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Occupational asthma due to spores of *Pleurotus ostreatus*

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Key words: Air Sentinel; occupational asthma; *Pleurotus*; specific inhalation challenge.

Basidiomycetes, the most morphologically advanced of the fungi, include mushrooms, puffballs, bracket fungi and toadstools. Exposure to basidiospores is known to give rise to allergic rhinoconjunctivitis, asthma, and hypersensitivity pneumonitis (1–4). *Pleurotus ostreatus* is one of the most important basidiomycetes.

A 49-year-old man, a nonatopic, had worked as a seller of fruits and vegetables for the last 30 years at one of the biggest market in Spain. In the last 3 years he had experienced dyspnea, cough, and wheezing 1 or 2 h after large quantities of different mushrooms were unload at the market. He had never had cutaneous or rhinoconjunctival symptoms. He had not experienced constitutional symptoms, such as fever, weight loss, or myalgia. He related his respiratory symptoms to environmental exposure to the mush-

room *P. ostreatus*, being symptom-free during holidays and when mushrooms were not available. He could eat all foodstuffs without any ill effect.

The blood tests revealed eosinophilia (547 eosinophils/mm³). Chest radiography and spirometry were normal. The patient was still working during the study. Skin prick tests to common aeroallergens were negative (including common environmental molds like *Aspergillus fumigatus*, *Alternaria alternata*, *Penicillium notatum*, and *Cladosporium herbarum*). Skin prick tests were positive to commercial *P. ostreatus* extract (10 mg/ml) (Bial-Aristegui, Bilbao, Spain) and to home-made *P. ostreatus* spore extract (10% w/v). Prick-prick with fresh *P. ostreatus* was also positive.

The methacholine inhalation test revealed bronchial hyperresponsiveness (PC₂₀ 0.39 mg/ml). Specific inhalation challenge was carried out by the tidal breathing method as previously described (5) with nebulized *P. ostreatus* spores extract. At a concentration of 1.25 mg/ml it elicited an early asthmatic response (maximum fall in FEV₁ from baseline of 31%) and an incipient late asthmatic reaction (16% fall in FEV₁) 9 h later.

Induced sputum when he was at work showed marked eosinophilia (56%). The

sputum obtained 24 h after the specific inhalation challenge showed a further increase in airway eosinophilia (70% eosinophils). Bronchial hyperresponsiveness to methacholine increased 24 h after the challenge (PC₂₀ < 0.125 mg/ml).

To verify the existence of airborne allergens that could be triggering the symptoms of our patient, environmental sampling was performed using a volumetric air sampler (Air Sentinel) located inside the cold chamber used to store the mushrooms during a regular working day. Airborne particles were collected onto polytetrafluoroethylene filters (6) and an extract was prepared for the *in vitro* study (immunoblotting and immunoblotting-inhibition assays).

Total serum IgE was 514 IU/ml. Determination of specific IgE measured by EAST (Enzyme Allergo Sorbent Test) technique was positive to *Pleurotus* spores extract (2.1 kU/l) and to the workplace air sample extract (0.6 kU/l). Specific IgE was negative for several molds (*A. fumigatus*, *A. alternata*, *P. notatum*, and *C. herbarum*).

Immunoblotting performed with the *Pleurotus* spores extract (Fig. 1A) showed several IgE-binding bands, ranging from 17 to 85 kDa. Immunoblotting-inhibition studies were carried out using the air sample extract as the solid

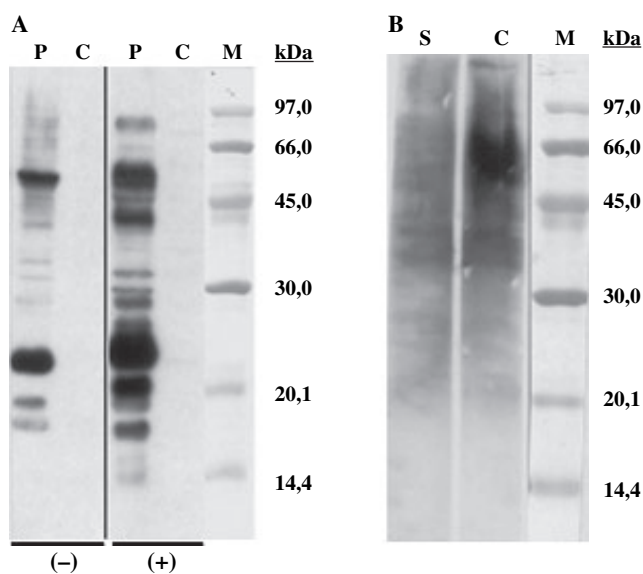


Figure 1. (A) SDS-PAGE immunoblotting for *Pleurotus* spores extract. P: Patient's serum. C: Control's serum. M: Molecular mass marker. (–): Non-reducing conditions. (+): Reducing conditions. (B) SDS-PAGE immunoblotting inhibition using the filter extract as the solid phase. Nonreducing conditions. S: Patient's serum pre-incubated with the *Pleurotus* spores extract. C: Patient's serum preincubated with lamb extract, as negative control. M: Molecular mass marker.

phase (Fig. 1B). When the patient's serum was preincubated with the *Pleurotus* spores extract, a wide IgE-binding band disappeared (around 66 kDa), whereas it was present when the patient's serum was preincubated with a nonrelated extract (lamb, as negative control).

In conclusion, the clinical history and the results of the allergologic work-up including the specific inhalation challenge, the variation of airway hyperresponsiveness and sputum eosinophilia post-challenge point out that this patient had developed occupational asthma caused by *P. ostreatus* spores. The environmental study demonstrated that airborne allergens from *Pleurotus* are present in the workplace.

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Accepted for publication 29 October 2006

Allergy 2007; 62:211–212

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DOI: 10.1111/j.1398-9995.2006.01286.x

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