

their hardness and dust-generating capacity must be studied.

We think that onion seeds can be considered as occupational allergens. Having proven that both *in vivo* and *in vitro* methods are useful tools for the diagnosis of this IgE-mediated disease, we expect to find new hypersensitivities to this allergen among workers in the same factory and to have information about the prevalence of sensitization. Other cases may be found elsewhere.

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Occupational asthma caused by grass juice

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Many individuals have rhinitis, conjunctivitis, or contact urticaria symptoms when they are exposed to grass or sit on a lawn. Some authors have related these symptoms with sensitivity to molds,¹ others with sensitivity to grass pollens, but not with molds or grass leaves,² others, in contrast, have related such symptoms with sensitivity to grass leaves caused by cross-reactivity to grass pollens in patients with hay fever.^{3,4}

We report on a gardener without hay fever who had occupational asthma caused by grass juice allergens aerosolized during mowing.

CASE REPORT

A 25-year-old man had worked as gardener for 4 years. Two years after he began working, he noticed episodes of runny nose, nasal itching, sneezing, cough, wheezing, and shortness of breath. The symptoms were

Abbreviations used

LE: Leaf extract
PE: Pollen extract

so intense that he occasionally required assistance in an emergency service. These episodes occurred only during lawn mowing, work that he performed at least 5 to 10 times a month. On holidays (even in the spring) the severity of his asthmatic symptoms decreased markedly until he was completely free of symptoms. He is a nonsmoker and has no other history of respiratory or allergic diseases, but he has a brother with extrinsic asthma. The patient was not receiving medication when he was admitted to our center. Results of physical examination, pulmonary function tests, and sinus and chest x-ray studies were normal. White blood cell count was 5500 cells/mm³, of which 5% were eosinophils. Total serum IgE was 246 IU/ml. Methacholine challenge showed slight bronchial hyperresponsiveness (methacholine PC₂₀ = 14 mg/ml).

Grass leaf extract (LE) was prepared from grass leaves of *Lolium perenne* (ryegrass) harvested by the patient during October. These leaves were carefully washed in water before they were liquefied (1:1 wt/vol). LE was filtered through a filter paper (Whatman Ltd.,

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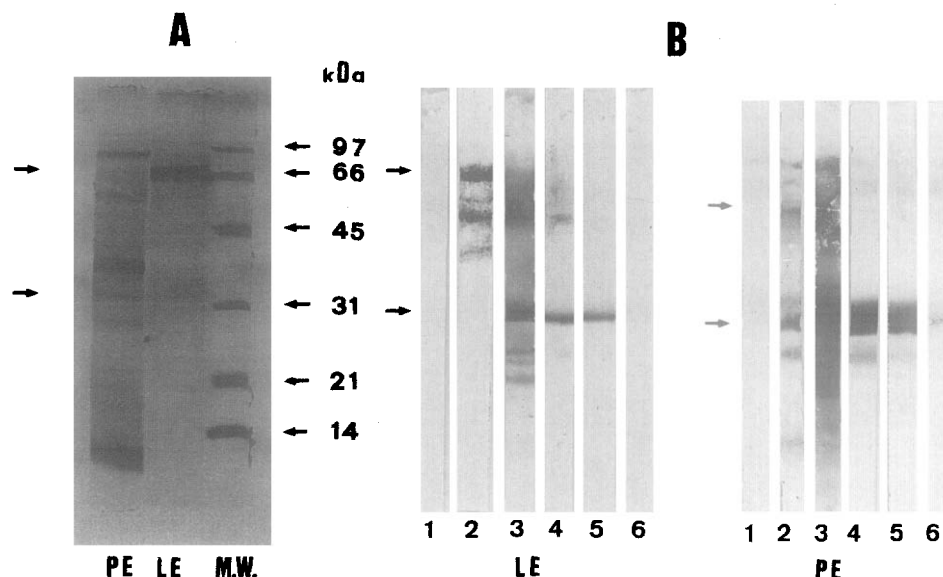


FIG. 1. A, 20% PAGE (nonreducing conditions) of PE and LE from *L. perenne* or protein standards (Coomassie blue staining). B, IgE reactivity against LE and PE by immunoblotting with the following sera: lane 1, Negative control; lane 2, patient; lanes 3 and 4, patients with hay fever who had symptoms when cutting lawns; lanes 5 and 6, patients with hay fever who did not have symptoms when cutting lawns. M.W., Molecular weight.

TABLE I. Patient's skin prick test and IgE measurement data

Extracts	Skin prick test wheal diameters (mm)	Specific IgE by ELISA	
		Patient's serum	Control sera*
<i>L. perenne</i> leaf	11 × 9	0.36 ± 0.0	0.12 ± 0.0
<i>L. perenne</i> pollen	7 × 5	0.97 ± 0.1	0.08 ± 0.0
None†		0.04 ± 0.0	0.03 ± 0.0

Results are expressed in optical densities at 492 nm (mean ± SD).

