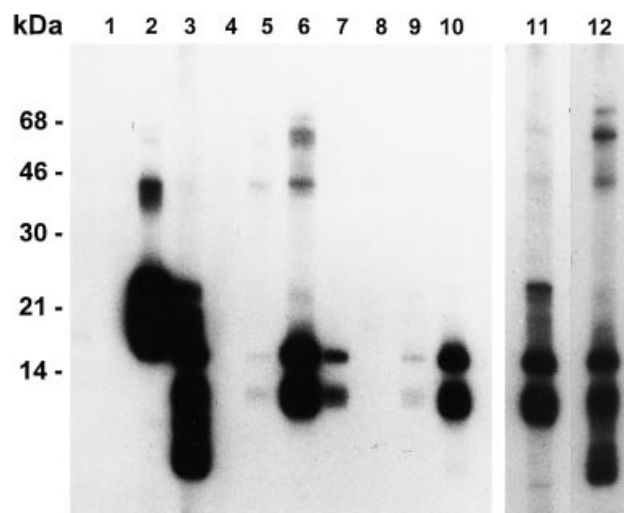


Figure 1. Lanes 1–10) IgE-binding to ash-pollen extracts separated by SDS-PAGE under reducing conditions in 10 randomly selected ash-pollen-allergic patients; lane 11) identification of ash-pollen profilin (13–16 kDa) by rabbit polyclonal antiserum (RP1) created against purified celery profilin; lane 12) atopic reference serum with known reactivity with birch profilin (Bet v 2). This serum also recognized Fra e 3 (Ca-binding protein) and HMW (glyco)proteins.

Table 1. Incidences of pollen sensitization among 5416 consecutive patients with positive skin prick tests to pollen allergens (observation period: 11/97–8/99)

Pollen	Average incidence (minimum/maximum)
Grasses	75.5 (60.9/86.8)
Fagales (birch)	53.1 (40.6/69.2)
Mugwort	20.3 (12.9/31.1)
Ash	17.6 (6.6/35.6)
Plantain	12.2 (8.4/18.0)
Ragweed	10.3 (3.3/19.1)
Nettle	6.0 (1.5/8.5)
Platanus	5.2 (1.0/9.0)
Oilseed rape (<i>Brassica</i>)	3.7 (1.0/6.5)

reduced ash-pollen proteins were used. A representative blot from 10 randomly selected patients is shown in Fig. 1. IgE binding occurred at 7/10 kDa (Ca-binding protein, Fra e 3), 13/16 kDa (profilin, Fra e 2), 17–23 kDa (Fra e 1), approximately 40 kDa (Fra e 1 dimer), and 40–80 kDa (putative HMW glycoproteins).

In 14 patients (30%), immunoblots were completely negative despite positive skin test and positive RAST. These patients had significantly lower RAST scores to ash (mean 2.57 kU/l, median 1.54) than positive patients (mean 9.00 kU/l, median 3.85; $P < 0.005$, two-tailed *t*-test) and also had lower total IgE levels (mean/median 144/85 kU/l vs 457/380 kU/l; $P < 0.0005$). However, negative immunoblot results were obtained in patients with RAST scores as high as 10 kU/l, while high immunoblot reactivity could be observed in some patients with specific IgE levels below 1 kU/l.

Identification of cross-reactive pollen panallergens

Ash-pollen profilin could be identified by the rabbit anticelery profilin antiserum as a double band running at approximately 16 and 13 kDa in SDS-PAGE (Fig. 1, lane 11). Additional binding occurred around 26 kDa, presumably identifying the profilin dimer. In a second allergen extract prepared from a different pollen batch, the two profilin fractions were poorly differentiated, and binding of the rabbit antiserum was predominantly seen with the 13-kDa protein (data not shown).

Reference sera recognizing Ca-binding proteins in alder (Aln g 4) and oilseed-rape pollen bound to a 10-kDa protein in extracts of olive pollen presumably corresponding with Ole e 3 (16). A protein of similar size was recognized by these sera in ash pollen (Fig. 2, lanes 1 and 2). One reference serum reacted also with Ole e 1, showing three distinct bands of 17 kDa (nonglycosylated Ole e 1), 20 kDa (glycosylated Ole e 1), and 23 kDa (highly glycosylated Ole e 1) and with Fra e 1 in ash pollen.

The reference sera recognizing periodate-sensitive carbohydrate epitopes on HMW glycoproteins of oilseed rape and grass pollen reacted with a similar protein cluster at 40–80 kDa in ash and olive pollen (Fig. 2, lanes 1 and 3). Both sera also recognized Fra e 1 and Ole e 1, respectively, but binding was restricted to the glycosylated proteins (20/23 kDa). RAST inhibition in one of these patients (lane 3) confirmed that IgE binding was entirely due to carbohydrate epitopes (see below).

