

Sensitization to oilseed rape is not due to cross-reactivity with grass pollen

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Summary

Background Oilseed rape is an important crop grown in the UK which can cause specific immunological sensitization with clinical symptoms in a relatively small number of the general population. Individuals with immunoglobulin (Ig) E-mediated allergy to oilseed rape have also been found to be sensitized to other pollen allergens, most frequently being grass pollen. Cross-reactivity between common grass and oilseed rape would have important implications, especially as their flowering period coincides.

Objective We have investigated whether the cosensitization found in individuals sensitized to both oilseed rape and grass pollen is due to cross-reactivity.

Methods Cross-reactivity between oilseed rape and grass pollen was determined using RAST, RAST inhibition, Western blotting and inhibition studies with Western blotting.

Results Competitive RAST inhibition studies between pollen of oilseed rape and grass failed to show any cross-reactivity between the pollen types. Self-inhibition with oilseed rape resulted in 90% inhibition, whereas there was less than 10% inhibition with grass pollen. Western blotting revealed allergens of similar molecular weight in both oilseed rape and grass pollen. Despite allergens of similar molecular weights being present in both pollen types, inhibition immunoblot studies confirmed that the allergens in the two allergens were immunologically distinct.

Conclusion The allergens of oilseed rape and grass pollen, although similar in molecular weights, are immunologically distinct and there is no evidence of cross-reactivity between them. Individuals allergic to grass pollen will not necessarily develop a specific nasal or airway response to inhaled oilseed rape pollens.

Keywords: cross-reactivity, grass pollen allergy, oilseed rape

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Introduction

Oilseed rape is an important crop in the United Kingdom, with about 400 000 hectares of arable land given over to its cultivation. The main species grown is *Brassica napus* with its distinctive yellow flowers in spring and late summer. The crop is mainly grown for its biodegradable oils which can be

used for the production of cooking oil, machine oil, diesel substitute and as a base oil for the plastics industry. The high protein seed residue following oil extraction provides a good source of animal feed.

With the increase in the acreage of the crop, concern has grown with regard to human health. There is concern that oilseed rape may in part be responsible for allergic symptoms during the peak flowering period. Oilseed rape is known to emit at least 22 volatile organic compounds, which could potentially act as irritants or allergens [1,2]. These volatile organic compounds are released to various

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degrees by other plants; however, the amount produced per unit area with oilseed rape crop may be largely due to the densely flowering heads of oilseed rape.

Oilseed rape can cause specific immunological sensitization with clinical symptoms [3,4]. The prevalence of immunoglobulin (Ig) E-mediated allergy to oilseed rape in the general population appears to be low [5], Hemmer *et al.* concluded from a very large data set of individuals attending an allergy clinic that there was a 3% prevalence of sensitivity to oilseed rape [6]. With increasing exposure to oilseed rape the rate of sensitization increases to 33% in occupationally exposed personnel [7].

The flowering period of oilseed rape coincides with the 'hay fever' season and a high proportion of individuals with IgE-mediated allergy to oilseed rape also have IgE antibodies to other pollen allergens, the most frequent being grass pollen [6]. Monoclonal antibodies raised to recombinant allergens of grass pollen have shown some cross-reactivity with proteins of oilseed rape [8].

We have investigated the possibility that cases of grass pollen allergy may have symptoms provoked by oilseed rape proteins which cross-react with IgE induced by grass pollen. Using RAST, RAST inhibition and Western blotting we have investigated the degree of cross-reactivity between proteins of oilseed rape pollen and proteins of grass pollen in individuals with IgE to both oilseed rape and grass pollen.

Materials and methods

Patient selection

Sera were obtained from three individuals who worked directly with oilseed rape (serum 1, 2 and 3).

Three other sera were obtained from individuals who were sensitized to grass pollen (serum 4, 5 and 6). Of the three grass-sensitized individuals, one worked with cereals but was also indirectly exposed to oilseed rape (serum 4), one drove a vehicle using diesel substitute made from oilseed rape (serum 5) and the third was not known to be exposed to oilseed rape (serum 6).

