

Occupational allergy caused by carnation (*Dianthus caryophyllus*)

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Background: Occupational respiratory symptoms caused by decorative flowers are seldom reported in the literature. In our area a large portion of the population works in carnation (*Dianthus caryophyllus*) winter quarters, and many workers have symptoms of rhinitis and asthma related to exposition.

Objective: The purposes of this study were to investigate whether the symptoms induced by carnation were IgE-mediated and to study the possible allergens involved.

Methods: A total of 16 subjects employed in indoor carnation cultivation with symptoms during exposition time were studied along with 15 patients with allergic asthma who were not exposed to carnations and 15 healthy carnation workers used as control subjects. Skin prick tests with carnation extract and RASTs were performed. Protein bands were isolated by SDS-PAGE, and afterwards immunoblotting was performed to characterize the extract. Specific nasal provocation and non-specific bronchial provocation tests were performed for all the asthmatic patients. Diurnal variation in peak expiratory flow was also measured.

Results: Skin prick test responses with carnation extract were positive in 15 of the 16 patients and negative in all control subjects. Nasal provocation test responses with carnation extract were positive in 13 of 16 patients. A significant correlation was seen between RAST and nasal provocation results ($P < .01$). Immunoblotting of sera from 13 patients showed 2 major IgE-binding fractions of 34 and 35 kd in most of the patients, which could constitute the major allergen. Methacholine PD₂₀ showed a variable degree of nonspecific bronchial hyperresponsiveness in all asthmatic subjects.

Conclusion: Data demonstrate the involvement of carnation in occupational allergy, mediated by an IgE-dependent mechanism. (*J Allergy Clin Immunol* 1999;104:181-5.)

Key words: Occupational asthma, *Dianthus caryophyllus*

Occupational asthma is a respiratory disease characterized by variable bronchial responsiveness induced by sensitization to specific agents inhaled in the workplace, which usually appears after a latency period.¹ The prevalence of occupational asthma has recently increased, becoming the most common work-related lung disease in developed countries.² However, epidemiologic studies underestimate the true incidence of this pathologic condition because many workers with respiratory problems must leave their work before diagnosis.

Abbreviations used

PEFR: Peak expiratory flow rate

PRU: Phadebas RAST unit

SPT: Skin prick test

More than 200 substances have been involved in producing occupational asthma.³ The ability of occupational agents to induce an allergic disease is influenced by physical-chemical properties of the substance, dose, duration, and route of exposition, as well as other factors such as industrial hygiene. Nevertheless, only a small proportion of exposed subjects experience the development of occupational asthma, which indicates the existence of predisposing factors in the host, such as atopy, nonspecific bronchial hyperresponsiveness, and smoking habit. Vegetable products are one of the most important sources of organic allergens, and, more rarely, some decorative flowers are implicated.⁴ Although the mechanisms by which many occupational agents may induce asthma are largely unknown, an established IgE-mediated sensitization has been demonstrated for some of these agents.

Several patients at the allergy consult had a suggestive clinical history of occupational rhinitis and asthma related to carnation exposure. The aim of this study was to prove by objective means the etiologic role of carnation in producing these respiratory symptoms.

METHODS

Patients

A total of 31 asthmatic patients, 16 employed in carnation cultivation with symptoms during exposure and 15 allergic asthmatic patients who were not exposed to carnations (control subjects), were studied. Medication used by patients included inhaled therapy (corticosteroids, inhaled β -agonists, or both on an as-needed basis). None of the patients was in acute respiratory distress at the time of the study or had required treatment with systemic corticosteroids for at least 6 months. Fifteen healthy carnation workers with no history of atopy and who had not taken any medication in the previous 3 months were used as additional control subjects. Table I summarizes the clinical characteristics of the patients included in this study.

Preparation of carnation extract

Carnations were collected and extracted by magnetic stirring (24 hours at 4°C) in 0.1 mol/L of phosphate buffer, pH 8.0, at 15% wt/vol. Extract was clarified by centrifugation at 5600g for 30 minutes, filtered through a 0.45- μ m pore diameter membrane, and dialyzed by ultracentrifugation in a Pellicon System (Millipore Corp,

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Received for publication Oct 5, 1998; revised Mar 26, 1999; accepted for publication Mar 29 1999.