Fra e 1 contains conformational IgE epitopes

Fifteen patients not reacting with reduced Fra e 1 (including the 14 completely negative patients), as well as another five patients with very weak reactivity, were reinvestigated by immunoblot employing nondenaturing/nonreducing conditions during electrophoresis.

Twelve of the 15 patients not binding with the reduced Fra e 1 reacted with the nonreduced Fra e 1; three remained negative. Of the five previously poorly reacting patients, three reacted much stronger with nonreduced Fra e 1, the binding remained poor in one patient, and in one patient, suspected to recognize Fra e 1 carbohydrates only, IgE-binding was restricted to HMW proteins. As a representative example, Fig. 3 shows immunoblot IgE-binding to reduced and nonreduced Fra e 1 in the six patients with isolated allergy to ash pollen; four of them reacted positively only under nonreducing conditions. In all, more than 50% of the investigated sera contained Fra e 1-specific IgE antibodies directed against conformation-dependent epitopes.

RAST inhibition with bromelain-BSA

Bromelain-BSA had no effect in 9/10 patients with Fra e 1 sensitization. One patient showed 43% inhibition by bromelain-BSA compared with 75% inhibition by ash pollen, indicating the presence of carbohydrate-specific IgE in this serum. Ash-pollen RAST in the CCD-specific reference serum (5.47 kU/l) was equivalently inhibited by ash-pollen extracts (83.5%) and bromelain-BSA (81.9%).

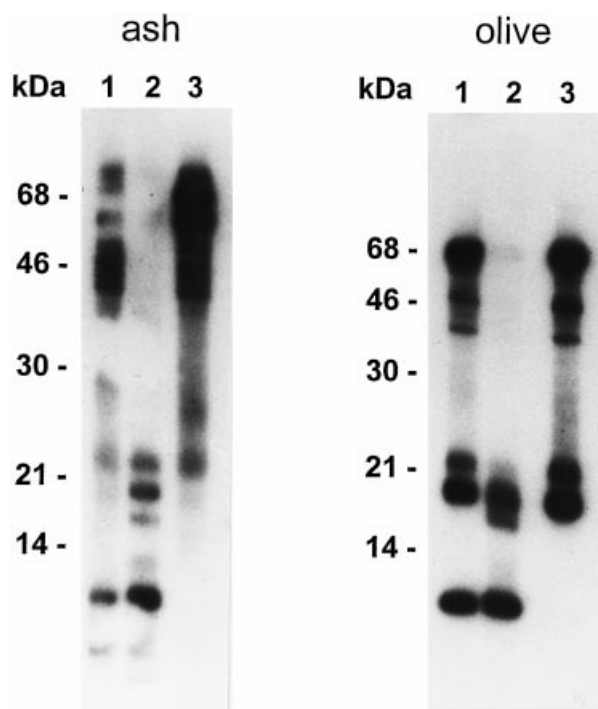


Figure 2. IgE-binding with ash (left panel) and olive pollen (right panel) extracts of three atopic reference sera selected for their known reactivity with Ca-binding proteins (lanes 1–2) and periodate-sensitive carbohydrate epitopes (lanes 1 and 3), respectively.

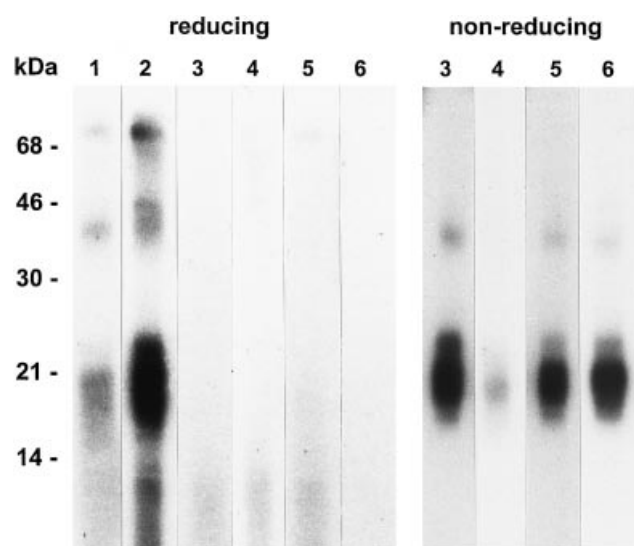


Figure 3. Conformation-dependent IgE epitopes in ash pollen. Sera from six patients with isolated sensitization to ash pollen were first incubated with blotted ash-pollen allergens separated by SDS-PAGE under reducing conditions (left panel, lanes 1–6). Four sera nonreacting with reduced proteins (lanes 3–6) were reinvestigated under nonreducing conditions (right panel).

