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***Allium cepa* seeds: A new occupational allergen**

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We present a case of occupational asthma caused by IgE-mediated sensitization to red onion (*Allium cepa*, Brunswick variety) seeds in a seed-packing factory worker. To our knowledge, there are no earlier reports of occupational allergy caused by Liliaceae plant seeds.

CASE REPORT

A 27-year-old female smoker without an atopic background was referred to us because of episodes of rhinoconjunctivitis, which had begun 1 year before evaluation. In the last few months before consultation, she had also complained of asthma attacks. The patient believed that her symptoms were caused by contact with red onion seeds. She had been working for 3 years in a room without an air extraction system in which a machine

Abbreviations used

SDS-PAGE: Sodium dodecylsulfate-polyacrylamide gel electrophoresis
SPT: Skin prick test

packed the seeds into bags; she had to put these bags in boxes. The patient had mild symptoms on contact with onion seeds other than red onion seeds. Handling other seeds, including those of the Liliaceae family (asparagus, leek) did not elicit any symptoms. She touched and ate raw and cooked onions without experiencing symptoms.

Results of physical examination, blood cell count, basic biochemistry, and chest and sinus roentgenograms were normal. Total IgE was 136 kU/L. Specific IgE to onion (CAP System; Pharmacia, Uppsala, Sweden) was negative. Basal spirometry revealed no abnormalities.

Extracts

Three 10% wt/vol extracts were prepared. For extract 1, 30 gm of crude red onion seeds in 30 ml of phosphate-buffered saline (PBS) were shaken for 24 hours at room temperature. The extract was then centrifuged for 15 minutes at 1500 g, and the supernatant was dialyzed against PBS and saline solution and finally filtered through a 0.22 µm filter. Extract 2 was made with

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incompletely crushed seeds, and the dialysis and filtration steps were omitted. The seeds were ground to obtain extract 3, which was also dialyzed and filtered. The protein concentration was less than 0.05 mg/ml in extracts 1 and 2; in extract 3, it was 0.35 mg/ml (Bio-Rad Protein Assay; Bio-Rad, Richmond, Calif.). Extracts of other Liliaceae and non-Liliaceae seeds (10% wt/vol) were prepared with prior grinding, and we used them for skin prick tests (SPTs) and immunoblotting.

Skin tests

Full-strength extracts were used to perform SPTs, with histamine 10 mg/ml (4×5 mm wheal) and saline solution as controls; they were read at 20 minutes. Red onion extract 3 and Italian and white onion seed extracts produced a 4×4 mm wheal, whereas a 4×6 mm wheal was obtained with the violet variety. Results were negative in 20 control subjects. SPT responses to red onion extract 1, leek, asparagus, and other non-Liliaceae seed extracts were negative. Responses to common aeroallergens were also negative.

Provocation tests

An end-point titration was done with extract 3, and then a bronchoprovocation test was performed according to the Townley intermittent inhalation method.¹ A ME.FAR MB3 breath-activated dosimeter (ME.FAR, Brescia, Italy) was used. The initial dilution was 1:1000 of the original extract, and the concentration was successively increased 10-fold. Ten minutes after the patient inhaled the 1:10 dilution, a fall in FEV₁ of 34% occurred. No late reaction was observed in an hourly peak expiratory flow rate control. No reaction was elicited in an atopic control subject.

Indirect ELISA

An indirect ELISA was performed with a flat-bottomed, high-affinity microtiter plate that was coated in duplicate with 1:10 and 1:20 dilutions of red onion extracts 1, 2, and 3 in PBS. Alkaline-phosphatase-labeled equine anti-human IgE was used. Extracts 2 and 3 produced positive values, corresponding to a class 3 of conventional RAST. Results for sera from seven non-atopic subjects' were negative.

Sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

Various gels were prepared according to the discontinuous system of Laemmli with a 16% acrylamide gel. No bands were observed in extract 1. One band was resolved in extract 2, with a molecular weight around 30 kD. In extract 3, 10 bands (from 18.5 to 80 kD) were separated.

Immunoblotting

Immunoblotting was carried out according to a method described by Petit et al.² with some modifications. The patient's serum (Fig. 1, A) recognized, in

extract 2, the only band that appeared in the SDS-PAGE, whereas in extract 3, six more bands, four of higher and two of lower molecular weight were recognized, in addition to the 30 kD band. Their intensity was weaker. When the assay was repeated with 20% methanol added to the buffers, a stronger band appeared in the 30 kD area in extracts 2 and 3. In the latter, the bands of higher molecular weight disappeared. The blotting assays with other seeds (Fig. 1, B) were carried out with methanol: a 30 kD band was observed for all onion seeds. A weak band was detected for leek seeds, but no bands appeared when other seeds were tested, including asparagus, a member of the Liliaceae family (data not shown).

DISCUSSION

Seed-derived dusts can be responsible for occupational asthma, usually caused by IgE-mediated mechanisms.³ We gave our patient a diagnosis of occupational asthma caused by sensitization to onion seeds because of the positive results of the skin and bronchoprovocation tests and the detection of specific IgE by means of ELISA and immunoblotting. The onion is a member of the Liliaceae family, which includes other foods, such as asparagus, chives, leeks, and garlic. There are previous reports of occupational asthma induced by garlic,⁴ but we have found no reports of Liliaceae seeds as sensitizers.

