

Allergens involved in occupational asthma caused by baby's breath (*Gypsophila paniculata*)

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Although the floral industry deals with many potential allergens, few examples of occupational asthma exist in this industry. A 22-year-old florist experienced symptoms of rhinitis, conjunctivitis, and asthma on exposure to baby's breath. To determine the contribution of baby's breath to the patient's symptoms, an extract of baby's breath was prepared. Prick skin tests with a 1:10⁻⁵ wt/vol concentration of the extract produced an immediate response, whereas nonexposed atopic and normal control subjects did not react. The patient's asthmatic response to baby's breath was confirmed by bronchial challenge that caused an immediate fall in FEV₁ of 26.2% from baseline after inhalation of 88 breath units of the extract. With a direct RAST, the patient's serum bound 38 times the amount of IgE bound by the negative control. IgE binding in the RAST was inhibited by the baby's breath extract but not by unrelated inhibitors (ragweed and tree pollens). Immunoblotting demonstrated IgE binding to 13 protein bands in the extract with molecular weights ranging from 11.5 to 68 kd. Serum from a patient previously reported to have sensitivity to baby's breath recognized five protein bands. Three proteins with molecular weights of 27, 31, and 37 kd were recognized by both patients' sera. We conclude that this patient developed IgE-mediated sensitivity to multiple allergens in baby's breath. This study confirms the importance of this plant as a potential cause of occupational asthma in the floral industry. (J ALLERGY CLIN IMMUNOL 1990;86:189-93.)

Occupational asthma, defined as reversible airflow obstruction occurring as the result of exposure to dusts, vapors, gases, or fumes in the workplace,¹ has been attributed to >200 different etiologic agents.² For some of these agents, a significant percentage of exposed individuals have been reported to develop asthma, whereas for other agents, only isolated cases of allergic reactions have been described. Although

Abbreviations used

SDS-PAGE:	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
TBS:	Tris-buffered saline
BSA:	Bovine serum albumin

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Received for publication April 20, 1989.

Revised Feb. 6, 1990.

Accepted for publication Feb. 14, 1990.

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**Dr. Robert K. Bush received general research funding for salaries of technicians and for supplies from the Veterans Administration.

1/1/21010

the floral industry deals with many potential allergens, there are few examples of occupational asthma within this industry. A single case of occupational asthma caused by exposure to the plant *Gypsophila paniculata*, commonly referred to as baby's breath, has been previously described by Twiggs et al.³ This plant is a tall, many-branched, perennial herb that has clusters of small fragrant white or pink flowers⁴ and is frequently used in floral arrangements. We report in this article the results of an evaluation of a second patient with occupational asthma caused by baby's breath. With immunoblotting techniques, we studied the allergens involved in these patients' immunologic response to baby's breath.

CASE REPORT

A 22-year-old woman, the owner of a floral shop, developed conjunctivitis, nasal congestion, clear rhinorrhea, and sneezing 1 year after opening her shop. About 6 months later, she also developed coughing, wheezing, and shortness of breath. Nasal symptoms were treated with a nasal decongestant spray used up to five times daily, and the pulmonary symptoms were treated with a time-release theophylline product. Nasal and eye symptoms would occur on each work day, beginning within 5 to 10 minutes after she would start to work with baby's breath and would improve within a few hours after she stopped handling the baby's breath. Pulmonary symptoms occurred two to three times weekly, would last for a few hours before clearing, and also had their onset in relationship to exposure to baby's breath. Although the patient worked with a variety of dried and fresh flowers and plants, only baby's breath exposure was temporally related to the occurrence of symptoms. The patient did not have asthma symptoms on days away from the store, and the nasal and eye symptoms were minimal. Before opening the floral shop, the patient had experienced only mild nasal symptoms in the springtime of each year, which did not require treatment. The patient did not smoke. Family history was only remotely positive for asthma in a maternal great-grandparent.

On physical examination there was moderate nasal congestion. Auscultation of the lungs revealed bilateral expiratory wheezes. Results of pulmonary function tests were normal; the FEV₁ was 3.2 L (100% of predicted), and the FVC was 3.8 L (103% of predicted). Skin tests by the prick method performed with 18 common allergens were significantly positive only to elm tree.

A crude extract of baby's breath was prepared by creating a mixture of fresh and dried baby's breath in saline. A prick skin test with this extract produced a wheal-and-flare response (mean diameter) of 9 mm and 46 mm, respectively. A nonexposed atopic individual tested with the same material had no response.

