

Primary sensitization to sweet bell pepper pollen in greenhouse workers with occupational allergy

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Summary

Background In a previous investigation, a high prevalence of allergy to sweet bell pepper pollen was found among exposed horticulture workers. Allergy to plant-derived food is often the consequence of primary sensitization to common pollen allergens.

Objective We therefore investigated the cross-reactivity between sweet bell pepper pollen and pollen from grass, birch or mugwort.

Method We selected 10 sera from greenhouse workers who had, besides specific IgE against sweet bell pepper pollen, also IgE to grass, birch or mugwort pollen. Cross-reactivity was tested by the inhibition of IgE binding to solid-phase coupled sweet bell pepper pollen extract. The 10 sera were also analysed for IgE binding to sweet bell pepper pollen by immunoblotting.

Results With these sera, no or small inhibition of IgE binding to sweet bell pepper pollen extract was observed with grass, birch and mugwort pollen. With immunoblotting, major IgE-binding structures were seen at 14, 29 and 69 kDa in sweet bell pepper pollen extract.

Conclusion The results of our study demonstrate that sweet bell pepper pollen contains allergens that have no or limited cross-reactivity with common pollen allergens. With sera from the 10 patients tested, sensitization to sweet bell pepper pollen was not the consequence of primary sensitization to common pollen allergens.

Keywords cross-reactivity, horticulture, occupational allergy, paprika, sweet bell pepper

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Introduction

About 10% of the greenhouse area in the Netherlands is used for growing sweet bell pepper fruit. The presence of IgE against sweet bell pepper pollen in greenhouse workers with symptoms of allergy upon contact with these plants has been described in a number of case reports [1–3]. Recently, the prevalence of occupational allergy to sweet bell pepper plants among 472 exposed horticulture workers was investigated. Skin prick tests with sweet bell pepper pollen extract were positive in one-third of the greenhouse workers [4]. A strong association was found between a positive skin test with grass pollen extract and sensitization to sweet bell pepper pollen [4].

IgE-mediated allergic reactions to plant-derived food are often a consequence of primary sensitization to common pollen allergens, like birch, grass and mugwort pollen [5, 6].

In the present study, we addressed the question of whether occupational allergy to sweet bell pepper pollen is also a consequence of primary sensitization to these common pollen allergens. The results of our study indicate, however, that

there is no or limited cross-reactivity between sweet bell pepper pollen and common pollen allergens.

Materials and methods

Sera

From the group of 472 employees who participated in the prevalence study [4], sera with IgE antibodies to sweet bell pepper pollen were selected that came from patients with positive skin tests to birch, grass and/or mugwort pollen (Table 1). IgE antibody levels against these pollens are shown in Table 2. All 10 selected patients experienced work-related rhinitis; work-related asthma was not reported by these 10 patients. The study was approved by the Medical Ethical Committee of the University Hospital Rotterdam; all employees gave their written informed consent.

Allergens

Pollen from flowers of the sweet bell pepper plant and from flowers of tomato plants were collected in greenhouses. Pollen of grass (*Dactylus glomerata*), birch (*Betula pendula*) and mugwort (*Artemisia vulgaris*) were obtained from ARTU Biologicals BV (Lelystad, the Netherlands).

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Table 1. Skin prick test results with GP: grass pollen, BP: birch pollen, MP: mugwort pollen and BPP: sweet bell pepper pollen extracts; the results are expressed as weal diameter, as described in [4]

Patient	Skin prick test, weal (mm)			
	GP	BP	MP	BPP
1	7	5	6	14
2	10	4	0	10
3	10	4	0	18
4	6	6	4	12
5	6	6	6	10
6	5	4	6	12
7	5	5	5	11
8	5	5	0	11
9	6	8	0	10
10	4	4	4	10

Table 2. Measurement of IgE against GP: grass pollen, BP: birch pollen, MP: mugwort pollen and BPP: sweet bell pepper pollen in sera from the 10 selected patients

