

Occupational asthma related to fresh *Gypsophila paniculata*

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Among the multiple pathogenetic agents of bronchial asthma in the work environment, the ornamental flower *Gypsophila paniculata* (*Gp*) – commonly used in flower arrangements and known as “baby’s breath” in the United States – has recently been reported to be a cause of bronchial asthma in the floral industry (5, 7). Furthermore, allergens involved in this sensitization have been studied by means of immunoblotting methods (5). This report documents another case of occupational asthma caused by *Gp*, as proved by skin tests, bronchial provocation tests, and *in vitro* studies – including direct ELISA and SDS–PAGE/immunoblotting.

Case report

A 26-year-old woman who had worked at a florist’s shop for the last 8 years, a nonsmoker with no prior clinical history, during the last 4 years had developed symptoms such as sneezing, nose and eye itching, and watery rhinorrhea, as well as dry coughing, chest tightness, and wheezing. These symptoms were episodic and showed no clear seasonal pattern. The patient had noticed a clinical improvement on weekends, and was free of symptoms during holidays. Moreover, she related her symptoms to the handling of certain plants in the workplace, especially fresh *Gp*. She had been diagnosed as suffering from intrinsic asthma and had been treated during the last

3 years with inhaled steroids, salbutamol, theophylline, and ipatropium bromide, resulting in partial improvement but unacceptable side-effects which led her to withdraw the medication for long periods of time. No other systemic or organic symptoms were present.

Anterior rhinoscopy showed a pale, exudative nasal mucosa; no polyps or other structural anomalies were present. Auscultation revealed bilateral inspiratory and expiratory wheezes. The rest of the physical examination was irrelevant. Basal spirometric data were the following: FVC 3930 (103%); FEV₁ 3000; FEV₁/FVC 76%; MEF₅₀ 2540 (71%). Blood counts and biochemistry were normal, as were urinalysis and chest and sinus (Waters) roentgenograms. Prick tests with grass and tree pollens, animal danders, molds, house-dust mites, and storage mites were negative.

Skin prick test with a crude, unpurified extract from fresh *Gp* (supplied by the patient) was performed. The fresh plant was extracted in Coca’s solution w/v 1:10, and the skin test showed an immediate wheal-and-flare reaction of 15 × 8 mm (mean diameter). The total protein content in the extract was not determined, but the same extract was tested on three control subjects and five atopic subjects with negative results. A similar extract was obtained from white carnations (w/v 1:10), and skin tests were done on the patient with again negative results.

Bronchial provocation test

A bronchial challenge test was subsequently done with different dilutions from the primary *Gp* extract, and according to procedures summarized by Cromwell et al. (3). Dilutions of 1:10 000 and 1:1000 failed to provoke any response in respiratory function, while a 1:100 dilution provoked an immediate 48% fall in FEV₁ that was fully recovered after 30-min salbutamol inhalation. No late asthmatic response was recorded. The same dilution failed to evoke any ventilatory changes when inhaled by two unexposed atopic, hyperreactive control subjects.

In vitro tests

All *in vitro* tests were kindly performed by Alergia e Inmunología Abelló, SA, Madrid, Spain.

A newly prepared extract from fresh *Gp* was used. This was obtained by the following procedures. Fresh flowers were frozen at -80°C , lyophilized, and then extracted in ammonium bicarbonate 20 mM, 15% w/v, for 4 h at $4-8^{\circ}\text{C}$. The preparation was centrifuged at 39 000 g for 20 min, a dialysis was made with distilled water, and the preparation was filtered through a 0.22- μm pore membrane. The total protein concentration of the extract – according to Lowry's method – was 9.40 mg/ml.

Total IgE was 320 kU/l. A direct ELISA (Phadezym RAST, Pharmacia, Uppsala, Sweden) was done, coupling the newly prepared extract to paper disks as solid phase; a significant amount of serum specific IgE (Class 2) was detected in the patient's serum, but not in those of two atopic controls. No specific IgE against grass pollen or house-dust mites was present in the patient's serum.

Proteins in the *Gp* extract were first dissociated with sodium dodecyl sulfate in a polyacrylamide gel (SDS-PAGE), and then polypeptides were separated by electrophoresis, with the usual standards as mol. mass markers (Fig. 1, band A). A portion of the gel was stained with Coomassie brilliant blue, showing several different protein bands (Fig. 1, band B). The remaining portion of the gel was subjected to electrotransference according to methods described by Towbin et al. (6), and immunodetection of IgE-binding components was done with the patient's serum and ^{125}I -labeled anti-IgE. Three IgE-binding bands were obtained, as indicated by their respective mol. mass in Fig. 1, band C. Control sera from grass-pollen-allergic subjects failed to reveal any IgE-binding bands (Fig. 1, band D).

