

Occupational Allergy to Flowers: Immunoblot Analysis of Allergens in Freesia, Gerbera and Chrysanthemum Pollen

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Abstract

High exposure to pollen from ornamental flowers can induce an IgE-mediated occupational allergy in florists and horticulture workers. We investigated IgE-binding antigens in chrysanthemum, freesia and gerbera pollen by immunoblot analysis and analysed the cross-reactivity of these pollen with birch, grass and mugwort pollen. In immunoblots with chrysanthemum pollen, major IgE-binding structures were seen with a molecular weight (MW) of approximately 25, 45 and 65 kD. In the immunoblots with freesia pollen, IgE from freesia pollen was directed against two proteins with an MW of approximately 15 kD. Most sera showed IgE binding to an approximately 15 kD band in gerbera pollen; with some sera additional bands were seen in the range of 30–50 kD. IgE binding to chrysanthemum pollen was inhibited by mugwort pollen only, whereas IgE binding to freesia pollen was suppressed by birch, grass and mugwort pollen. The inhibitory activity of birch and grass pollen extract on IgE binding to gerbera pollen extract was serum dependent and ranged from no inhibition to complete inhibition. Occupational exposure to many different flowers induced IgE against all three types of pollen. Exposure in greenhouses to gerbera flowers elicited mainly IgE against gerbera pollen. Mugwort pollen extract inhibited IgE binding to pollen from all three flowers.

Introduction

In contrast with occupational contact dermatitis [1, 2], occupational respiratory diseases caused by decorative flowers were infrequently reported when Piirilä *et al.* [3] reviewed this subject in 1994. These authors described four such patients whom they had examined in Finland. Since then, more case reports have been published which were summarized by De Jong *et al.* [4] and more recently by Baur and Bakehe [5]. Goldberg *et al.* [6] found that 17% of patients in Israel referred for allergic evaluation had positive skin prick test (SPT) responses to pollen of various ornamental plants. They suggested that in many patients, the primary sensitization to decorative flowers is caused by the pollen of wild plants of the *Asteraceae* family.

Initial descriptions of occupational allergy to freesia and gerbera flowers [7, 8] did not address the IgE response against individual allergens in these flowers. In the present study, we used immunoblotting to analyse the IgE response against freesia (*Freesia hybrida*) and gerbera (*Gerbera hybrida*) pollen in more detail. We included

chrysanthemum (*Chrysanthemum indicum*) pollen in this analysis because it is one of the most popular cut flowers in the Netherlands. *Chrysanthemum* and *Gerbera* both belong to the *Asteraceae* plant family, and *Freesia* is a member of the *Iridaceae* plant family.

Sera and methods

Sera were obtained from patients who were referred to the Department of Allergy of the Erasmus University Medical Center because of symptoms associated with their occupational handling of flowers. After total IgE and allergen-specific IgE antibodies were analysed, sera were stored at -20°C . Seven sera were selected that contained specific IgE against chrysanthemum, freesia and/or gerbera pollen. The study was approved by the Institutional Review Board of Erasmus University Medical Center.

Freesia and gerbera flowers were collected as fresh flowers in greenhouses. Bunches of gerbera flowers were kept in water in our laboratory for collection of pollen; the gerbera flowers were gently shaken over a petri dish once a

week. The complete stamen was removed from the freesia flowers. Chrysanthemum pollen was obtained from ALK Diephuis (now ALK Abelló), Groningen, the Netherlands, because the chrysanthemum flowers that we collected in a greenhouse did not produce sufficient pollen.

Specific IgE against pollen from gerbera, freesia, and chrysanthemum flowers was determined using Sepharose beads as allergen support [9], as described previously [7]. Freesia stamen, chrysanthemum pollen or gerbera pollen (50 mg each) were extracted for 1 h in coupling buffer (0.1 M NaHCO₃, 0.5 M NaCl). After centrifugation, the protein in the supernatant was covalently coupled to 50 mg CNBr-activated Sepharose 4B according to the manufacturer's instructions (Sigma-Aldrich, Zwijndrecht, the Netherlands). After three cycles of acid-base washings, the pollen-Sepharose preparations 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). For each test, 1 mg pollen-Sepharose was incubated for at least 4 h with 0.05 ml of patient serum. After three washes with 0.2% Tween-20 in physiological saline and one wash with RAST buffer, the pollen-Sepharose was incubated overnight with the same batch of radioiodinated anti-IgE antibodies that was used in routine specific IgE antibody analysis with the ImmunoCAP system (Phadia, now Thermo Fischer, Nieuwegein, the Netherlands). After four washes with 0.2% Tween-20 in physiological saline, bound radioactivity was counted in a gamma counter. The amounts of pollen-specific IgE antibodies in sera were read from the ImmunoCAP standard curve as described previously [10, 11]. Total IgE and specific IgE against common allergens were determined with the ImmunoCAP system (Phadia) according to the manufacturer's instructions.