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0091-6749/99 \$8.00 + 0 1/1/99016

TABLE I. Patient characteristics and SPT results

Patient	Sex	Symptoms	Other sensitization	Carnation SPT response
1	F	R, A	Pollens	Positive
2	M	R, A	Negative	Positive
3	F	U	Negative	Negative
4	M	R, A	Negative	Positive
5	F	R, A	Negative	Positive
6	F	R, A	Negative	Positive
7	F	R, A	Pollens and dust mite	Positive
8	F	R, A	Pollens	Positive
9	M	R, A	Molds	Positive
10	F	R, A	Pollens and danders	Positive
11	F	R, A	Negative	Positive
12	F	R, A	Pollens	Positive
13	F	R, A	Pollens and danders	Positive
14	F	R, A	Negative	Positive
15	F	R, A	Pollens	Positive
16	M	R, A	Pollens and dust mite	Positive

R, Rhinitis; A, asthma; U, urticaria.

Milford, Mass) with a 5000-d cutoff. The dialyzed extract was sterilized by filtration through a 0.22- μ m pore diameter membrane and freeze-dried.

The protein concentration estimated by the method of Bradford⁵ was 11% in relation to raw material. Aliquots of lyophilized extract of the same batch were used for all in vivo and in vitro tests performed in the patients identified as having a positive reaction by the crude extract and in control subjects.

Skin tests

Prick tests with carnation extract (20 mg/mL) were performed both in patients and control subjects. Routine skin prick tests (SPTs) were performed with 2 grass pollens, 2 tree pollens, 5 weed pollens, 2 house dust mite allergens, 5 common molds, and 3 animal danders. Histamine hydrochloride, 10 mg/mL, and saline solution 0.9% were used as positive and negative controls, respectively. The test results were read after 15 minutes, and only a wheal with a flare higher than 5 mm was considered a positive result.

RAST

Solid-phase was obtained by coupling the extract solution (2 mg of allergen per disk) to the 6-mm diameter cyanogen bromide-activated paper discs (Bial-Aristegui), according to the method of Ceska and Lundqvist.⁶ RAST was performed according to the method of Wide et al⁷ with Phadezym Trazer reagents (Kabi Pharmacia), according to the manufacturer's instructions. Results were expressed as class 0 (<0.35 Phadebas RAST unit [PRU]/mL), class 1 (0.35 to 0.7 PRU/mL), class 2 (0.7 to 3.5 PRU/mL), class 3 (3.5 to 17.5 PRU/mL), or class 4 (>17.5 PRU/mL). A pool of atopic patients who were not sensitized to carnation was used as negative control subjects.

SDS-PAGE

SDS-PAGE was performed by the method of Laemmli.⁸ Polyacrylamide concentration (12.5%) was used for stacking gels. After electrophoresis, gel proteins were stained with Coomassie Brilliant Blue R-250 or argentic solutions, according to needs. It was applied to a parallel protein pattern curve with known molecular weights (Pharmacia Biotech): phosphorylase b (94.0 kd), bovine serum albumin (67.0 kd), ovalbumin (43.0 kd), carbonic anhydrase (30.0 kd), trypsin inhibitor (20.1 kd), and α -lactalbumin (14.4 kd). It was

added to 10 μ L/lane of patron and sample, corresponding to 20 pg of protein when Coomassie is used and 1 μ g when argentic staining is used, respectively. Argentic staining was made with Silver Stain Plus kit (BIO-RAD), following the manufacturer's recommendations.

SDS-PAGE immunoblotting

Isolated protein bands by SDS-PAGE were electrophoretically transferred to polyvinylidene difluoride membranes (Immobilon-P) and, afterwards, incubated with patient serum. The bound-IgE bands were identified with 1:25 peroxidase-conjugated rabbit immunoglobulins to human ϵ -chains. Bound peroxidase was revealed by the addition of freshly prepared 0.1% Amido Black 10 B solution. The measurement of the obtained bands (molecular weights calculation) for SDS-PAGE and immunoblotting was realized with a BIO-IMAGE analyzer (Millipore).