Case histories of occupationally exposed workers to oilseed rape

The three individuals who were occupationally exposed to oilseed rape worked for a company developing modified strains of oilseed rape for the agricultural market. Their job included the manual cross-pollination and inspection of large numbers of oilseed rape plants grown under glass, a task giving rise to close contact with oilseed rape pollen. Three or four crops a year of oilseed rape are grown in this way.

Case 1

Case 1, a 21-year-old man, reported a history of spring and summer rhinorrhea, without lower respiratory symptoms, since teenage. His symptoms were worse and more prolonged during occupational exposure to oilseed rape pollen; in addition he developed an itchy skin rash and some exercise-induced wheeze. He had immediate skin prick test responses to grass pollens (9 mm weal) and house dust mite (5 mm). Specific IgE to oilseed rape as measured by RAST was 29%. A single measurement of his FEV₁ was normal, without change after salbutamol. Serial measurement of his peak flow, however, suggested mild, work-related asthma. His symptoms improved with the use of laminar-flow respiratory protection during oilseed rape pollination; and resolved entirely when he moved to a different job.

Case 2

The second case, a woman of 27 years, reported episodes of asthma induced by house dust mite, cold weather and respiratory infections since the age of 10; she did not describe a past history of seasonal rhinitis. Three years after starting work with oilseed rape she developed rhinitis, itching and swelling of her eyes, an itchy skin rash and exacerbations of her asthma during exposure to oilseed rape pollen. During the winter months her symptoms resolved away from work; during spring they improved but not entirely. Skin prick tests were positive to house dust mite (6 mm weal) and mixed grass pollen (7 mm); serum specific IgE to oilseed rape showed 37% binding by RAST. Her FEV₁ was 2.9 L, rising to 3.3 L after inhalation of salbutamol. Her nasal symptoms resolved completely when she moved to an administrative post.

Case 3

The third case, a woman of 23 years, gave a history of moderately severe hay fever (summer rhinitis and eye itching) since childhood; she had no past history of wheezing. We were asked to advise on the suitability of her employment with oilseed rape pollen. Since starting work with oilseed rape, 6 months prior to her referral, she had experienced no new or work-related symptoms. This situation is unchanged a year later. Skin prick tests confirmed her sensitivity to grass pollens (9 mm weal); serum-specific IgE to oilseed rape were negative as measured by RAST (0.7% binding).

Preparation of allergen extracts

Rape pollen and anthers were collected from the place of work of the occupationally exposed workers. Individual

grasses within the grass mix used for the Grass CAP (Pharmacia and Upjohn, Milton Keynes, UK), namely *Poa*, *Phleum*, *Lolium*, *Dactylis* and *Festuca* were obtained from Allergon, Angelholm, Sweden. Rye, wheat and maize pollen were obtained from Sigma, Poole, UK.

Aqueous extracts were prepared by shaking 1 g allergen in 10 mL 0.1 mol/L ammonium carbonate overnight at 4°C. Extracts were centrifuged for 10 min at 500 g at 4°C, the supernatants dialysed for 3 days against distilled water, lyophilized, and stored at -20°C until used.

Specific IgE determination

Specific IgE to common silver birch, grass mix, *Alternaria alternata* and *Botrytis cinerea* were measured using CAP assay according to the manufacturer's instructions (Pharmacia and Upjohn). Specific IgE to rape pollen, rye pollen, maize pollen, wheat pollen and barley was carried out using a RAST assay. Briefly, 3 mg of allergen was coupled to 300 mg cyanogen-activated paper discs according to the method of Ceska *et al.* [9]. For the assay, serum (50 µL) was added to a disc. Following incubation at room temperature for 16 h, the disc was washed and added to 50 µL ¹²⁵I-antihuman IgE. After 16 h at room temperature, the disc was washed and counted in a gamma scintillation counter. The amount of antigen-specific IgE was expressed as the percentage of the added c.p.m. that remained bound to the disc (percentage binding). A 2% binding was chosen as the positive cutoff point. Cord blood was used as a negative control.

