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Identification of Allergens in Oilseed Rape (*Brassica napus*) Pollen

Abstract

Background: Pollen from oilseed rape (OSR), *Brassica napus*, an increasingly cultivated oilplant from the Brassicaceae, has been recognized as a potential cause of allergic sensitization. Allergens have been hardly investigated. **Methods:** We characterized IgE binding proteins in OSR pollen by immunoblot, immunoblot inhibition and specific monoclonal antibodies using sera from 89 patients sensitized to OSR. **Results:** Two low-molecular-weight allergens of 6/8 kD and 14 kD as well as a high molecular-weight cluster (27–69 kD) comprising six cross-reactive peptides could be identified. The three allergens were recognized by 50, 34 and 80% of patients, respectively. Immunoblot IgE binding to OSR could be totally inhibited by rye pollen and moderately by birch pollen (6/8 and 14 kD) while mugwort had little effect. An anti-profilin-specific monoclonal antibody bound specifically to a 14-kD protein in OSR. Binding to the 6/8-kD rape allergen could be effectively inhibited by rAhn g 2, a calcium-binding protein from alder. Periodate treatment led to a significant reduction in IgE binding to the 27 to 69-kD OSR allergens indicating that carbohydrate determinants are involved in IgE binding. OSR proteins were capable to quench IgE binding to timothy grass pollen proteins of ≥ 60 kD suggesting that grass pollen group 4 allergens cross-react with the 27 to 69-kD cluster in OSR. **Conclusions:** The data demonstrate that OSR pollen is allergenic and indicate that the identified allergens represent cross-reacting homologues of well-known pollen allergens, i.e. calcium-binding proteins, profilins, and high-molecular-weight glycoproteins. Via cross-reactivity, exposure to OSR pollen may be a prolonging and aggravating factor in underlying birch and grass pollen allergy.

Key Words

Oilseed rape
Rapeseed
Brassica napus
Brassicaceae
Pollen allergens
Allergens
Cross-reactivity

Introduction

Oilseed rape (rapeseed, *Brassica napus*, Brassicaceae; OSR) is a partly wind-pollinated oilplant which has been increasingly cultivated in Europe and overseas for a decade or longer. For some time now there is increasing awareness that the cultivation of this crop may be associated with health adverse effects although there is still controversy concerning the magnitude of this impact and the mechanisms involved [1–3]. While complaints attributed to OSR cultivation have been considered to be caused by irritant

rape volatiles, such as terpenes, which are produced in high quantities by the plant for insect attraction or natural pathogen defense [3, 4], it has been known for nearly 50 years that OSR pollen may sensitize and provoke allergic symptoms [5]. Recent studies revealed conflicting results regarding the prevalence of OSR allergy but consistently proved the possibility of allergic sensitization to its pollen [1, 2, 6, 7]. The cultivation of the closely related species *B. rapa* (= *campestris*, turnip rape) has been recognized for years as a cause of allergic disease in India [8], and sensitization was also described from Japan [9].

Table 1. Specificity of monoclonal IgG antibodies against birch pollen, mugwort pollen and celery proteins

Antibody	Species	Allergen	Size, kD	Reference No.
BIP 1	birch	<i>Bet v 1</i>	17	13
BIP 3	birch		28, 30, 32	13
BIP 4	birch	<i>Bet v 1</i>	17	14
MUP 6	mugwort		28	unpublished
MUP 9	mugwort		10, 40	unpublished
MUP 12	mugwort		68	unpublished
MUP 15	mugwort		22, 32, 35	unpublished
Anti-profilin	celery	profilin	15	15

The allergens in OSR pollen have not yet been properly identified. Some limited data exist on *B. rapa* pollen [8] and IgE binding to one recombinant *Brassica* protein [9]. A more precise insight into IgE binding Brassicaceae pollen proteins using a limited number of sensitized sera was presented in our recent paper [7] showing allergens between 6 and 69 kD. These preliminary data also suggested potential cross-reactivity with birch pollen, while cross-reactivity with grass pollens has been indicated by others [10]. The aim of the present study was to identify major and minor allergens in OSR pollen in the sera of 89 rape-sensitive individuals by immunoblot, and to investigate possible cross-reactivities with other pollens by immunoblot inhibition and specific monoclonal antibodies (mAbs).