Only 13 of 16 subjects were evaluated by this method because one of the other 3 subjects had negative skin test results, and 2 patients were lost to follow-up.

Specific nasal provocation test

Nasal provocation tests were performed at 9 AM in all subjects of the study. After spraying diluent as a control substance, 0.2 mL of increasing concentrations (5, 10, and 20 mg/mL) of allergen were sprayed into the nostril every 15 minutes until resistances doubled that induced by the diluent. Nasal resistances were measured with the youlten peak nasal inspiratory flow meter (Clement Clarke International, London, UK).

Nonspecific bronchial provocation test

A methacholine challenge test was performed for all the asthmatic patients. A breath-activated dosimeter connected to a nebulizer (De Vilbiss 646; De Vilbiss Co, Somerset, Pa) was used. Methacholine was administered, beginning with a dose of 0.095 mg/mL (0.095, 0.19, 0.39, 0.78, 1.56, 3.12, 6.15, 12.5, and 25 mg/mL). FEV₁ values recorded after phenolated saline buffer (control reagent) inhalation were considered as baseline. FEV₁ values were recorded 5 minutes later, followed by the next dose. The challenge sequence terminated when FEV₁ values had decreased by 20% or more or when a maximum dose (25 mg/mL) had been reached. The methacholine PD₂₀ was obtained from the dose-response curve plotted in a semilogarithmic scale; the abscissa represents logarithmic methacholine doses, and the ordinate shows the percentage of decrease of FEV₁. The challenge was performed with buffered lyophilized methacholine (phenol buffer; Bial-Aristegui, Bilbao, Spain). The dosimeter delivered methacholine by means of an air compressor.

Diurnal variability in peak expiratory flow

During follow-up, peak expiratory flow rate (PEFR) was recorded by a Mini-Wright peak flowmeter (Clement Clarke International, London, UK) 3 times each day for 2 weeks during work and 2 weeks during holidays. Diurnal variability in PEFR was calculated as (PEFR high – PEFR low)/PEFR average.

Statistical analysis

To compare differences among the 3 study groups, a 1-way analysis of variance was performed and completed by the use of a 2-tailed unpaired Student *t* test. Only *P* values below .05 were considered significant.

RESULTS

SPT results with carnation were positive in 15 of the 16 patients and negative in all the asymptomatic carna-

TABLE II. Specific IgE to *Dianthus* by RAST, provocation tests, and peak flow variations

Patient	RAST (PRU/mL)	RAST class	Nasal provocation (mg/mL)	Methacholine test PC ₂₀ (mg/dL)	PEFR daily variations (%)
1	2.34	2	Positive, 10	Positive, 1.2	>20
2	2.68	2	Positive, 10	Positive, 2.3	>20
3	< 0.35	0	Negative	Negative	NR
4	4.40	3	Positive, 10	Positive, 0.7	>20
5	0.45	1	Positive, 20	Positive, 3.7	>20
6	0.63	1	Positive, 10	Positive, 1.3	> 20
7	1.61	2	Positive, 10	Positive, 0.8	>20
8	< 0.35	0	Negative	Positive, 2.2	>20
9	< 0.35	0	Positive, 10	Positive, 0.6	>20
10	0.61	1	Positive, 20	Positive, 6.1	<20
11	3.22	2	Positive, 10	Positive, 1.3	>20
12	0.37	1	Positive, 20	Positive, 2.2	>20
13	< 0.35	0	Positive, 20	Positive, 1.9	>20
14	0.97	2	Positive, 10	Positive, 0.1	Abandoned
15	0.35	0	Negative	Positive, 2.4	>20
16	0.53	1	Positive, 20	Positive, 0.8	>20

NR, Nonrealized because the patient did not have asthma.

tion workers and nonworker control subjects. The only patient with a negative result had urticaria and no respiratory symptoms. Data of aeroallergen and carnation extract prick tests are presented in Table I.