The significance of Fra e 1 in relation to pollen panallergens in ash-pollen allergy

To estimate the significance of Fra e 1 in ash-pollen allergy, we compared the frequency of Fra e 1-specific IgE antibodies between the different patient groups, considering patients studied both under reducing and nonreducing conditions, as well as those already reacting with the reduced protein. The data suggest that Fra e 1 sensitization is mandatory in mono- and oligosensitization, while its frequency is below 50% in subjects with multiple pollen allergies (Table 2). On the other hand, sensitization to pollen panallergens was significantly more frequent in polysensitized individuals than in those mono- and oligosensitized (Table 3).

Pollen counts

Fraxinus pollen counts for Vienna were available from 1976 to 1999, except for 1996. Loads varied considerably between years, with total counts ranging from 229 to 5351 grains and yearly peak concentration between

Table 2. Frequency of IgE antibodies against Fra e 1 in mono-, oligo-, and polysensitized ash-pollen-sensitized patients according to immunoblot data. All patients ($n=36$) were first studied under reducing conditions and, in case of negative result, reinvestigated under nonreducing conditions. Statistical analysis was done by chi-square-test

Patient group	<i>n</i>	Fra e 1 sensitization		<i>P</i> <0.001
		Yes	No	
Isolated ash-pollen sensitization	6	6 (100%)	0	
Pollen oligosensitization	14	13 (93%)	1	
Pollen polysensitization	16	7 (44%)	9	

Table 3. Frequency of IgE-binding to pollen panallergens in crude ash-pollen extracts in 47 ash-pollen-sensitized patients according to Western blotting

Patient group	<i>n</i>	IgE against		
		7/10 kDa Ca-binding protein	13/16 kDa profilin	40–80 kDa HMW allergens
Isolated ash-pollen sensitization	6	0	0	1 (17%)
Pollen oligosensitization	16	0	3 (19%)	5 (31%)
Pollen polysensitization	25	8 (32%)	18 (72%)	15 (60%)
Chi-square-test iso-oligo- vs poly-		<i>P</i> <0.005	<i>P</i> <0.005	NS

23 and 837 grains/m³/24 h (Table 4). There was a trend toward increasing pollen loads from 1976 to 1999 ($r=0.42$ for total counts).

Discussion

The present study evaluated the incidence of sensitization to ash pollen and patterns of concomitant pollen allergies in an atopic population from eastern Austria, and focused on the question of to what extent sensitivity to ash pollen in this population reflects cross-sensitization to pollen panallergens. Two major conclusions can be drawn from the present study. Firstly, our data show that sensitization to ash pollen is not at all uncommon within the atopic population; secondly, our data demonstrate that in the majority of cases this sensitization is due to the ash-unique allergen Fra e 1. Pollen panallergens are implicated only in

Table 4. Yearly total counts and peak concentrations (grains/m³/24 h) of *Fraxinus* pollen in Vienna city, 1976–99. Arrows indicate years with pollen loads above average

Year	Annual total	Peak value	Days > 50 grains	Days > 100 grains
1976	247	60	1	0
1977	→ 2474	450	11	7
1978	1020	152	6	1
1979	656	119	2	1
1980	459	70	1	0
1981	229	23	0	0
1982	→ 3896	356	25	13
1983	365	55	1	0
1984	1171	159	8	4
1985	257	35	0	0
1986	→ 2355	354	15	8
1987	1054	200	7	3
1988	1224	258	8	4
1989	1060	226	6	3
1990	→ 2770	333	18	10
1991	→ 5351	837	16	13
1992	1709	126	15	2
1993	1192	130	7	3
1994	→ 2821	530	15	6
1995	→ 2595	476	12	8
1997	570	51	1	0
1998	1658	190	9	4
1999	→ 5289	613	18	13
Average	1757	252	8.8	4.5

subjects with multiple allergies. As a third point, we could confirm previous observations (7, 8, 18) that the ash-pollen load may vary extensively between years. In this connection, our data for 1998 and 1999 might indicate a positive correlation between pollen load and the incidence of sensitization, perhaps due to stronger boosting of ash-specific B-cell clones and selective referral of patients with clinical ash-pollen allergy in years of high atmospheric ash-pollen concentrations. By and large, we obtained stronger skin test reactions and higher RAST scores to ash in 1999 than 1998; however, other factors, such as composition of the allergen extracts employed for *in vivo* and *in vitro* testing, may also account for this difference.