Material and Methods

Allergen Extracts

1 g of *B. napus* pollen (Kabi-Pharmacia-Allergon, Ängelholm, Sweden) was extracted overnight at 4°C in 10 ml phosphate-buffered saline (pH 7.4). The supernatant was recovered after centrifugation (12,000 rpm for 30 min at 4°C), dialyzed against distilled water at 4°C for 3 days, filtered through a 0.22-µm membrane (Millipore Intertech, Bedford, Mass., USA), lyophilized and stored at -20°C. Allergens from other pollens (birch, mugwort, rye, timothy grass) and proteins from crushed Brassicaceae seeds (*B. napus*, *Sinapis alba*/yellow mustard) were prepared according to the same protocol.

Patients

A skin prick test extract from OSR pollen (ALK Soluprick, Hørsholm, Denmark) was routinely included into a screening series for inhalant allergies. 89 patients with a positive skin prick test (wheal diameter >3 mm) and specific IgE to OSR (Pharmacia, positive cut-off ≥class 2) were selected for further investigation. Nearly all of these patients were also sensitized to another inhalant allergen (predominantly grass pollen) and about 75% were atopic with sensitization to a broad range of seasonal and perennial allergens [7]. Rhinitis and rhinoconjunctivitis were the predominant symptoms in 67 patients, 11 patients suffered from bronchial asthma and 11 patients suffered from recurrent bronchitis or recurrent cough.

IgE binding of OSR proteins was also studied in 17 patients monosensitive to grass pollen, in 5 patients monosensitive to mug-

wort, and in 5 atopic high IgE responders (total IgE levels >1,000 KU/l, Pharmacia PRIST).

SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE) and Protein Transfer

SDS-PAGE was carried out under reducing conditions [11] using 13.5% homogenous 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). 120 µg protein were loaded onto a single gel representing approximately 6 µg protein per lane. Transfer to nitrocellulose (Schleicher & Schüll, Dassel, Germany) was performed according to Towbin et al. [12].

IgE Immunoblots

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 or 1:20) at 4°C under continuous shaking, washed in buffer for 30 min and incubated overnight with ¹²⁵I-labelled rabbit anti-human IgE (Pharmacia) diluted to achieve 200,000 cpm/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.

mAbs and Recombinant *Aln g 2*

Seven mouse mAbs created against various birch and mugwort pollen antigens and a rabbit anti-celery profilin-specific mAb (table 1) were investigated for their reactivity with OSR proteins. Nitrocellulose strips were incubated with concentrated hybridoma supernatants under continuous shaking at 4°C overnight. After a wash with the above-mentioned buffer, the strips were incubated with ¹²⁵I-labelled sheep anti-mouse or donkey anti-rabbit IgG (Amersham), respectively, for 1 h. *Aln g 2* has been identified as a two EF-hand calcium-binding protein in mature alder (*Alnus glutinosa*) pollen running at 7.5 kD in SDS-PAGE. An alder pollen cDNA library was established and *rAln g 2* expressed as a non-fusion protein in *Escherichia coli* BL21 as described by Hayek et al. [16]. A rabbit was immunized with *rAln g 2* and the pre-immune and immune serum used to identify cross-reacting proteins in OSR pollen.

Immunoblot Inhibition

Immunoblot inhibition experiments were carried out either using pooled or individual sera. Sera were diluted 1:10 or 1:20 and preincubated overnight with ≥25 µg/ml protein when using whole pollen ex-

tracts, and 1 µg/ml of rAln g 2, respectively. 25 µg BSA/ml serum and buffer alone were used as controls. Subsequent steps were the same as described above.

Identification of Glycoproteins by Protein Periodate Treatment
PAS Staining. Identification of glycoproteins in electrophoretically separated OSR pollen extracts on SDS-PAGE was performed by PAS staining. Gels were fixed in 5% trichloroacetic acid (6 h), washed twice in a mixture of isopropanol:acetic acid:water (1st wash 25:10:65, 2nd wash 10:10:80) and finally in 10% acetic acid. Sugar residues were oxidized with 1% periodic acid in 5% acetic acid (1 h, 20°C). The gel was washed twice for 10 min with 0.2% sodium-metabisulfite in 5% acetic acid and incubated overnight with Schiff's reagent at 4°C. The gel was finally washed with 0.1% sodium-metabisulfite/0.01 N HCl until bands became visible.