Discussion

Occupational asthma caused by several plant-derived materials has been reported, mainly in rela-

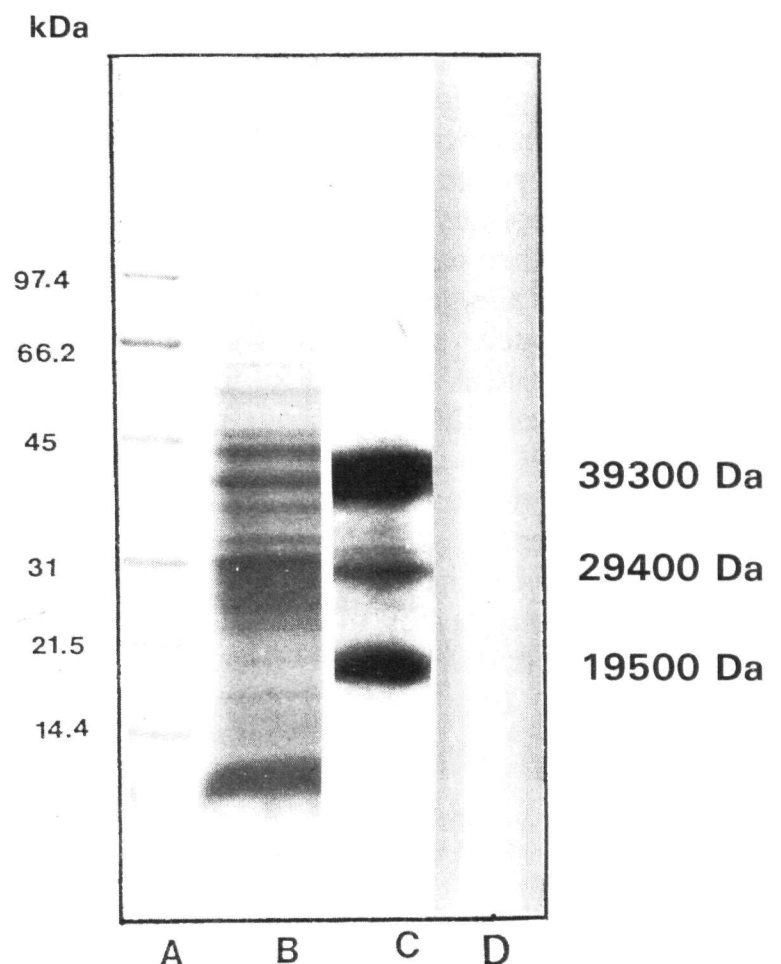


Fig. 1. Electrophoresis in polyacrylamide gel of *Gp* extract dissociated with SDS-PAGE, and immune detection of IgE-binding components in patient's serum (see text).

tion to wood and grain dust, plant-derived rubber, and some enzymes of vegetal origin (2). To our knowledge, there are four previous reports on occupational asthma related to the floral industry. One of these reports deals with *Ficus benjamina* (weeping fig) (1), and another with the ornamental dried plant *Limonium tartaricum* (4), while both the other cases appear to be related to the handling of "baby's breath" in the workplace (5, 7). *G. paniculata* is a perennial herb which belongs to the Caryophyllaceae family and is often used in flower arrangements. As suggested in a previous article (5), and as it is a dried plant that crumbles easily, sensitization is probably achieved by the inhalation of airborne dust particles from the plant. However, our patient suffered from symptoms even when handling the fresh plant.

We report a characteristic case of occupational sensitization to *Gp*, as demonstrated by skin tests, serum specific IgE, and bronchial challenge test. In addition, the antigenic structure of the proteins in the extract is studied, with a demonstration of three IgE-binding bands when incubated with the patient's serum. These IgE-binding components appear to have mol. mass of 20–40 kDa, as in the reported cases of sensitization to *Gp* by Schroeckenstein et al. (5), with minor differences in the blotting results that might be due to differences in the extracts themselves; as stated before, our extracts were always

made from *fresh* plants, not dry herbs, and this circumstance could have modified the results to some extent. Interestingly, the range of mol. mass is significantly higher than expected; major allergenic fractions in many other occupational allergens are known to be small polypeptides functioning as haptens only. Nevertheless, further study is needed to identify and standardize these allergenic fractions, including several methods (such as high-performance liquid chromatography) that might help to identify the single fractions according to various physical properties.

In conclusion, the authors would like to confirm the role of this herb as a potential cause of occupational sensitization in the floral industry, and, finally, to emphasize the necessity of careful occupational anamnesis in bronchial asthma.

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