RAST inhibition assays

Competitive RAST inhibition assays were conducted to determine the inhibition of binding of IgE to oilseed rape using grass pollen and also the inhibition of binding of IgE to grass pollen using oilseed rape. The inhibitions were carried out using pooled sera from cases 1 and 2 who had specific IgE to both oilseed rape and grass pollen. The pooled serum was diluted 1 : 32 to give an optimal binding to oilseed rape (13% binding) and grass pollen (14% binding). Fourfold dilutions of the inhibiting allergen were used, ranging from 1 mg/mL to 0.015 mg/mL. Grass pollen comprised of equal concentrations of *Poa*, *Phleum*, *Lolium*, *Dactylis* and *Festuca*. Briefly 50 µL of serum and inhibitor were incubated overnight at room temperature and incubated for 16 h with the disc. The disc was washed and processed as above in the RAST assay.

Electrophoresis

Oilseed rape (preparation from pollen and anthers), *Poa*, *Phleum*, *Lolium*, *Dactylis* and *Festuca* (Allergon) were separated by SDS-PAGE using 12.5% acrylamide gel

according to the method of Laemmli [10]. Samples (40 µg) were electrophoresed in parallel with molecular weight markers (Pharmacia and Upjohn). The relative mobilities of the marker proteins were plotted against the log molecular weight (in kilodaltons), and the molecular weight of the protein bands were interpolated manually.

Western blotting

Forty micrograms of oilseed rape and a grass mix comprising of equal concentrations of *Poa*, *Phleum*, *Lolium*, *Dactylis* and *Festuca* (Allergon) were separated by SDS-PAGE using 12.5% acrylamide gel and were then transferred onto nitrocellulose membranes (BioRad, Hemel Hempstead, UK) using the method of Trowbin *et al.* [11]. The membranes were cut into 0.5 cm wide strips and probed with 2-mL aliquots of sera for 16 h at room temperature with shaking. Where necessary the sera were diluted to give a RAST of between 10 and 15% binding. Those sera with less than 10% binding were used neat. The strips were given three 10-min washes with PBS 0.5% Tween 20 and once with H₂O. The washed strips were probed with ¹²⁵I antihuman IgE (Pharmacia and Upjohn) which was diluted 1 : 4 with PBS 0.1% HSA for 16 h at room temperature. The strips were given three washes, dried and exposed to Hyperfilm MP (Amersham, UK) at -70°C for 24 and 48 h.

Inhibition studies were carried out by incubating inhibitor at a concentration of 1 mg/mL with the sera for 1 h prior to incubation with the nitrocellulose membrane strips.

Results

Specific IgE to oilseed rape and other pollen allergens

Specific IgE measurements were carried out against oilseed rape, grass pollen mix and other allergens likely to be present during the oilseed rape season namely, wheat, barley, maize, common silver birch. Fungi associated with oilseed rape such as *Alternaria alternata* and *Botrytis cinerea* were also investigated (Table 1). The two patients with occupational allergy had specific IgE to oilseed rape; none of the others had specific IgE to oilseed rape. All six cases were sensitized to grass pollen. The oilseed rape-sensitized individuals were also found to have IgE antibodies to the whole panel of allergens.

RAST inhibitions between oilseed rape and grass pollen

Competitive RAST inhibition between grass pollens and oilseed rape suggests there is no cross-reactivity between oilseed rape and grass pollens (Figs 1,2). Self-inhibition with oilseed rape results in a significant 90% inhibition at 1 mg/mL, however, grass pollens at 1 mg/mL have less

Table 1. Specific IgE to oilseed rape and other pollen allergens

Sera	OSR (% b)	Grass pollen (kU/L)	Wheat (% b)	Barley (% b)	Rye (% b)	Maize (% b)	Botrytis (kU/L)	Alternaria (kU/L)	Silver birch (kU/L)
1*	28.9	>100	21.6	15.7	38.5	50.0	1.8	44.3	9.9
2*	36.7	>100	16.2	8.0	39.5	60.1	1.4	0.6	16.1
3*	0.7	>100	5.1	3.1	27.0	50.4	<0.35	N.D.	0.5
4	1.4	6.7	1.8	3.3	21.0	17.1	<0.35	1.0	<0.35
5	0.4	0.5	0.9	0.8	1.0	1.5	<0.35	1.0	<0.35
6	1.8	>100	3.2	4.5	31.3	53.4	0.4	0.4	<0.35

*OSR occupationally exposed.

than 10% inhibition. This suggests that oilseed rape and grass pollens have distinct allergens.