Serum-specific IgE levels to carnation (RAST) ranged from 0.35 to 4.4 PRU/mL. RAST class 3 was found in 1 serum, although 5 sera demonstrated RAST class 2, 5 sera demonstrated RAST class 1, and 5 demonstrated RAST class 0 (Table II).

Methacholine PD₂₀ showed a variable degree of non-specific bronchial hyperresponsiveness in all asthmatic subjects (Table II).

It was found that 86.66% of patients with a positive history of bronchial asthma showed diurnal variations in peak expiratory flow greater than 20% during working days, as compared with the days away from work.

Nasal provocation test results with carnation extract were positive, with nasal obstruction, itching, sneezing, and nose running in 13 of 16 patients. Eight patients had positive nasal test results with the concentration of 10 mg/mL, and 5 patients had positive nasal test results with 20 mg/mL. None of the patients had a positive reaction with the lowest concentration administered (Table II). Specific nasal provocation test results were negative in all control subjects.

A statistically significant correlation was found between RAST and nasal challenge test results ($P < .01$).

Molecular mass proteins of carnation extract separated by SDS-PAGE under reducing and nonreducing conditions are shown in Fig 1.

Results of SDS-PAGE immunoblotting with or without β -mercaptoethanol showed similar data, which indicates that epitopes recognized by IgE antibodies are not altered by this reducer denaturalizing agent. This assay revealed the existence of IgE-binding bands with molecular weights of 34 and 35 kd bands, which suggests that they could constitute the major allergen (Fig 2).

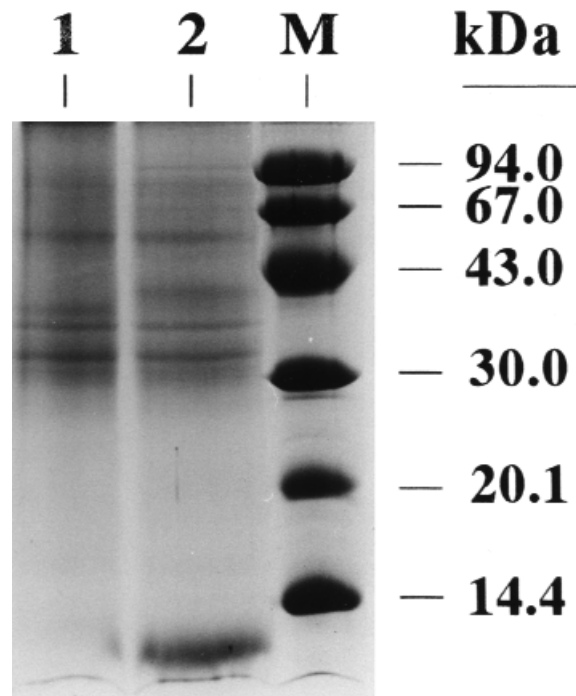


FIG 1. SDS-PAGE after Coomassie blue staining. Line 1, *D Caryophyllus* extract without β -mercaptoethanol; line 2, *D Caryophyllus* extract with β -mercaptoethanol; line M, molecular mass patron.

DISCUSSION

Carnation (*D caryophyllus*) is an angiosperma dicotiledonea plant, which belongs to the Caryophyllacea family, together with *Gypsophila paniculata* and *Saponaria officinalis*. At least 250 different species are known to be cultivated indoors, and therefore many