The effect of periodate treatment on the IgE binding capacity of OSR pollen allergens was studied in 5 individual patients. Single nitrocellulose strips with electroblotted OSR proteins were oxidized with periodate (10 mM periodic acid in 0.1 M sodium acetate buffer, pH 5.5, 1 h, 20°C) and subsequently the IgE binding patterns compared with those from untreated strips.

Results

IgE Binding Proteins in OSR Pollen

Sixty-two of the 89 patients were positive in the immunoblot. Nine IgE-binding proteins with molecular masses of 6, 8, 14, 27, 33, 42, 51, 60 and 69 kD could be identified (fig. 1). IgE binding was most prominent with the high-molecular-weight allergens above 33 kD, while less than 50% of patients reacted with allergens below 27 kD. The calculated frequencies of IgE binding by the different allergens are shown in table 2.

The diversity of individual IgE binding patterns was found limited due to obvious linkage of some allergens. Three independent groups of allergens could be distinguished: (1) a 6/8-kD double band; (2) a 14-kD protein occasionally showing a 12/14-kD double-band structure and (3) a high-molecular-weight protein cluster between 27 and 69 kD showing decreasing IgE reactivity from 60/69 towards 27 kD. Only 2 patients reacted with just one protein near 42 kD (fig. 1, lane 2).

Immunoblot Inhibition with Birch, Rye and Mugwort Pollen Extracts

Preincubation of a serum pool with OSR proteins (25–100 µg protein/lane) completely inhibited IgE reactivity in the Western blot (fig. 2, lanes 1–4). Preincubation with birch pollen extract blocked IgE binding to the 14-kD protein and quenched binding at 6/8 kD (lane 5). No inhibition with mugwort could be detected apart from a weak reduction in binding at 6 kD (lane 6). Preincubated rye pollen ex-

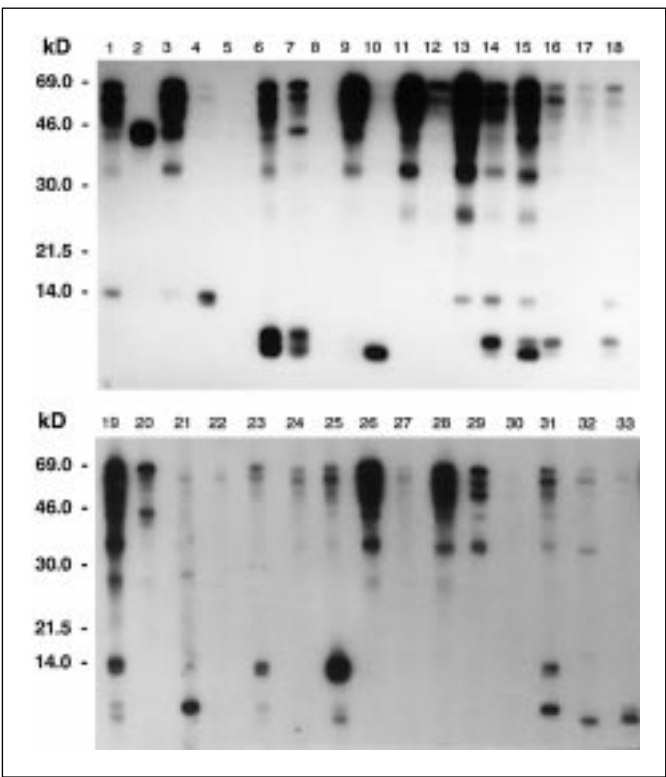


Fig. 1. IgE immunoblot to OSR pollen extracts of 33 representative patients with positive skin prick test and positive RAST to OSR pollen. Three groups of IgE binding proteins with molecular masses of 6/8, 14 and 27–69 kD can be distinguished.

Table 2. Frequency of IgE antibodies against different OSR pollen allergens in 62 patients positive in Western blots

kD	6	8	14	27	33	42	51	60	69
Frequency, %	37	43	34	35	52	56	61	80	76
	50								

tracts were as effective as OSR extracts, leading to an almost complete inhibition of IgE binding. Weak reactivity remained to the 6-kD protein (lane 7).

IgE Reactivity to OSR Pollen Allergens of Patients with Grass, Mugwort and Multiple Pollen Sensitivity

No IgE antibody binding to OSR proteins was detected in the 5 mugwort monosensitive sera and in the 5 polysensitized high IgE responders. Three of the 17 grass-pollen-allergic patients (17.6%) had IgE antibodies against the 27 to 69-kD cluster (data not shown).