For immunoblotting, pollen or complete stamens (freesia) were extracted (10% w/v) in demineralized water for 1 h at 4 °C with shaking as described by Peltre *et al.* [12]. After centrifugation, the supernatant was frozen in small aliquots at -20 °C. These supernatants contained 1.6–4.4 mg protein/ml, when analysed by the pyrogallol red method

[13]. Before electrophoresis, 1:10 dilutions [in sample buffer, including sodium dodecyl sulphate (SDS) and dithiothreitol] of these supernatants were heated at 100 °C for 5 min. SDS polyacrylamide gel electrophoresis and electroblotting were performed with the Mini Protean II system according to the manufacturer's instructions (Bio-Rad Laboratories, Venendaal, the Netherlands). Western blots were incubated with patient sera in the Mini Protean Multiscreen apparatus (Bio-Rad). For electrophoresis, a 12% resolving gel and a 4% stacking gel were used; for protein blotting, a 0.45 micron pore size nitrocellulose membrane (Bio-Rad) was used. Bound IgE was, after incubation with radioiodinated anti-IgE antibodies (Phadia), visualized by autoradiography at -70 °C as described previously [10].

For IgE inhibition experiments, 10 mg of 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. An aliquot (0.05 ml) of this mixture was incubated with pollen-Sepharose as described above. Sera with a low level of specific IgE against a particular pollen were not tested in the inhibition of IgE binding to that pollen type.

Results

Symptoms on exposure to flowers are shown in Table 1. Patients 1 and 2 worked in greenhouses; patients 3, 5, 7 and 8 worked in florist shops, and patient 6 was a student at a florist school. Patient 4 was a patient with grass pollen allergy and an increased total IgE level, who had no symptoms of occupational allergy, but was included as a control patient in the immunoblot experiments. Patients 1 and 2 were involved in the culture of gerbera plants only. Allergy symptoms disappeared in patient 1 after switching her work from gerbera to roses. The diagnosis of an IgE-mediated allergy to decorative flowers drove patient 6 to drop out of florist school and patient 7 to stop working in a florist shop. Patients 2, 3 and 5–8 all had positive results in intracutaneous skin tests for grass, birch and mugwort pollen extract; the case report of

Table 1 Serum IgE analysis and skin prick test results.

Patient	Gender	Symptoms	Total IgE kU/l	sIgE chry. p. kUa/l	sIgE free. p. kUa/l	sIgE gerb. p. kUa/l	SPT chry.p.	SPT free.p.	SPT gerb.p.	ImmCAP Bet v2 kUa/l
1	f	rc	100	<0.35	<0.35	20.4	nd	nd	pos	<0.35
2	f	rc, a	185	2.1	0.45	59.4	nd	nd	pos	2.3
3	m	rh	185	10.9	3.2	4.2	pos	nd	nd	4.2
4			4753	2.3	1.2	1.3	nd	nd	nd	nd
5	m	rh	251	34.7	9.4	9.7	pos	nd	pos	19.5
6	f	rh, a	385	29.0	17.3	13.4	neg	pos	neg	16.0
7	f	rh	267	24.3	4.7	10.2	pos	nd	dubious	7.2
8	f	rc, a	955	68.0	7.1	35.5	pos	neg	pos	19.3

chry, chrysanthemum; free, freesia; gerb, gerbera; p, pollen; f, female; m, male; rc, rhinoconjunctivitis; a, asthma; rh, rhinitis; nd, not done; pos, positive; neg, negative; ImmCAP, ImmunoCAP. Serum 4 is a control serum with an increased total IgE level.