MATERIAL AND METHODS

Preparation of baby's breath extracts

Samples of fresh and dried baby's breath supplied by the patient were mixed together and ground with a mortar and pestle. The mixture was then defatted by soaking in acetone for 18 hours at room temperature. After siphoning the acetone, the samples were rinsed with acetone and allowed to soak in acetone for 1 additional hour. After siphoning once again, the samples were air-dried for 24 hours. Each sample was then placed in phosphate-buffered saline, pH 7.4, at a concentration of 1:10 wt/vol. After overnight incubation with constant stirring at 4° C, the samples were centrifuged at 900 g for 15 minutes, and the supernatants were stored at -20° C until use. Protein concentration of the extract, determined according to the method of Bradford⁵ with a commercial kit (Bio-Rad Laboratories, Richmond, Calif.), was 0.35 mg/ml. Because of the low protein content, the

extract was concentrated by filtering through a YM5 filter (Amicon Corp., Lexington, Mass.). After this procedure, the protein concentration was 2.2 mg/ml; the amount of protein lost in the filtrate was determined to be 40 µg/ml.

Skin tests

The stock solution of baby's breath (1:10 wt/vol) was filtered through a 0.45 µm Millipore filter (Millipore Corp., Bedford, Mass.). Serial tenfold dilutions were prepared with sterile albumin saline with phenol (Hollister-Stier Laboratories, Spokane, Wash.), and dilutions were used for prick skin testing of the patient and a nonatopic control subject.

Bronchial challenge

The patient had bronchial challenge to baby's breath with the dilutions prepared for skin testing. Challenge solutions were administered via an Ailife Misty nebulizer (American Hospital Supply, Brown Deer, Wis.) driven by a Mistogen air compressor (model C6605, Allied Health Care Products, St. Louis, Mo.), which delivered a flow rate of 9.5 L/min. FEV₁ was measured with a spirometer (model S, Vitalograph Medical Instruments, Lenexa, Kan.). Responsiveness to baby's breath was tested by measuring the patient's FEV₁ before and after five tidal breaths of sterile albumin saline with phenol (Hollister-Stier) and then after increasing cumulative breath units of baby's breath administered at 10-minute intervals. One breath unit equaled one inhalation of a 1:5000 wt/vol dilution of the baby's breath extract. The challenge was stopped after a fall in FEV₁ of at least 20% from baseline. Nonexposed atopic and nonatopic control subjects were challenged with the same extracts to determine the specificity of the bronchial challenge results.

Direct RAST measurement

The proteins in the extract of baby's breath were coupled to cyanogen bromide-activated Sepharose 4B (Pharmacia, Piscataway, N.J.) at a concentration of 2 mg of protein per milliliter of swollen gel, with methods previously described.⁶ Coupling efficiency was 95.5%.

All assays were performed in duplicate. The average background count was subtracted from each result before data analysis. Results of the RAST test are expressed as the ratio of radioactive binding in the serum sample to that in a negative control sample (3% sorbent plus RAST buffer; no serum added). A ratio of 2:1 or more is considered significant.⁷

RAST-inhibition measurements

To determine the specificity of IgE binding in the direct RAST, RAST inhibition was conducted according to the method of Gleich et al.⁸ Serum from the patient was diluted 1:5 in RAST buffer. Diluted serum (0.1 ml), 0.5 ml 3% sorbent, and varying quantities of the following inhibitors were allowed to react with constant rotation at room temperature for 18 hours: (1) baby's breath, 1.16 mg/ml (protein content); (2) ragweed, 10,000 PNU/ml (Greer Labo-

ratories, Lenoir, N.C.); and (3) six eastern tree mix, 10,000 PNU/ml (Greer). The remainder of the procedure was the same as for the direct RAST. The amount of radioactivity bound to the Sepharose particles was compared to that of a control to which inhibitor had not been added.⁹ In all tests the amount of nonspecific binding of radioactivity was taken to be the amount bound by a blank sample.

SDS-PAGE/immunoblotting

Immunoblotting was performed with methods similar to methods previously described.¹⁰ Proteins in the baby's breath extract were first separated by SDS-PAGE with a 3.9% polyacrylamide stacking gel and a 12.5% resolving gel. The fractions were treated at 100° C for 2 minutes in the presence of SDS and 2-mercaptoethanol. Twenty-five micrograms of protein was applied to each lane of the gel. Electrophoresis was performed at 30 mA per gel with constant current until the bromphenol blue tracking dye was about 1 cm from the bottom of the gel. Portions of the gel were sliced and stained with Coomassie brilliant blue R-250 or periodic acid-Schiff, and the remaining portion was used for the immunoblotting experiments.