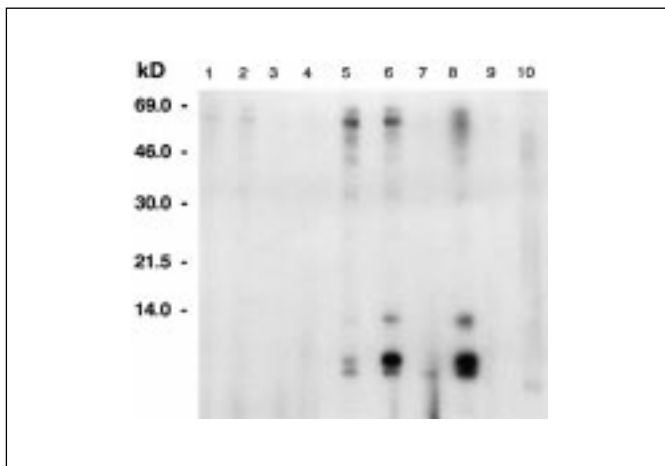


Fig. 2. Inhibition of IgE binding to OSR pollen allergens by serum preincubation with pollen extracts from OSR (lanes 1–4: 25–50–75–100 µg), birch (lane 5), mugwort (lane 6) and rye (lane 7); lane 8: positive control, lane 9: non-atopic human serum, lane 10: buffer control. 25 µg of OSR pollen or rye pollen protein completely inhibited IgE binding. Birch pollen extracts blocked binding to the 14-kD rape protein. All extracts to a variable degree reduced IgE binding to the 6/8-kD double band.

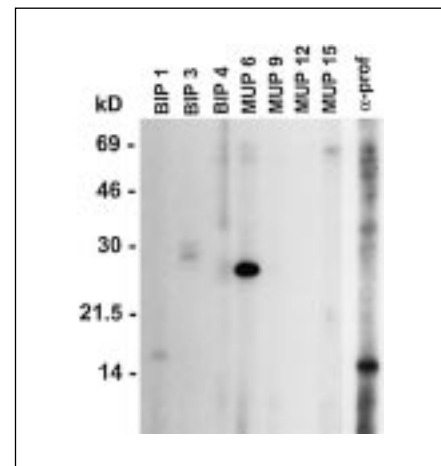


Fig. 3. Reactivity of monoclonal IgG antibodies raised against birch pollen proteins (lanes 1–3), mugwort pollen proteins (lanes 4–7), and celery profilin (lane 8) with proteins from OSR pollen. Lanes 1–3: BIP 1, BIP 3, BIP 4; lanes 4–7: MUP 6, MUP 9, MUP 12, MUP 15; lane 8: anti-celery profilin. BIP 1 weakly recognizes a putative *Bet v 1* homologue at 17 kD, BIP 3 (anti-birch 28/30/32 kD) binds to comparable OSR proteins of 30/32 kD. Of the mugwort-specific mAbs, MUP 6 (anti-mugwort 28 kD) strongly binds to a 27-kD rape protein. The anti-profilin mAb reacts with a putative rape profilin of 14 kD.

Immunoblot Reactivity of mAbs Directed against Birch and Mugwort pollen Allergens

BIP 1 raised against the major birch pollen allergen *Bet v 1* bound weakly to a 17-kD protein. BIP 3, recognizing 26/28/30 kD in birch, bound to a 30/32-kD double band in OSR. Among the mugwort-specific mAbs tested, MUP 6 strongly recognized a rape protein of 27 kD (fig. 3). The anti-celery profilin-specific mAb bound specifically to a 14-kD protein; weak unspecific binding seen with other proteins was considered to be due to antibody overload. The remaining mAbs did not bind.

IgE Cross-Reactivity between Pollen and Seed Extracts

IgE binding to OSR seed extracts was very similar to the pattern obtained in pollen extracts except for the lack of IgE binding at 6/8 kD. Reactivity with the mustard seed was slightly weaker. No binding was observed at 6/8 and 14 kD; instead, a band near 20 kD was noted. Both seed extracts showed an additional double band of approximately 29/31 kD (fig. 4).