Specific IgE (sIgE) against chrysanthemum, freesia and gerbera pollen was determined with the Sepharose bead procedure.

patient 1 did not mention skin test results with these three types of pollen. Skin prick tests (SPTs) with extracts of chrysanthemum, freesia and gerbera pollen were performed as described previously [4] at the Department of Allergy; the results are shown in Table 1.

Whereas SPTs had not been conducted for all three flower types, the seven sera from the patients with symptoms of occupational allergy were all tested for IgE against pollen from the three flowers (Table 1). The two greenhouse workers (patients 1 and 2) predominantly showed IgE binding to gerbera pollen. Four patients (3, 5, 7 and 8) who worked in florist shops and a student in floriculture (patient 6) all had more IgE against chrysanthemum pollen than against freesia and gerbera pollen. Four sera with total IgE levels of 490, 510, 719 and 1080 kU/l from patients without (<0.35 kUa/l) specific IgE against birch, grass and mugwort pollen were negative (<0.35 kUa/l) for specific IgE against chrysanthemum, freesia and gerbera pollen (data not shown).

In immunoblotting experiments, sera from two greenhouse workers, who were primarily exposed to gerbera flowers, showed IgE binding only to gerbera pollen (Fig. 1). Both sera showed IgE binding to two proteins of approximately 45 kD, whereas serum 2 showed additional IgE binding to two proteins of approximately 15 kD. The other five patient sera showed IgE binding in all three pollen blots. In immunoblots with chrysanthemum pollen, major IgE-binding structures were seen with a MW of approximately 25, 45 and 65 kD. In the immunoblots with freesia pollen, IgE from sera 3 and 5–8 was primarily directed against two proteins with a molecular weight of approximately 15 kD. Other than serum 1, all sera showed IgE binding to an approximately 15 kD band in gerbera pollen; with some sera, additional bands were seen in the range 30–50 kD. A control serum (patient 4) showed no IgE binding in the three pollen immunoblots.

In IgE inhibition experiments (Fig. 2), only mugwort pollen extract could substantially inhibit the IgE binding to Sepharose-coupled chrysanthemum pollen extract; grass and birch pollen had considerably less activity. In contrast, birch, grass and mugwort pollen extract were all able to inhibit IgE binding to Sepharose-coupled freesia pollen extract. Mugwort pollen extract could substantially inhibit IgE binding to gerbera pollen extract in all six sera tested. The inhibitory activity of birch and grass pollen extract on the IgE binding to gerbera pollen extract was serum dependent and ranged from no inhibition to complete inhibition.

Discussion

IgE-mediated clinical allergy to pollen in the general population is usually restricted to pollen from wind-pollinated plants. High exposure to pollen from insect-pollinated flowers can also induce symptoms of allergy in horticulture workers and employees in florist shops [3–6].