Patient	kUa/L				Total IgE
	GP	BP	MP	BPP	
1	3.83	3.67	2.37	30.1	279
2	3.57	1.67	<0.35	30.2	231
3	3.26	<0.35	<0.35	4.75	42
4	1.09	0.72	0.38	25.5	146
5	7.4	1.09	1.91	14.8	320
6	1.54	1.1	2.78	27.1	191
7	6.15	6.31	1.73	45.8	486
8	<0.35	4.04	<0.35	3.56	123
9	0.55	4.04	0.92	14.4	319
10	nd	nd	nd	8.73	223

IgE antibody measurements

Specific IgE against sweet bell pepper, grass, birch and mugwort pollen was determined using agarose beads as allergen support, as previously described [1, 3]; 10 mg of sweet bell pepper, grass, birch or mugwort pollen were extracted with 2 mL coupling buffer (0.1 M NaHCO₃ and 0.5 mol/L NaCl, pH 8.5) for 1 h at room temperature. After centrifugation for 10 min at 1400g, protein in the supernatant was covalently coupled with 100 mg of CNBr-activated Sepharose 4B (Sigma Chemical Co., St Louis, USA). The sweet bell pepper, grass, birch and mugwort pollen sepharose conjugates were suspended in 25 mL RAST buffer (0.82% NaCl, 10 mM phosphate, 0.1% NaN₃, 0.2% Tween 20, 0.3% bovine serum albumin, pH 7.4). In all, 2 mg per test of sweet bell pepper, grass, birch or mugwort pollen sepharose conjugates, suspended in 0.25 mL RAST buffer, was incubated overnight with 0.05 mL patient serum. After four washes, the agarose beads were further incubated overnight with 0.05 mL radioiodinated anti-IgE antibodies (Pharmacia & Upjohn, Uppsala, Sweden). After another four washes, bound radioactivity was measured and expressed in kUa/L when read from the Pharmacia CAP standard curve.

IgE inhibition

For IgE inhibition experiments, 20 mg pollen was extracted with 1 mL RAST buffer under rotation for 1 h. After centrifugation, 0.05 mL supernatant was incubated overnight at room temperature with 0.05 mL serum (diluted in RAST buffer). Sera 3, 8 and 10 were incubated undiluted, sera 5 and 9 were 2 × diluted, sera 1, 2, 4 and 6 were 3 × diluted, and serum 7 was 4 × diluted. A volume of 0.05 mL of this mixture was incubated with pollen sepharose as described above.

For immunoblotting, sweet bell pepper pollen was extracted (10 mg/mL) in distilled water for 1 h at room temperature, under continuous rotation. After centrifugation, the supernatant was frozen in aliquots at -20 °C. The protein content of this supernatant contained 40 mg/L, when analysed with the benzethonium chloride method [7] on the Hitachi 911 routine clinical chemistry analyser, according to the manufacturer's instructions (Boehringer/Roche, Almere, the Netherlands). Before electrophoresis, this extract, diluted in sample buffer, including sodium dodecylsulphate and dithiothreitol, was placed at 100 °C for 5 min.

Immunoblotting

SDS-PAGE (12% homogeneous gel 100 × 70 × 1 mm 5% stacking gel) and electrotransfer to nitrocellulose were performed with the Mini Protean II system (BioRAD Laboratories, Veenendaal, the Netherlands). After electrotransfer, the nitrocellulose strips (BioRAD Laboratories, Veenendaal, the Netherlands) were saturated at 37 °C with RAST buffer for 1 h. Sera were diluted (1:2) with the same buffer and nitrocellulose strips were incubated with 0.5 mL diluted serum under continuous shaking overnight at room temperature. After the removal of unbound compounds by washing (PBS/0.2% Tween 20), radiolabelled anti-IgE (Pharmacia & Upjohn, Uppsala, Sweden) was added. Bound IgE was visualized by autoradiography.

Results

Whereas IgE binding to agarose-bound sweet bell pepper pollen extract could be largely inhibited by pre-incubation of the 10 selected sera with sweet bell pepper pollen extract, the extracts of tomato, grass, birch and mugwort pollen had no or only a small inhibitory effect (Fig. 1). The amounts of grass, birch and mugwort pollen extract, used for the inhibition of IgE binding to agarose-bound sweet bell pepper pollen extract, were able to inhibit IgE binding to homologous UniCAP reagents (data not shown).