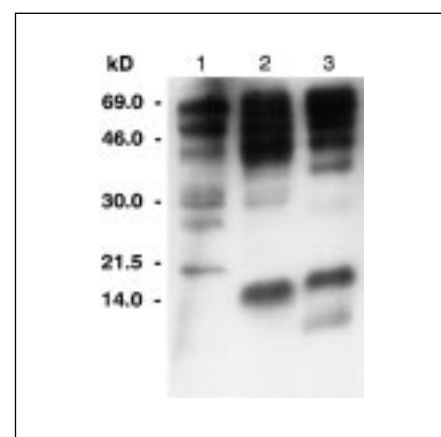


Fig. 4. IgE binding patterns obtained with pooled rape-pollen-sensitive sera ($n = 10$) to OSR pollen proteins (lane 3) and seed extracts from yellow mustard (lane 1) and OSR (lane 2).

Identification of Allergenic High-Molecular-Weight Glycoproteins in OSR Pollen

Direct staining for carbohydrate moieties of OSR pollen proteins on polyacrylamide gels by PAS revealed only weak staining around 46 and over 60 kD (data not shown). Periodate treatment of OSR proteins led to a dramatic reduction of IgE binding to the high-molecular-weight allergens in 3

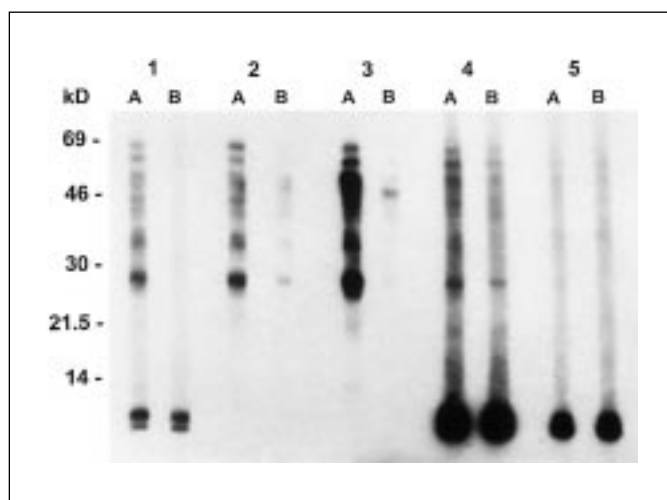


Fig. 5. IgE binding patterns of 5 selected patients to proteins from OSR pollen before (lanes A) and after (lanes B) protein oxidation by periodate. Reactivity with the high-molecular-weight allergen cluster is largely lost after periodate treatment in patients 1–3. Binding to the 6/8-kD double band is not influenced by the treatment.

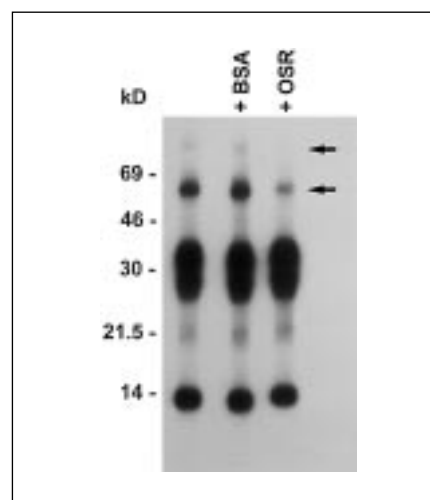


Fig. 6. Locating of allergens in timothy grass pollen cross-reacting with high-molecular-weight OSR glycoproteins. Pooled sera of 3 patients with IgE antibodies against the OSR 27- to 69-kD allergen cluster were preincubated with 50 µg of BSA (middle lane) and OSR pollen proteins (right lane), respectively, and subsequently tested on a timothy grass pollen extract. Rape proteins were capable to quench IgE binding to grass pollen allergens around 60 kD and higher. No obvious inhibition could be seen with group 1 + 5 allergens and low-molecular-weight allergens.

of 4 individuals recognizing these allergens in untreated extracts (fig. 5, patients 1–3) indicating that carbohydrates represent the IgE binding determinants or are involved in the conformation of allergenic epitopes in these allergens. A less clear reduction in IgE binding to the high-molecular-weight allergens was seen in patient 4. Binding to the 6/8- and 14-kD allergens was not affected by periodate treatment. To identify in grass pollen cross-reactive homologues of the OSR 27- to 69-kD cluster, we inhibited IgE binding of a OSR-sensitive serum pool to timothy grass pollen extracts with OSR extract (50 µg/ml serum). OSR proteins were able to quench IgE binding to *Phleum* proteins of approximately 60 kD and to extinct a weak additional band around 90 kD (fig. 6).