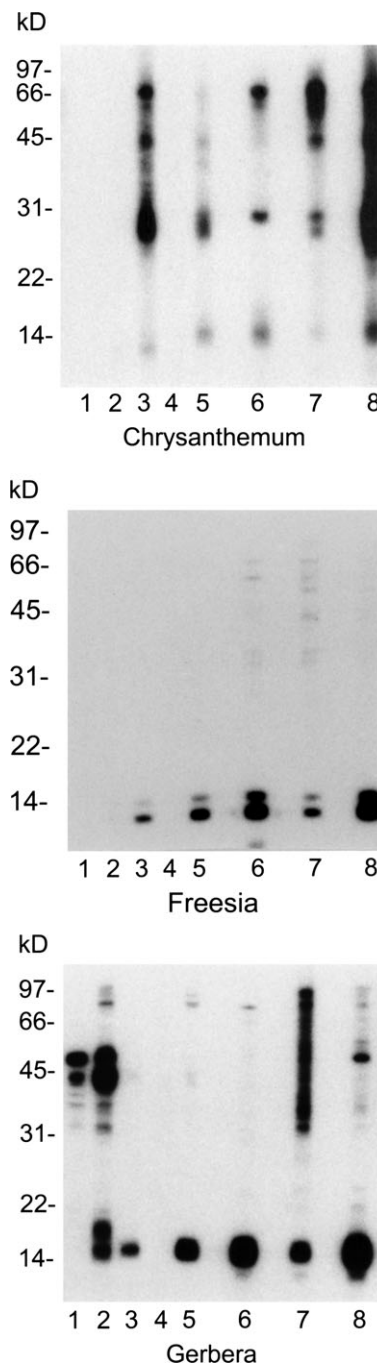


Figure 1 Pattern of IgE binding to SDS-PAGE separated extracts of freesia, gerbera and chrysanthemum pollen. All lanes were exposed for 7 days. Serum 1 was incubated undiluted, sera 2 and 3 were 1/2 diluted, and sera 4–8 were 1/4 diluted during incubation with the blotted proteins. Serum 4 is a control serum with an increased total IgE level.

Emberlin *et al.* [14] showed that in florist shops, high concentrations of pollen from decorative flowers can be found that may even exceed those of wind-dispersed pollen in ambient air.

In 2012, there were approximately 4000 flower shops in the Netherlands, with a workforce of approximately 13,000

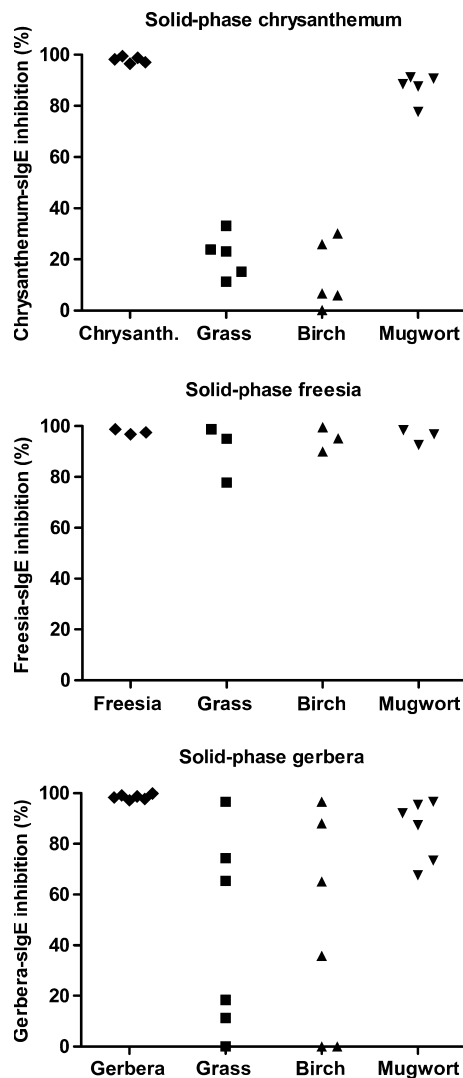


Figure 2 Inhibition of IgE binding to Sepharose-bound flower pollen extract by pre-incubation of sera with extracts of grass, birch, mugwort or flower pollen extract. Sera with a low level of specific IgE (sIgE) were not tested in inhibition experiments.

people. The total greenhouse area for cut flowers was 2138 hectares (ha), of which 480 ha was used for the culture of chrysanthemum, 180 ha for gerbera and 98 ha for freesia, divided among 200, 65 and 36 greenhouses, respectively. An estimated 1500 greenhouse workers were mainly exposed to chrysanthemum, gerbera or freesia. In 2002, Groenewoud *et al.* [15] reported a high prevalence of work-related symptoms in chrysanthemum greenhouses. Sensitization to chrysanthemum pollen was found in 21 of 104 employees tested. When 20% or more of workers in flower shops are sensitized to pollen of decorative flowers, at least 2600 people of this workforce are at risk to develop symptoms of occupational allergy.

In sera from patients 1 and 2, who worked in greenhouses where gerbera flowers were cultivated, IgE against chrysanthemum pollen was absent or very low. This finding

indicates that IgE-binding allergens in gerbera pollen occur that have no or limited cross-reactivity with allergens in chrysanthemum pollen. Unfortunately, not enough serum was available for analysis of the cross-reactivity between chrysanthemum and gerbera pollen by mutual inhibition of IgE binding. Pre-incubation of sera from patients 1 and 2 with mugwort pollen extract inhibited approximately 70% of the IgE binding to solid-phase gerbera (Fig. 2), which indicates that both patients were sensitized to mugwort pollen and which also could indicate that there are no or few unique allergen components in gerbera pollen that are not represented in mugwort pollen.