SDS-PAGE of rape and grass pollens

Gel electrophoresis of oilseed rape stained with Coomassie blue reveals a number of proteins ranging between 67 and 94 kDa. Grass pollen extracts have proteins ranging between 14 and 94 kDa. (Fig. 3).

Western blotting

The sera of those with OSR-sensitization bound predominantly to an allergen of 14 kDa in OSR and also to two further allergens at 21 and 33 kDa (Fig. 4). There was no IgE-binding to oilseed rape in serum which was negative in RAST assay for oilseed rape.

All six sera used within this study had specific IgE to grass pollen, and all of the sera showed IgE-binding to grass pollen in the immunoblot. There was strong binding to grass pollen allergens of molecular weights at 14 and 17 kDa and at 32 and 39 kDa.

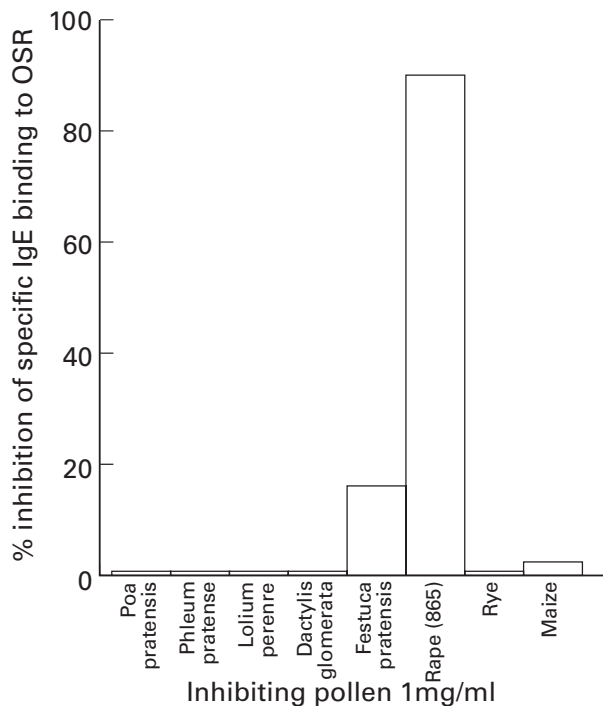


Fig. 1. Inhibition of specific IgE-binding to oilseed rape by various pollen at 1 mg/mL.

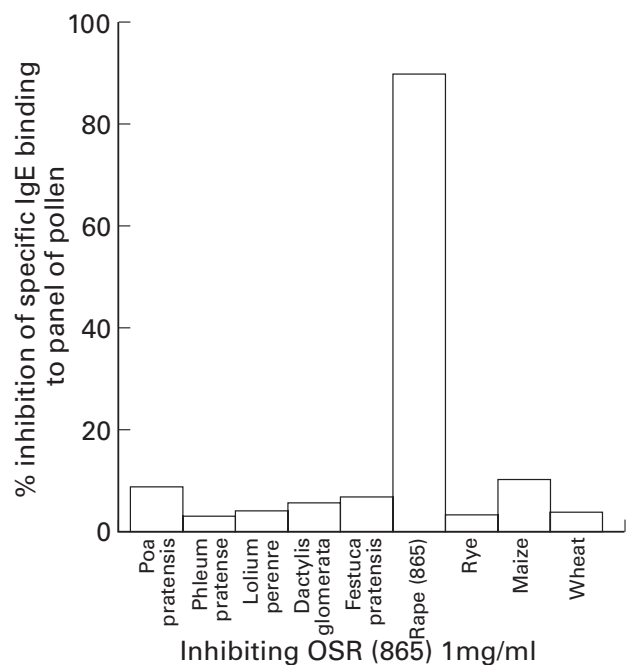


Fig. 2. Inhibition of specific IgE-binding to various pollen by oilseed rape at 1 mg/mL.