Identification of the 6/8-kD OSR Allergen as a Calcium-Binding Protein

IgE binding to the 6/8 kD OSR allergen could be inhibited to a variable degree by all pollen extracts used (birch, rye, mugwort; fig. 2) suggesting that this allergen could belong to a recently identified class of Ca-binding pollen proteins. To further test this hypothesis, *rAln g 2*, a calcium-binding protein from alder pollen, was employed in immunoblot inhibition experiments, and the IgE reactivity of OSR-allergic patients with this protein was tested. In 5

selected patients with antibodies against the 6/8-kD OSR allergen, IgE binding could be effectively inhibited by serum preincubation with 1 µg/ml *rAln g 2* leading either to quenching or complete loss of IgE reactivity (fig. 7, lanes 1A–5B). All these patients also reacted with the immunoblotted *rAln g 2* (fig. 8, lanes A). In 4 of the 5 patients, IgE binding to the *rAln g 2* could be essentially inhibited by a whole timothy grass pollen extract (fig. 8, lanes B). In patient 3, IgE binding to *rAln g 2* could not be inhibited by grass pollen extract (fig. 8, lanes 3A, 3B) and *rAln g 2* only blocked IgE antibodies against the 8-kD but not those directed against the 6-kD OSR peptide (fig. 7, lanes 3A, 3B). (Pre)immune serum from a rabbit immunized with recombinant *Aln g 2* bound selectively to the 6/8-kD double-band in OSR pollen (fig. 8, lanes 6, 7).

Discussion

In the present study we identified nine different IgE binding proteins in pollen from OSR and demonstrated IgE and IgG cross-reactivity with proteins from other pollens. IgE binding peptides in OSR may be classified into three allergen groups, i.e. 6/8, 14 and a high-molecular-weight cluster comprising several cross-reactive peptides with

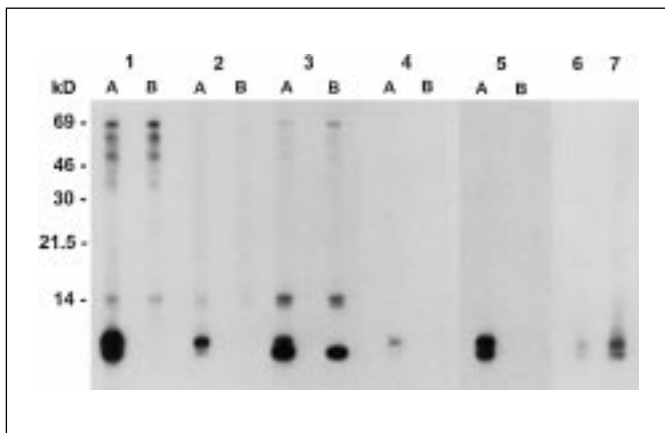


Fig. 7. Inhibition of IgE binding to the 6/8-kD OSR allergen by recombinant *Aln g 2*, a calcium-binding protein from alder pollen. Preincubation of 5 selected individual sera with 1 µg r*Aln g 2*/ml led to a total (patients 1, 2, 4, 5) or partial (patient 3) extinction of IgE binding to the rape allergen. A rabbit anti-serum against r*Aln g 2* recognizes the same 6/8-kD double band (lane 6: pre-immune serum, lane 7: immune serum). Lanes A: not inhibited, lanes B: inhibited with r*Aln g 2*.

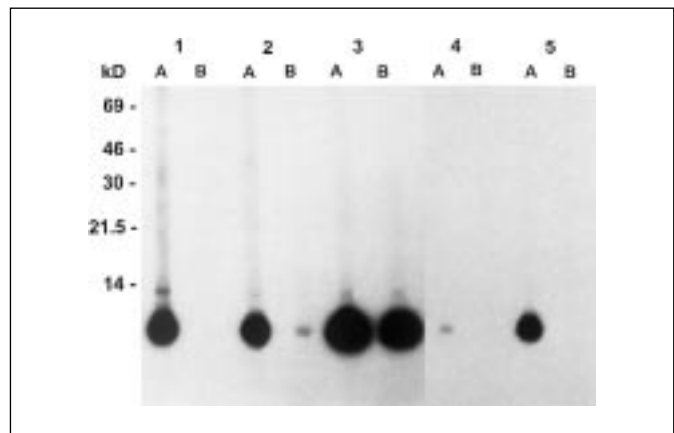


Fig. 8. Reactivity of five rape-sensitive patients (same as in fig. 7) with electroblotted recombinant *Aln g 2* (lanes A) and inhibition of antibody binding by total timothy grass pollen extract (lanes B). All patients reacted with the recombinant alder protein equivalently to their binding to the 6/8 rape allergen, and in all but patient 3 could the IgE binding be effectively inhibited by *Phleum* proteins.