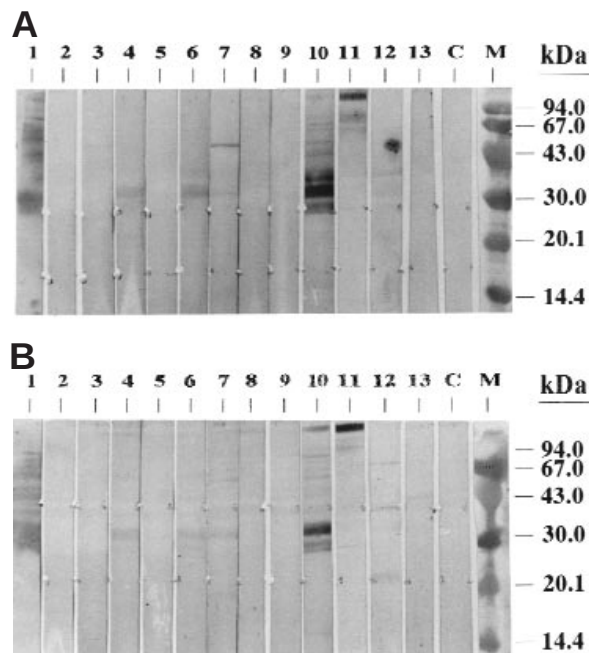


FIG 2. SDS-PAGE-immunoblotting to *D Caryophyllus* (**A**, without β -mercaptoethanol; **B**, with β -mercaptoethanol) incubated with sera from 13 subjects of the study. Two major IgE-binding fractions of 34 and 35 kD were present in most of the patients.

workers may be exposed. Allergic disorders induced by ornamental flower exposure are usually manifested by dermatologic symptoms (eczema, urticaria, and contact dermatitis) associated or not with respiratory manifestations,⁹⁻¹¹ but sometimes exclusively respiratory symptoms are seen.^{4,12,13}

The crucial points in occupational asthma diagnosis are to prove that the patient does indeed have asthma and to objectively demonstrate the causal relationship between asthma and work exposure. In the patients in this study, medical and employment history and positive bronchial hyperresponsiveness to methacholine confirmed the existence of asthma, although positive SPT and RAST results demonstrated the presence of specific IgE antibodies to *D caryophyllus*. A causal relationship between allergen exposure and symptom appearance was proven by the presence of variable airflow limitation at work, determined by PEFR monitoring, and positive specific nasal challenge results. Some authors suggest that the measurement of specific bronchial challenge test results is not necessary,^{2,14} and therefore we did not perform such measurements because it is potentially dangerous, being a potential trigger for severe asthmatic reactions. All these results demonstrate the existence of a type I hypersensitivity mechanism responsible for the episodes of rhinitis and asthma presented by the carnation workers; additionally irritating or pharmacologic mechanisms have been reasonably excluded by the negative cutaneous and challenge test results in asthmatic control subjects.

Results of SDS-PAGE immunoblotting revealed the existence of IgE-binding bands with molecular weights of 34 and 35 kD, which could constitute the major allergen. High molecular weight occupational agents produce asthma in a similar way to aeroallergens, acting as complete antigens through an IgE-mediated mechanism.^{15,16} Thus it has been described that atopy seems to be a predisposing factor to the development of occupational asthma to these substances,¹⁷ which justify the finding that 9 patients of the study (56.25%) exhibited positive SPTs to standard aeroallergens. Moreover, high molecular weight allergens induce an immediate response preferentially with or without a late response,¹⁸ which is in agreement with the fact that an immediate response without late symptoms was observed in all the specific nasal challenges that we performed.

Until now, only isolated cases of occupational asthma induced by gladiolus, carnation, and *G paniculata* sensitization have been reported in florist subjects.^{13,19-21} In this study, collective occupational rhinitis and asthma by sensitization to carnation has been demonstrated by the presence of positive SPT response, specific IgE detection in serum, and positive specific challenge result. Therefore this article reveals that *D caryophyllus* is able to induce both sensitization and disease under natural conditions, and therefore this flower should be taken in account as a cause of respiratory allergic disease at least in our area. As described with other allergens,^{22,23} 7 patients from our study had negative SPT results to a standard battery of aeroallergens. This indicates that some patients diagnosed as having intrinsic rhinitis or asthma may, in fact, be sensitized to unknown allergens not included in skin test batteries of common aeroallergens.

In summary, we have demonstrated the involvement of carnation in occupational allergy, mediated by IgE-mediated hypersensitivity; therefore the inhalation of material derived from carnations may cause respiratory allergy in exposed workers.

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