IgE binding to sweet bell pepper was further analysed by immunoblotting. With seven sera, strong IgE binding occurred to a component of about 14 kDa. A heterogeneous pattern of IgE binding to other proteins was found with prominent IgE binding to 21 kDa (serum 4), 29 kDa (sera 1 and 2), 69 kDa (sera 5 and 9), and 56 and 43 kDa proteins (serum 9) (Fig. 2).

Three of these 10 sera were pre-incubated with sweet bell pepper pollen extract, before they were added to agarose-bound grass, birch or mugwort pollen extract. Sweet bell pepper pollen extract could inhibit the IgE binding to grass and birch pollen

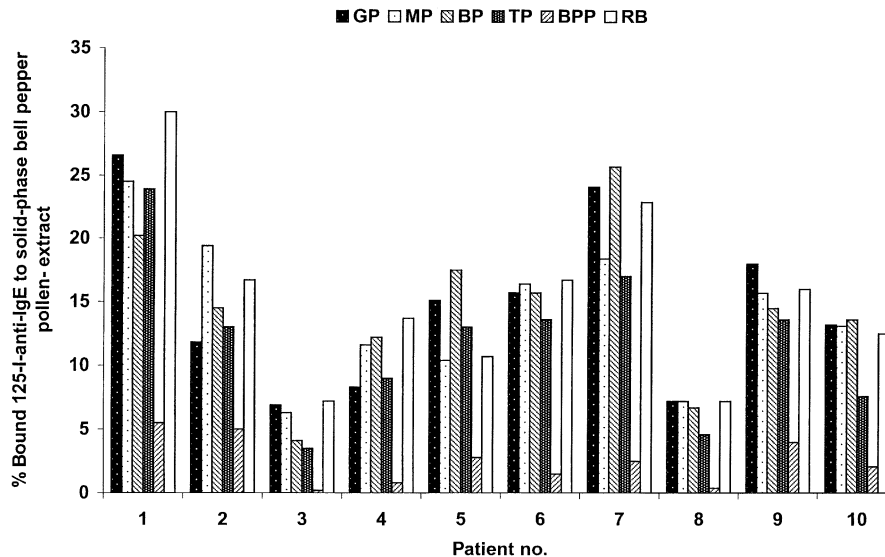


Fig. 1. Inhibition of IgE binding to sepharose-bound sweet bell pepper pollen extract with – from left to right – extracts of grass pollen (GP), mugwort pollen (MP), birch pollen (BP), tomato pollen (TP) or sweet bell pepper pollen (BPP), and with RAST buffer (RB) as a control.

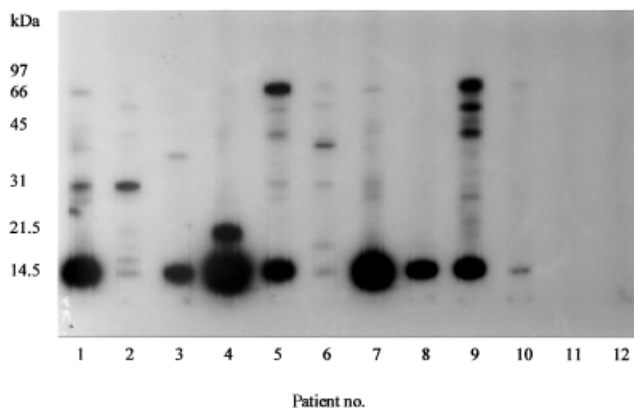


Fig. 2. IgE binding to SDS-PAGE separated sweet bell pepper pollen extract after incubation with 10 sera from greenhouse workers with IgE against sweet bell pepper pollen (lanes 1–10), serum of a patient with no IgE against common pollen (lane 11) and buffer control (lane 12).