molecular masses between 27 and 69 kD. Although the sample size was much bigger than in our previous study [7], we could not definitely identify new allergens. Additional allergens might be detected by two-dimensional techniques or, considering genetic heterogeneity between different cultivars [17, 18], with extracts from other OSR varieties. Furthermore, people with very high exposure to rape pollen, e.g. occupationally exposed individuals, might react with other allergens. The 6/8-kD allergen as well as the 27- to 69-kD cluster were recognized by at least 50% of our patients and may be considered major rape pollen allergens therefore. Our data also indicate that allergen profiles seem to be similar among *Brassica* species as comparable allergens (67, 60 and 14 kD) have been recently described from the closely related species *B. rapa* (= *campestris*, turnip rape) [8].

IgE binding to the 6/8-kD double-band may be restricted to either 6 or 8 kD alone but mostly occurs to both peptides suggesting sequence homology between them. As there is no evidence from PAS staining and periodate treatment that the 8-kD peptide represents a glycosylated peptide, these peptides more likely represent naturally occurring transcription or splicing variants with both shared and unique allergenic epitopes. Recently, Toriyama et al. [9] reported IgE binding to recombinant *Brassica* peptides of 8 kD with sequence homology to calmodulin in an OSR-allergic individual. In the present study, we demonstrated that patients

reacting with the 6/8-kD rape allergen also react with recombinant *Aln g 2*, a calcium-binding protein from alder pollen. We also showed that IgE binding to the 6/8-kD OSR allergen may be effectively inhibited by serum preincubation with r*Aln g 2*. Furthermore, serum from a rabbit immunized with the r*Aln g 2* selectively bound to the 6/8-kD double band in OSR extracts. These data corroborate the findings of Toriyama et al. [9] and provide evidence from a higher number of individuals that the 6/8-kD allergen in OSR pollen is a calcium-binding protein sharing IgE and IgG epitopes with homologous proteins in other pollens. Calmodulin-like proteins have been increasingly recognized as pollen allergens within the last years [16, 19–21] and have been proposed to define another class of cross-reacting panallergens among plant pollens due to conserved amino acid sequences within their Ca-binding domains. Our inhibition results support the conception of a broad cross-reactivity within this protein family. Likewise, cross-reactivity has been recently shown between *Cyn d 7*, a Ca-binding allergen from Bermuda grass, and *Bra r 1*, a Ca-binding protein from turnip rape (*Brassica rapa*) [22]. On the other hand, specific IgE antibodies against the OSR calcium-binding protein in patient 3 (fig. 7, 8) could be only marginally or not at all inhibited by *Aln g 2* and *Phleum*, respectively, indicating the existence of species-specific epitopes.

The IgE-binding peptides within the 27- to 69-kD cluster in OSR seem to be closely related although minor individual differences in IgE binding intensity within the cluster suggest that different IgE epitopes are involved. In only 2 of 62 patients were IgE antibodies directed against the 42-kD protein alone (e.g. fig. 1, lane 2), but it remains to be elucidated whether the latter protein belongs to the 27- to 69-kD cluster or rather represents a separate allergen. We have shown by Western blot inhibition that IgE binding to the 27- to 69-kD allergens is totally inhibited by rye pollen proteins but merely by birch and mugwort proteins. This fact might explain why OSR sensitization is so commonly associated with grass pollen allergy [7]. To identify cross-reacting allergens in the Gramineae we investigated the inhibitory capacity of OSR proteins on IgE binding to timothy grass pollen allergens in Western blot and we could locate a group of cross-reacting proteins in the high-molecular-weight range ≥ 60 kD, presumably representing group 4 grass pollen allergens. These findings are consistent with recent data by Astwood et al. [10] who found that mAbs against group 4 and group 9 grass pollen allergens specifically bind with high-molecular-weight proteins (55/65 kD) in OSR pollen. The fact that as little as 25 μ g of rye pollen extract was able to totally extinct IgE binding to blotted OSR proteins while 50 μ g of OSR pollen extract only moderately inhibited binding to grass pollen allergens favors the idea that grass pollen was the primary sensitizer in these patients.