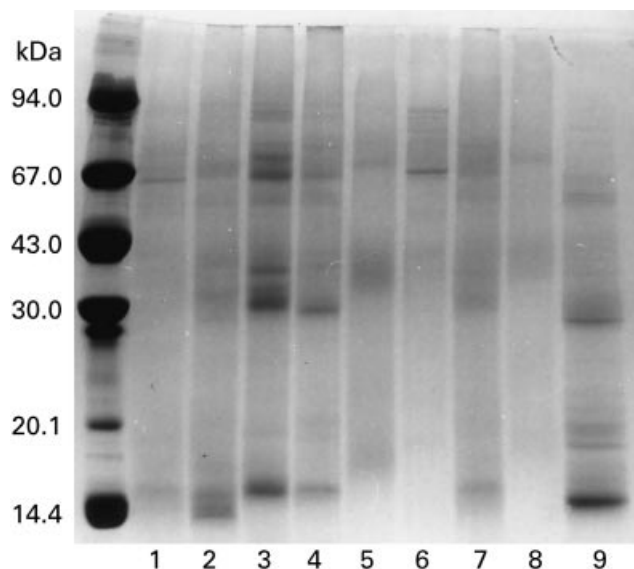


Fig. 3. SDS-PAGE analysis of protein extracts, Lane 1 *Poa pratensis*, Lane 2 *Phleum pratense*, Lane 3 *Lolium pratense*, Lane 4 *Dactylis glomerata*, Lane 5 *Festuca pratensis*, Lane 6 Oilseed rape pollen, Lane 7 Grass pollen mix, Lane 8 Rye pollen, Lane 9 Maize pollen

Inhibition immunoblot

To distinguish whether the similar molecular weight proteins found in the Western blotting of both oilseed rape and grass pollen allergens were immunologically similar we performed cross inhibition studies. Grass pollen was unable to inhibit the binding of IgE to oilseed rape, although there was complete self-inhibition. Thus despite allergens of similar molecular weight being present in both pollen types, which might suggest common allergens, inhibition immunoblot studies confirmed that the allergens in the two pollens were immunologically distinct.

Discussion

Sensitization rates to oilseed rape are considered to be generally low [5]; even in individuals attending an allergy clinic, the sensitization rate was only 3% [6]. Interestingly patients attending the allergy clinic who were sensitized to oilseed rape were also found to have a broad cross-reactivity with grass pollen allergens [4,6]. In our study, the two oilseed rape-sensitized individuals had specific IgE to the panel of allergens tested, which included grass, cereals and fungi, all present during the peak flowering period of oilseed rape. This raised the possibility that oilseed rape does not itself induce specific IgE, and that symptoms provoked by oilseed rape are due to the presence of cross-reactive allergens in grass pollen. Thus being sensitized to