Proteins were transferred electrophoretically from the SDS gel to nitrocellulose. IgE binding to the transferred proteins was detected as follows: Unoccupied sites of the nitrocellulose were blocked with 3% BSA in 10 mmol/L of Tris HCl, 0.9% NaCl, pH 7.4 (TBS) plus 0.02% NaN₃ for 1 hour at 40° C. The nitrocellulose was cut into individual strips that were incubated overnight in 5 ml of a solution of the patient's serum diluted 1:5 in TBS plus BSA plus NaN₃. Serum from another patient previously reported to have sensitivity to baby's breath¹ (kindly provided by Dr. John Yunginger, Mayo Clinic, Rochester, Minn.) was also used for immunoblotting studies. Control strips without antigen were blocked and incubated with TBS plus BSA plus NaN₃ only. After extensive washing,¹⁰ the strips were then incubated overnight with ¹²⁵I-labeled antihuman IgE (Pharmacia). After a final set of washes, the strips were wrapped in plastic film and placed on XAR-5 x-ray film (Eastman Kodak, Rochester, N.Y.) for autoradiography at -70° C for 1 to 3 days.

RESULTS

Skin tests

The patient was prick skin tested with increasing tenfold concentrations of the nonglycerinated extract. With increasing protein concentration there was a corresponding increase in the size of the skin test reaction from 10⁻⁵ wt/vol to 10⁻³ wt/vol. A nonexposed non-atopic control subject had a negative response to the stock 1:10 wt/vol solution.

Bronchial challenge

The bronchial challenge was started at a dilution of the baby's breath extract of 10⁻⁷ wt/vol because this

dilution was believed to be a concentration that would be unlikely to cause a response based on the negative skin test response to the 10⁻⁶ wt/vol dilution. The patient developed an immediate asthmatic response after a cumulative dose of 88 breath units (80 minutes after initiating the challenge). The patient's FEV₁ fell from a baseline of 3.05 L to 2.25 L; this fall represents a 26.2% decrease in the FEV₁ from baseline. After inhalation of two breaths of isoproterenol, the FEV₁ returned to the baseline value. Similar bronchial challenges performed on nonexposed atopic and nonatopic control subjects demonstrated no change from baseline values after inhalation of a total dose of 250 breath units (data not presented).

Direct RAST/RAST inhibition

The patient's serum had a significant elevation of IgE antibody directed against baby's breath, binding 38 times the amount of IgE of the negative control. Four nonexposed control subjects had IgE binding of 0.88 ± 0.11 (mean ± SD) times the negative control subject (range, 0.74 to 1.0).

In the RAST-inhibition assay, baby's breath caused significant inhibition. Unrelated inhibitors, ragweed, and an Eastern tree mix failed to cause any significant inhibition, confirming the specificity of the IgE binding measured in the RAST.

SDS-PAGE/immunoblotting

The protein separation pattern of the baby's breath extract after staining with Coomassie brilliant blue is illustrated in Fig. 1 demonstrating several distinct protein bands. Periodic acid-Schiff staining demonstrated no protein bands, indicating that bands were not glycoproteins (figure not illustrated).

The autoradiograph is illustrated in Fig. 2 of the patient's serum and serum from the individual previously reported to have sensitivity to baby's breath³ incubated with nitrocellulose strips containing the baby's breath proteins. The patient in this study demonstrated IgE binding to 13 proteins, whereas the individual previously reported demonstrated IgE binding to only five proteins. Both patients had IgE binding to proteins with molecular weights of approximately 37, 31, and 27 kd. Nonexposed normal and atopic control sera did not demonstrate any IgE binding to the baby's breath extracts.

DISCUSSION

Numerous examples of occupational asthma caused by plant-derived materials have been described²; included are studies of sensitivity to the dusts of plants,

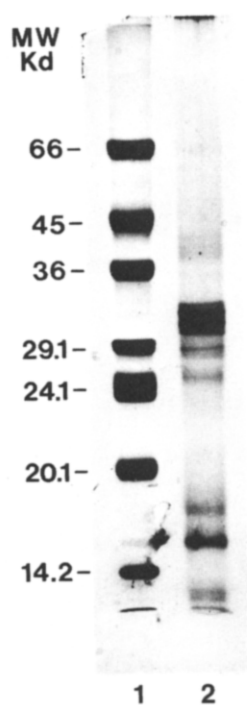


FIG. 1. SDS-PAGE of baby's breath extract, 25 μ g per lane, stained with Coomassie brilliant blue. *Lane 1*, standard molecular weight markers; *lane 2*, baby's breath extract. Molecular weight (MW) expressed in kilodaltons.

particularly cotton and grain dusts. Furthermore, allergies have been attributed to the fruits, seeds, leaves, and pollens of various plants. Additionally, extracts from plants may also cause asthma, such as plicatic acid in western red cedar asthma.¹¹ Lacking from such lists are examples of occupational allergies in the floral industry. In fact, there is a single case report of asthma caused by handling baby's breath in a florist who experienced conjunctivitis, rhinitis, and asthma.³