Taking into account the observed patterns of pollen sensitivities and results from Western blotting, approximately 80% of ash-pollen-sensitized individuals will have Fra e 1-specific IgE. This is important to know with regard to immunotherapy in patients with spring pollinosis sensitized to birch and ash. Since cross-reactivity between these pollens is limited and confined to minor allergens (10, 17, 31), desensitization with birch pollen alone will poorly affect ash-pollen-induced symptoms. On the other hand, recent clinical studies in subjects monosensitized to olive pollen revealed that pollen loads as high as 400 grains/m³ are necessary to induce at least moderate allergic symptoms (41). Accordingly, the ash-pollen load would be clinically important only on a few days of the season. In our study, we observed a significant coincidence of sensitization to ash and birch pollen, while no such linkage existed between ash and other plants. This finding may indicate that pre-existent birch-pollen allergy represents a risk factor for subsequent sensitization to ash pollen, or vice versa, and may also imply that ash pollen may be more effective in provoking symptoms in the presence of allergic inflammation due to Fagales pollens. Specific immunotherapy with birch pollen may thereby have a certain impact on ash-pollen allergy. Future studies should investigate clinical threshold levels of airborne *Fraxinus* pollen and the influence of concomitant sensitization to ash pollen on the clinical efficacy of immunotherapy with Fagales pollens in patients suffering from spring pollinosis.

Our current knowledge about ash-pollen allergens comes mainly from *in vitro* studies focusing on cross-reactivities within the Oleaceae in subjects with primary allergy to olive pollen (12, 17, 21, 27), whereas patients with primary allergy to ash pollen have hardly been investigated (10). The present immunoblot results from 47 selected patients are largely consistent with published data on Oleaceae allergens (11, 12, 16, 17, 28–33). With the help of a profilin-specific mouse antiserum and selected human reference sera, we were able to assign most of the observed IgE binding in our sample to the following four different allergens:

- 1) the major ash-pollen allergen Fra e 1 (17/20/23 kDa)
- 2) ash-pollen profilin (Fra e 2, 13/16 kDa)
- 3) Fra e 3 (Ca-binding protein, 10 kDa)
- 4) HMW (glyco)proteins of 40 kDa and higher.

In contrast to some studies in olive-pollen-allergic patients, IgE-binding to other proteins was very rare in our sample.

IgE-binding to HMW allergens in Oleaceae is still poorly understood. We found that reactivity may be confined to a single band around 40 kDa. This pattern was only seen in patients with Fra e 1 sensitization and is probably due to the allergen dimer (1, 27–29), as binding was more pronounced under nonreducing conditions. In contrast, reactivity with a cluster of proteins of 40–80 kDa seems to be different from binding to the Fra e 1 dimer. We could demonstrate that two reference sera selected for their known reactivity with carbohydrate IgE epitopes on glycoproteins of grass and oilseed-rape pollen heavily bound to such proteins in ash and in olive pollen, and also selectively bound with the glycosylated Fra e 1 and Ole e 1 peptides. RAST inhibition in one of these patients revealed that IgE-binding can be totally inhibited by a neoglycoprotein displaying the xylose- and fucose-containing bromelain glycan. This supports the view that carbohydrate determinants represent ubiquitous, although perhaps clinically less relevant (34–36) cross-reactive structures in many pollens. In agreement with this, HMW allergens have been recently described as major cross-reacting structures between ash and birch pollen (10), and we suppose that carbohydrates are also involved in the observed cross-reactivity with grass pollens (11, 15, 37–40).

On average, about 70% of our patients had IgE antibodies against the major allergen of ash pollen, Fra e 1; however, half of them reacted only with nonreduced Fra e 1 in the Western blot. Provided that reduced and nonreduced proteins interact similarly with the nitrocellulose membranes, these findings suggest that IgE antibodies in these patients are directed against nonlinear epitopes. Conformation-dependent epitopes have been previously proposed to be also present on Ole e 1, since three of eight generated mouse monoclonal antibodies were found to bind specifically with the native protein in ELISA, but not with the reduced protein in Western blots (27). Accordingly, it is surprising that in olive-pollen-allergic patients, Ole e 1 has been repeatedly described to bind with 80–90% of tested sera in the Western blot despite denaturing and reducing conditions in all these studies (11, 28–30). Similar to our findings, in a recent immunoblot investigation in ash-pollen-allergic patients using standard reducing SDS-PAGE, around half of the tested sera were negative in the blot (10). It seems unlikely that

conformation epitopes play a different role in Fra e 1 and Ole e 1 in view of the high sequence identity between these proteins. One explanation is that much of the Ole e 1 reactivity seen in olive-pollen-allergic patients may be due to the Ole e 1 glycans, while carbohydrates do not seem to play a major role in Fra e 1-binding in ash-pollen-allergic subjects from temperate zones. In agreement with this view, the binding patterns obtained with our carbohydrate-specific reference sera (Fig. 2, lanes 1 and 3) indicate the possibility that Ole e 1 is much more highly glycosylated than its counterpart in ash pollen, Fra e 1.

In conclusion, our data indicate that ash pollen should be considered a potentially relevant source of

spring pollinosis. According to immunoblot data, the majority of patients appear to have been actively sensitized by ash pollen, while cross-sensitization to pollen panallergens is confined to the highly atopic population.

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