*Control sera were obtained from five atopic patients without hay fever who had serum IgE levels between 150 and 700 IU/ml.

†ELISA wells were not coated with any extract.

Maidstone, England) and sterilized by 0.22 µm filtration (Millipore, Molsheim, France) to obtain a final concentration of 700 µg/ml in protein, as determined by Bio-Rad protein assay (Bio-Rad Laboratories, Richmond, Calif.). Also, as previously described,⁵ we prepared a 1:20 wt/vol *L. perenne* pollen extract (PE) (350 µg/ml in protein). Furthermore, both extracts were adjusted in protein to a concentration of 350 µg/ml.

The patient exhibited an immediate positive reaction after skin prick testing with both extracts (Table I), with positive histamine control (5 mm × 5 mm), negative saline control, and negative skin test responses to other common aeroallergens, *Dermatophagoides pteronyssinus*, danders, and molds (Aristegui Laboratory, Bilbao, Spain). Five nonatopic persons who served as control subjects for the skin tests did not exhibit any cutaneous response to LE or PE. In contrast, four patients with hay fever who also served

as control subjects showed a positive skin test reaction to PE. Two of the latter patients with hay fever, who had rhinitis symptoms when they mowed their lawns, also exhibited an immediate skin test response to the same LE (wheals of 4 × 3 mm and 5 × 5 mm).

A bronchial provocation test with LE was performed in the patient, as previously described.⁵ On the control day no significant variation in FEV₁ was observed up to 8 hours after inhalation of phosphate-buffered saline. On the contrary, a 49% fall from baseline in FEV₁ was observed at 20 minutes after the challenge with 1:1 wt/vol LE. Five hours later, FEV₁ returned to baseline; but 8 hours after challenge, FEV₁ again decreased by 21%.

Results of the same inhalation challenges were negative in two patients with intrinsic asthma who served as control subjects.

Specific IgE for LE and PE were determined by ELISA⁵ and immunoblotting,⁶ the results of which are shown in Table I and Fig. 1.

DISCUSSION

The onset of respiratory symptoms within minutes of exposure to cut grass at work and the significant improvement away from work suggest the occupational nature of this patient's asthma. This conclusion is supported by the presence of a significant bronchial response after double-blind challenge with LE and by positive prick test results and the presence of specific IgE to the LE (Table I). The negative results obtained in the nonatopic subjects support the specificity of these findings.

It may be argued, however, that the asthma could have been related to the agitation of molds deposited on the grass.¹ Nevertheless, this is unlikely because the patient showed negative skin test responses to molds, and in addition, he did not have symptoms (noncutting days) when, for example, he raked leaves or carried out other similar garden activities associated with a high exposure to molds.¹

Morrow Brown³ demonstrated positive skin test reactions to grass juice or to extracts of grass leaves in patients with grass pollen allergy whose skin reacted to contact with grass at any time of year. Fernandez Caldas et al.,⁴ by performing RAST inhibition assays with sera of patients with grass pollen allergy, found that *L. perenne* pollen allergens are present in the leaves of some, but not all, grass species.

In our patient more than 50% of IgE reactivity to PE was readily inhibited by LE, as determined by inhibition ELISA, supporting the existence of such cross-reactivity between the two extracts. This cross-reactivity would help to explain the positive skin test response and specific IgE to PE found in this patient (Table I), who in contrast, did not experience hay fever symptoms.

Fig. 1, A shows the protein pattern of each extract as detected by sodium dodecylsulfate–polyacrylamide gel electrophoresis. As can be seen, this pattern did differ substantially between leaf and pollen; major proteins appeared as 60 kd and 34 kd bands, respectively.

As determined by immunoblotting, our patient's serum (Fig. 1, lane 2) reacted with the major protein and other bands of relatively high molecular weight (50 to 80 kd) present in LE (Fig. 1, B). Interestingly, two patients with hay fever (lanes 3 and 4) who had symptoms while cutting the lawn

did react with these bands as well, in contrast and another two patients with hay fever (lanes 5 and 6) who did not have symptoms when cutting the lawn. As can be seen, patient sera corresponding to lanes 3, 4, and 5 reacted, in addition, with a low molecular weight band (approximately 35 kd) present in LE (Fig. 1, B). This was a minor band of this extract, which could be related to the major antigen (Lol p 1) of PE (Fig. 1, A). This was likely because all three sera reacted strongly with the 34 kd band present in PE (Fig. 1, B).

On the other hand, this latter extract also contained bands of high molecular weight, reacting with some sera (lanes 2 and 3), which might therefore correspond to similar bands found in LE.

Taken together these results suggest that although antigens are present in both extracts, accounting for the cross-reactivity found, great quantitative differences among them are apparent. We therefore hypothesize that these differences might explain why, depending on the sensitizing agent (pollen or leaf juice), symptoms appear after exposure to one, but not necessarily to the other. In this regard, it should be emphasized that our patient only had symptoms in natural conditions when the grass juice was aerosolized during mowing, and not on exposure to airborne grass pollen.

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