As group 4 allergens are highly glycosylated proteins [23], we investigated the potential role of carbohydrate determinants in IgE binding to the high-molecular-weight OSR allergens. PAS treatment of OSR proteins on polyacrylamide gels revealed only weak staining, but periodate treatment led to a nearly complete extinction of IgE binding to the 27- to 69-kD allergens in 3 of 4 patients indicating that carbohydrate moieties either represent the IgE binding epitopes in these allergens or play a pivotal role in the maintenance of conformational peptide epitopes. However, as periodate treatment did not totally inhibit antibody binding or was not equally effective in all patients, the role of non-carbohydrate epitopes is currently under investigation with a higher number of patients. Among the mAbs used in this study, BIP 3, directed against proteins of 26/28/30 kD in birch pollen, and MUP 6, directed against a 28-kD mugwort protein, cross-reacted with rape proteins of comparable size. It seems less likely, however, that the peptides bound by these mAbs are part of the 27- to 69-kD cluster as both birch and mugwort extracts could not inhibit IgE binding to these allergens.

The strong IgE binding seen with blotted extracts from rapeseeds and mustard seeds demonstrates that many *Bras-*

sica allergens are common in both, pollen and seeds. The clinical relevance of these in vitro findings remains unclear at this time. No adverse effects were reported by any of our patients when eating mustard or vegetables from the Brassicaceae family but we think that these observations should be subjected to further investigation. In clinical mustard allergy, seed storage proteins around 14 kD, called napins, have been identified as responsible allergens [24, 25]. There is evidence that napin genes are also expressed during *Brassica* microspore embryogenesis [26], suggesting that the 14-kD allergen detected in OSR pollen could be a napin. In accordance with this evidence, positive skin prick test reactions to *Brassica* pollens have been described in patients with adverse effects to mustard and Brassicaceae food [27], but, on the other hand, serum IgE from a patient with proven napin allergy did not bind to OSR pollen proteins [28]. We have now shown using a serum pool of OSR-sensitive patients that IgE binding to the 14-kD OSR pollen allergen is considerably inhibited by birch and rye pollen extracts and that a mAb directed against celery profilin specifically binds to a 14-kD protein in OSR. Although this is not conclusive evidence, it makes us assume that the 14-kD OSR pollen allergen identified in our patients is not napin-like but a profilin cross-reacting with *Bet v 2* (birch profilin) and grass pollen profilin [15].

The cultivation of OSR has been blamed to be associated with adverse health effects in the general population, particularly in the UK. Allergic sensitization to OSR pollen, however, was found to be uncommon in most studies [2, 6, 7], thus leading to an increasing interest in the potential role of OSR volatiles [4, 29]. However, epidemiological studies have shown that more than 40% of those reporting symptoms during the rape flowering season are atopic [29] suggesting that, albeit sensitization against rape pollen is usually not demonstrated, allergic mechanisms do play a role in OSR-associated adverse effects in a substantial part of the affected population. Our present findings indicate that allergens in OSR pollen, as identified by our study population, represent cross-reactive homologues of well-known pollen allergens, i.e. (1) calcium-binding proteins, (2) profilins and (3) glycoproteins cross-reacting via their carbohydrate moieties. We could not identify OSR-unique allergens so far, and there is no good evidence from our patients for active sensitization by OSR pollen at all. It should not be excluded therefore that OSR pollen via one of its cross-reacting allergens may cause moderate symptoms during the rape flowering season (April–May) in a subset of atopic individuals despite a negative allergy test to OSR. 10% of patients with IgE antibodies against *Cyn d 7*, a calcium-binding protein from Bermuda grass, have been reported to complain of a

‘nonspecific hyperreactivity’ during the entire pollen season [22] underlining the potential role of cross-reactive pollen panallergens. Furthermore, it has to be kept in mind that commercially available OSR pollen extracts are not standardized. Particularly, low-molecular-weight allergens may be underrepresented in these extracts and may thus produce false-negative test results.

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