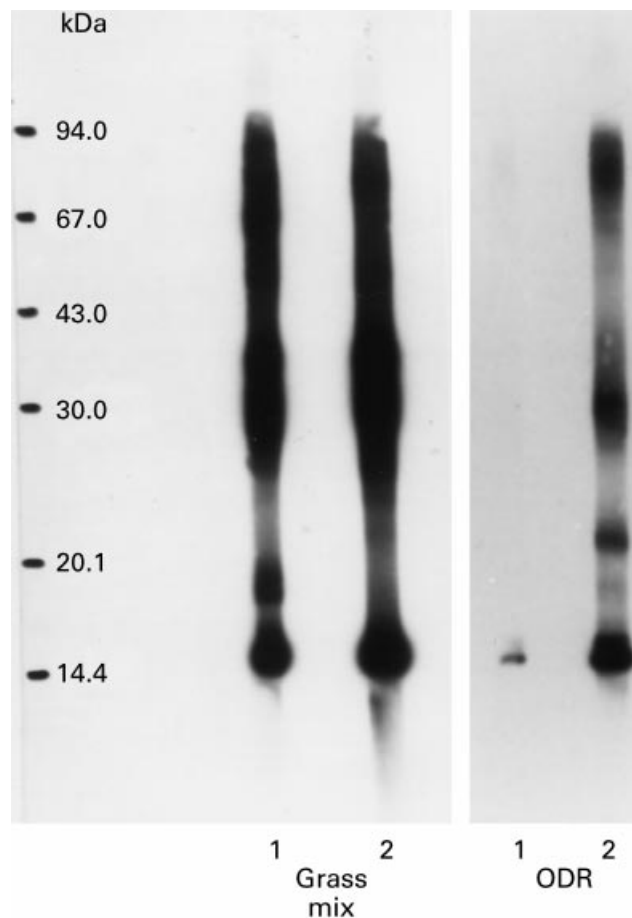


Fig. 4. Western blotting of serum 1 & 2 who were sensitized to both oilseed rape and grass pollen

grass may be a significant risk factor for those occupationally exposed to oilseed rape.

Individuals occupationally exposed to oilseed rape are at an increased risk of developing specific IgE and associated symptoms: 35% of specialist plant breeders working closely with oilseed rape developed sensitization [7]. This increase in sensitization in occupationally exposed personnel in comparison with the general population is presumably due to the level of pollen exposure; the general population are exposed to generally low levels of oilseed rape pollen, peaking at around 90 grains/m³/24 h during the few days of the pollen season [7]. Occupationally exposed individuals in this study were probably exposed to high levels of oilseed rape pollen as they worked in close contact with the oilseed rape plant. Very little airborne pollen is transported over long distances.

The possibility of similar allergens being present in oilseed rape and grass pollen becomes an important issue in grass-sensitized individuals working in close contact with

oilseed rape. Hemmer reported that oilseed rape occurs in individuals with multiple allergies to other pollen antigens and that grass pollen was the most frequent concomitant allergen followed by weeds and birch pollen [6]. Oilseed rape sensitization was found in 7.1% of pollen-allergic individuals. Allergens of similar molecular weights were reported in grass and oilseed pollen [6] and it was suggested that the oilseed rape-allergic pool is mainly a subset of grass pollen-allergic patients recognizing cross-reacting epitopes in oilseed rape [4]. Other indicators which may suggest cross-reactivity between grass and oilseed rape pollen come from studies showing that monoclonal anti-Lol p 4 (57 kDa) and anti-Poa p 9 (35, 28 kDa) binds proteins from oilseed rape [8].

In the three individuals in this study with heavy occupational exposure to oilseed rape, two of the individuals had respiratory and skin symptoms consistent with a workplace allergy and had IgE specific to oilseed rape. All three individuals were sensitized to grass pollen. We found no evidence of cross-reactivity between oilseed rape and grass pollen from the RAST inhibition studies. Western blotting of the two individuals sensitized to oilseed rape, showed a predominant binding of IgE antibodies to an allergen of 14 kDa in oilseed rape and also to two further allergens at 21 and 33 kDa. There was strong binding to grass pollen allergens of molecular weights at 14 and 17 kDa and at 32 and 39 kDa. Despite allergens of similar molecular weights being present in both pollen types, which may suggest common allergens, inhibition immunoblot studies confirmed that the allergens in the two pollens were immunologically distinct. Grass pollen was unable to inhibit the binding of IgE to oilseed rape and similarly oilseed rape did not inhibit the binding of IgE to grass pollen, although there was complete self-inhibition. Thus there was no evidence of any cross-reactivity with grass pollen. The third individual who was sensitized to grass pollen, had no IgE to oilseed rape, despite being occupationally exposed to oilseed rape for 18 months.

In this study of occupationally exposed individuals who were sensitized to grass pollen and exposed to oilseed rape, we were able to demonstrate that the allergens of grass pollen and oilseed rape are immunologically distinct. This is

an important finding showing that individuals sensitized to grass pollen will not necessarily develop a specific nasal or airway response to inhaled oilseed rape pollens.

Acknowledgements

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