Our patient was not atopic. This was consistent with the negative results of the common allergen skin tests. Nevertheless, results of SPTs with onion seed extracts were positive. It is noteworthy that extract 1 was negative and extract 3 was positive (extract 2 was only used for *in vitro* purposes). Results of SPTs were consistent with results of *in vitro* studies. In the SDS-PAGE of extract 2, only a 30 kD band was resolved. Interestingly, extract 2 was positive in the ELISA, and the same band appeared on the immunoblotting of extract 2. Thus considering that its extraction method was gentle and of poor efficacy in terms of protein concentration (<0.05 mg/ml), it seems that the allergen is easily diffusible. Extract 1, which showed no bands on electrophoresis, was negative in ELISA, as it was on immunoblotting. Extract 3 was also positive in the ELISA assay. Its immunoblotting showed recognition of higher and lower molecular weight bands.

Towbin et al.⁵ included the use of methanol in their original immunoblotting description. Thus we performed a new assay, adding 20% methanol to the equilibrating and transfer buffers; the observation of a better definition of the 30 kD band and the disappearance of the high molecular weight bands

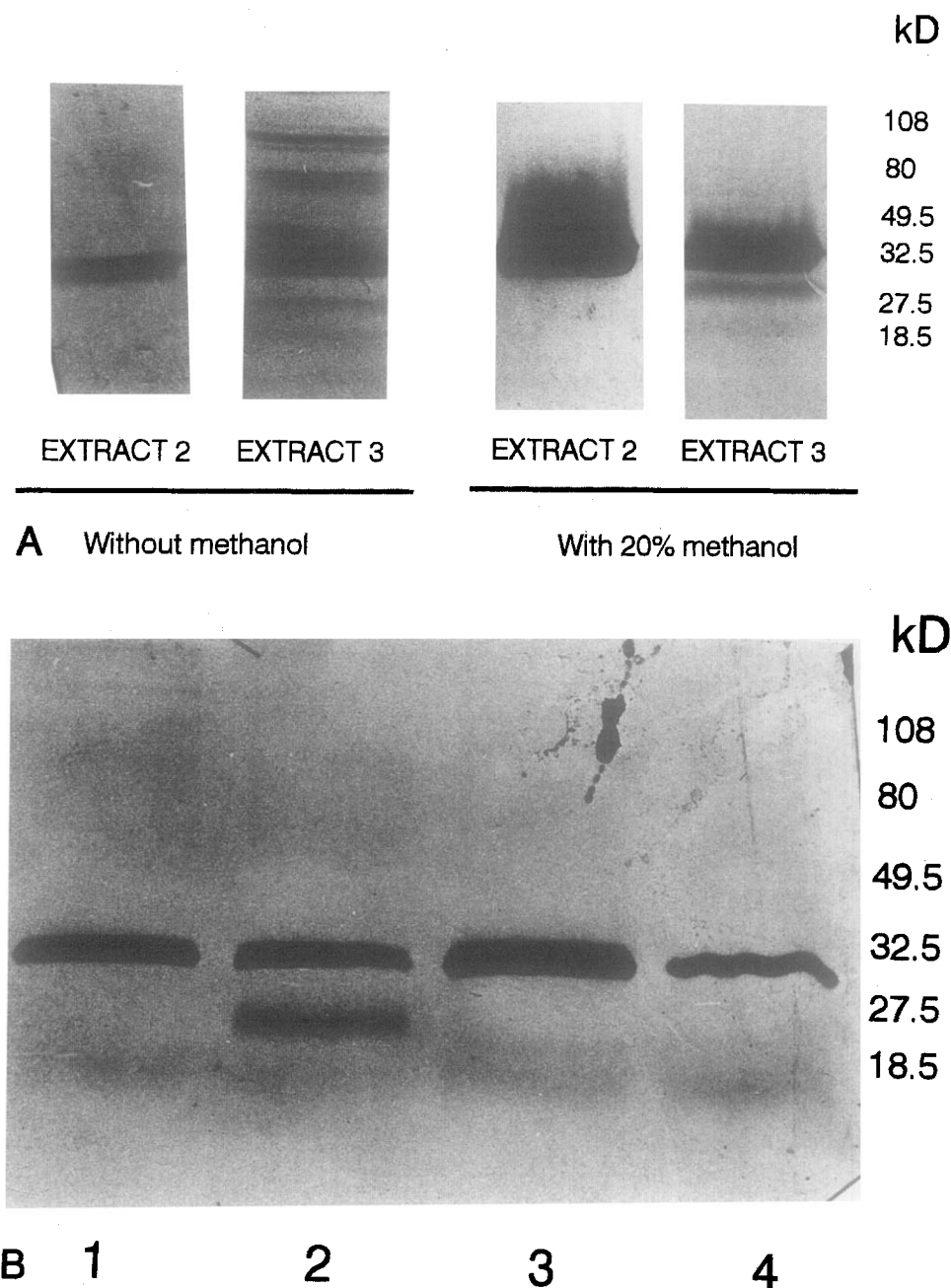


FIG. 1. A, Immunoblotting of red onion seed extracts 2 and 3. A band in the 30 kD area is observed in all assays. **B,** Immunoblotting of various onion seeds. *Lane 1*, Italian onion seeds; *lane 2*, red onion seeds; *lane 3*, violet onion seeds; *lane 4*, white onion seeds. The same band is recognized in all extracts.

suggest that methanol caused some kind of conformational changes in the proteins before they were transferred, hiding some epitopes and exposing others. We therefore considered the use of methanol to be our best immunoblotting condition and studied other seeds by using this method. The results indicated that our patient's IgE recognized the same band in all the onion

seeds and also in leek seeds, but only in red onion seed extract could a lower molecular weight band, which did not appear in other extracts, be seen. This may be related to the fact that the patient had the most severe symptoms when handling red onion seeds, whereas contact with other onion seeds caused only mild complaints. In any case, exposure conditions for each seed might be different, and

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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