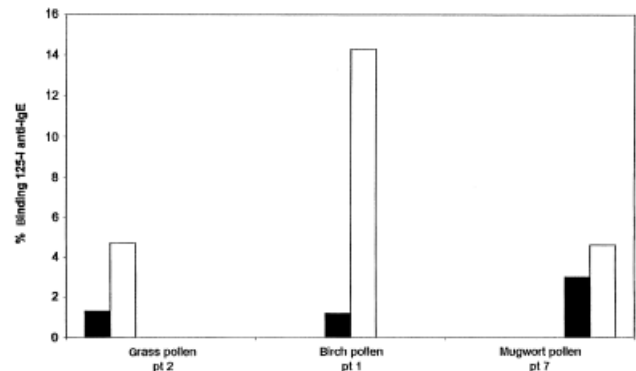


Fig. 3. Inhibition of IgE binding to sepharose-bound birch, grass or mugwort pollen extract with an extract of bell pepper pollen. Three sera from Table 2 were tested. White columns: pre-incubation with buffer; black columns: pre-incubation with bell pepper pollen extract.

(Fig. 3). Not enough serum was available to incubate all 10 sera from Fig. 1 with birch, grass or mugwort pollen sepharose, after pre-incubation with sweet bell pepper pollen extract.

Discussion

Because of the strong association between positive skin tests to sweet bell pepper and grass pollen in our recent study [4], cross-reactivity between these pollens was expected. Furthermore, extensive IgE cross-reactivity between tomato fruit and grass pollen allergens was found by Petersen et al. [8]; sweet bell pepper and tomato both belong to the Solanaceae plant family. The results shown in Fig. 1, however, indicate that most IgE-binding structures in sweet bell pepper pollen do not cross-react with allergens in grass, birch or mugwort pollen. In the 10 selected sera, the average IgE levels against grass, birch or mugwort pollen were at least seven times lower

than the IgE levels against sweet bell pepper pollen (Table 2). The results in Fig. 3 rather suggest that the IgE binding to common pollen extracts in sera with relatively low levels of IgE against these pollens can actually be caused by primary sensitization to sweet bell pepper pollen. Groenewoud et al. [4] found that 51 of 187 patients with positive skin tests to sweet bell pepper pollen were negative in skin tests with grass, birch or mugwort pollen extract. This further indicates that sweet bell pepper pollen contains IgE-binding structures that are not present in grass, birch or mugwort pollen. Pre-incubation of the 10 sera with tomato pollen extract showed no inhibition of IgE binding to solid-phase-coupled sweet bell pepper pollen extract (Fig. 1). In a previous study [3], the absence of cross-reactivity between bell pepper and tomato pollen towards IgE from two greenhouse workers was also seen; one of these two patients had IgE against sweet bell pepper pollen, but no IgE against tomato pollen. In the same report [3], IgE from a greenhouse worker with symptoms of

rhinitis and asthma upon contact with tomato plants displayed partial cross-reactivity between tomato and sweet bell pepper pollen: IgE binding to tomato pollen could only partially be inhibited by sweet bell pepper pollen extract. In contrast, pre-incubation of serum from this patient with tomato pollen extract showed complete inhibition of the IgE binding to solid-phase-coupled sweet bell pepper pollen extract.

In immunoblotting, major IgE binding was seen to a protein of about 14 kDa. In a previous case report, no substantial IgE binding to a 14 kDa protein was seen [3]. Similar to these cases, [3] sera 2 and 6 in Fig. 2 also show more IgE binding to another protein than to the 14 kDa band. Yu et al. [9] isolated profilin cDNA from tomato pollen and calculated the molecular weight of the protein as 14.435 kDa. This may suggest that the 14 kDa band in the figure is a sweet bell pepper pollen profilin. Jensen-Jarolim et al. [10], who analysed IgE-binding structures in bell pepper fruit, could inhibit IgE binding to a 14 kDa band in SDS-PAGE separated and nitrocellulose blotted sweet bell fruit pepper extract by pre-incubation of sera from food-allergic patients with recombinant birch pollen profilin. Not enough serum was available to extend the experiments, shown in Fig. 1, with sera, pre-incubated with recombinant birch, pollen profilin.

The lack of cross-reactivity between bell pepper pollen and common environmental pollen has practical consequences for the diagnosis of occupational allergy: a negative result of skin tests with, or IgE antibody measurements against, common inhalant allergens does not exclude the presence of an IgE-mediated occupational allergy. In her review on spice allergy, Niinimäki [11] quotes three case reports of inhalant allergy to powdered spices, all three with negative skin test results to birch, grass and mugwort pollen.

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