The patient described in the present study experienced similar symptoms with occupational exposure to baby's breath; specific sensitivity was confirmed by skin tests, bronchial challenge, and the demonstration of specific IgE antibodies to baby's breath by the direct RAST. The patient's IgE response was specific to baby's breath, as demonstrated by a lack of inhibition of the baby's breath RAST by other plant-derived proteins, including ragweed and trees. Immunoblotting was used to characterize further the allergens involved in the patient's IgE-mediated response to baby's breath. With our patient's serum, 13 protein bands that bound IgE were recognized with molecular weights ranging from 11.5 to 68 kD. Serum from the patient reported by Twiggs et al.³ recognized only five protein bands ranging from 24 to 37 kD; however, both sera bound IgE to proteins with mo-

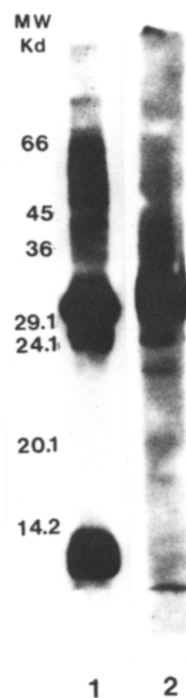


FIG. 2. Autoradiograph of the baby's breath extract after immunoblotting with individual patients' sera; *lane 1*, patient's serum; *lane 2*, serum from individual previously reported to have sensitivity to baby's breath.³ Molecular weight (MW) of IgE-binding antigens in kilodaltons.

lecular weights of approximately 27, 31, and 37 kD, suggesting that the patients' immunologic responses were similar.

The specific source of the allergenic proteins present in baby's breath is not clear from our study, since the whole plant was used to prepare the extracts. Twiggs et al.³ found no predominant pollen in baby's breath when it was examined microscopically. When it is used in floral arrangements, baby's breath is often dry and brittle and therefore can crumble easily when it is handled. It is likely therefore that the patients were sensitized to baby's breath by inhaling airborne dust particles from the plant rather than its pollen.

This study confirms that baby's breath may be an important occupational allergen in the floral industry. Although it is estimated that approximately 2% of all cases of asthma in the United States are occupational in origin,¹² the allergens involved in IgE-mediated occupational asthma have generally not been well characterized. We have used immunoblotting to identify and characterize, at least partially, allergenic proteins in baby's breath. The use of immunoblotting and other immunologic methods may allow better identification and standardization of allergens, thereby improving the diagnosis of IgE-mediated occupational allergy.

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Interaction of platelet-activating factor with interferon- γ in the stimulation of interleukin-1 production by human monocytes

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*Platelet-activating factor (PAF) produces both early and late inflammatory responses in vivo. The late and persistent responses to PAF may result from the production of cytokines, such as interleukin-1 (IL-1). The effects of PAF on IL-1 release by human monocytes were studied with PAF alone and in combination with interferon- γ . These studies involved the use of D10.G4.1 cells in a proliferation assay to determine the actual concentration of active IL-1 in monocyte supernatant and pellet fractions. These studies confirmed that PAF stimulates IL-1 release at two different ranges of PAF concentrations, 100 fM to 1 pM and 100 pM to 1 nM. PAF inhibited IL-1 release at 1 or 10 pM. PAF effects on IL-1 release were specific to the form of PAF that is biologically active in most systems; (S)-PAF and lyso-PAF had no effect. When monocytes were incubated with both PAF and interferon- γ , IL-1 release was greatly stimulated at the same two ranges of PAF concentrations, 100 fM to 1 pM and 100 pM to 1 nM of PAF. PAF and interferon- γ interacted synergistically to enhance significantly the release of IL-1. (*J ALLERGY CLIN IMMUNOL* 1990;86:193-201.)*

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Supported by the Veterans Administration, American Cancer Society Grant IM522, and National Institutes of Health Grants AI19784 and AI22141.

Received for publication Dec. 5, 1989.

Revised March 27, 1990.

Accepted for publication April 11, 1990.

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*Dr. R. Barthelson was supported in part by a fellowship from the American Lung Association of California.

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IL-1 is a key mediator of inflammation.¹ Monocytes, the principal source of IL-1,² release IL-1 in response to adherence of the cell to a foreign surface or in response to endotoxin, interferon- γ , TNF, substance P, or bradykinin.¹⁻⁵ There are two species of IL-1, designated IL-1- α and IL-1- β , that have similar biologic effects. Although IL-1- α and IL-1- β are different proteins, they have similar sizes (17,500 MW), and both are produced as larger precursors of ~31,000 MW before they are cleaved and secreted.^{1, 2}

PAF is a potent inflammatory mediator produced not only by monocytes but also by neutrophils, ba-