Paulsen *et al.* [16] described three patients with a positive skin test for gerbera; one patient had negative SPTs for common inhalant allergens (including mugwort pollen). Goldberg *et al.* [6] found that 8% of flower growers had positive SPT responses to the flowers without having any positive SPT responses to all other allergens. Other patients described by Paulsen *et al.* [16] and Van der Waal *et al.* [17], besides being sensitized to gerbera, also had positive skin tests for birch, grass and mugwort pollen. Similarly patient 2 from this study was sensitized to both gerbera and all three common pollen types. Estlander *et al.* [18] described a researcher in plant molecular biology with a positive SPT and serum IgE test to gerbera; SPTs for florist's chrysanthemum and freesia were positive as well. This patient was SPT positive to birch and grass pollen, although the patient experienced atopic symptoms only after having been sensitized to gerbera.

Patient 6 in Table 1 had negative results in SPTs for gerbera and chrysanthemum pollen, but in her serum, substantial levels of IgE against both gerbera and chrysanthemum pollen could be demonstrated, in addition to IgE against freesia pollen. The source material of the SPT extracts, prepared by de Jong *et al.* [4], was obtained from a florist shop. A disadvantage of this source material is that a substantial amount of pollen may have been lost during the route from the greenhouse to florist shops. De Jong *et al.* [4] could not always collect sufficient pollen from such flowers for preparation of SPT extracts; in those cases, they used the heart of the flowers, which, as stated by de Jong *et al.* [4], may have led to less potent extracts. The source material used in specific IgE analysis and immunoblotting was preferably collected in greenhouses. The chrysanthemum flowers, however, that we collected in a greenhouse did not produce sufficient pollen. Extraction of the hearts from these flowers, as described above for pollen, gave an extract with a much lower protein content (0.05 g/l) than the pollen extracts (approximately 2.5 g/l). Therefore, we used chrysanthemum pollen from a commercial supplier.

Mugwort pollen extract inhibited IgE binding to pollen from all three flowers. De Jong *et al.* [4] and Groenewoud *et al.* [15] suggested a strong cross-sensitization to chrysanthemum and mugwort, which was later confirmed by Lee *et al.* [19].

The cross-reactivity between mugwort pollen and pollen from the three flowers supports the suggestion by De Jong *et al.* [4] that testing for sensitization to mugwort pollen may have predictive value in the diagnosis of occupational allergy to flowers. Furthermore, this cross-reactivity holds promise for the treatment of allergy to these flowers by means of immunotherapy with mugwort pollen extract.

The pattern of IgE binding to chrysanthemum pollen (Fig. 1) resembles the pattern of IgE reactivity against pollen from sunflower [20], another member of the *Asteraceae* plant family.

The pattern of IgE binding to freesia pollen (Fig. 1) is similar to the immunoblot pattern of pollen from saffron, which also belongs to the *Iridaceae* plant family [21]. The description [21] of a 15.5 kD protein with profilin nature in saffron pollen prompted us to test the seven sera for IgE against birch pollen profilin: all five sera with IgE against freesia pollen were positive in the ImmunoCAP assay for IgE against Bet v2 (Table 1).

Most variation in immunoblot pattern (Fig. 1) and inhibition of IgE binding (Fig. 2) by grass and birch pollen extract for the individual sera was seen with gerbera pollen. An explanation for this variation might be the existence of hundreds of gerbera cultivars [22].

A limitation of the presented results is that the inhibition experiments were performed with only one concentration of birch, grass or mugwort pollen extract, because not enough serum was available for dose–response inhibition curves. Likewise, immunoblot inhibition experiments might have given qualitative information about the cross-reacting allergens in the pollen studied. At the dose tested, birch, grass and mugwort pollen extract do not cause non-specific inhibition of IgE binding [10].

In summary, this paper describes the pattern of IgE binding to allergens in freesia, gerbera and chrysanthemum pollen. Occupational exposure to many different flowers induced IgE against chrysanthemum, freesia and gerbera flowers. Exposure to gerbera flowers in greenhouses elicited mainly IgE against gerbera flowers.

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Conflicts of interest

None.

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