

Aspergillus fumigatus is the causative agent of hypersensitivity pneumonitis caused by esparto dust

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Esparto grass (*Stipa tenacissima*) is a herbaceous plant commonly found in the Mediterranean countries. It is mainly used for the manufacture of rope, matting, paper pulp, and plaster. Although esparto grass fiber has been reported as a cause of hypersensitivity pneumonitis (HP), the causative antigen has not yet been identified.¹⁻⁴

CASE REPORT

A 48-year-old man, who was a nonsmoker, had been working as a plasterer for 30 years. During the last 12 years he had recurrent episodes of fever, cough, dyspnea, and chest pain, requiring emergency treatment on several occasions. He was first seen in our department in June 1997 with symptoms of weight loss, anorexia, malaise, persistent dry cough, and dyspnea. The patient associated these symptoms with the manipulation of esparto fibers, and he showed marked relief by avoiding exposure to this material.

He was admitted to the hospital to assess the possible role of esparto fibers in the symptoms reported. Crackles were heard on chest auscultation. Blood differential count showed 7300 leukocytes/mm³. Arterial blood gas determinations revealed a pH of 7.43, a PaCO₂ of 38.3 mmHg, and a PaO₂ of 64 mmHg. A chest roentgenogram showed bilateral micronodular infiltrates (Fig. 1). Tuberculosis and connective tissue disorders were ruled out. Spirometry revealed a forced vital capacity (FVC) of 3.27 L (81.2% of predicted value), FEV₁ of 2.76 L (83.7% of predicted value), and FEV₁/FVC of 84.3%. Total lung capacity, as determined by plethysmography, was 6.46 L (103% of predicted value). Single-breath carbon monoxide diffusion test revealed a carbon monoxide transfer lung capacity of 8.82 mmol/min/kPa (94.3% of predicted value). Gallium-67 lung scanning showed an uptake of 46.7% (normal value, <60%). High-resolution computed tomography revealed a diffuse "ground glass" pattern with septal thickening and small centrilobular nodules. Total IgE (20 kU/L) and serum immunoglobulins were within normal limits (IgG, 1370 mg/dl; IgA, 284 mg/dl; and IgM, 71 mg/dl). *Rhizopus* spp. and *Aspergillus fumigatus* were isolated from different samples of esparto fibers.

Skin prick tests to common aeroallergens, *A. fumigatus* and *Rhizopus nigricans* 1% wt/vol (ALK-Abelló, Madrid, Spain), and esparto (10% wt/vol) were negative. Intradermal tests with esparto and *A. fumigatus* extracts were positive at 15 minutes and 6 hours, whereas no reaction was observed to *R. nigricans*. Strongly positive precipitins to both esparto fibers and *A. fumigatus* were found in the patient's serum by using the double immunodiffusion technique (Fig. 2). Specific IgG antibodies against extracts of *A. fumigatus*, *R. nigricans* and esparto

Abbreviations used

FVC: Forced vital capacity
HP: Hypersensitivity pneumonitis
PBS: Phosphate-buffered saline
PIPT: Pulmonary inhalation provocation test

were found in the patient's serum by use of the ELISA method.⁴ These serologic test results were negative in 10 nonexposed control subjects. *A. fumigatus* and *R. nigricans* as liquid-phase antigens were able to inhibit by 50% and 70%, respectively, esparto IgG-ELISA activity.

Pulmonary inhalation provocation tests (PIPT) were performed on different days with esparto extract (10% wt/vol), *R. nigricans* (1% wt/vol), and *A. fumigatus* (1% wt/vol) following the method previously described.⁴ The patient's symptoms and signs were monitored for 24 hours after PIPT, and measurements were made of leukocyte count, serum C-reactive protein level, and pulmonary function tests. Positive clinical (dyspnea, cough, fever, and malaise), physiologic (decrease >20% of FVC and FEV₁ from baseline), and laboratory responses (marked leukocytosis and significant increase of C-reactive protein) was observed 6 hours after PIPT to both esparto and *A. fumigatus* extracts. Symptoms and signs resolved 24 hours after PIPT. The same extracts were inhaled by two healthy subjects, and no response was observed. Bronchoalveolar lavage performed 20 hours after PIPT showed a total cell count of 66 × 10⁶ with marked lymphocytosis (66%). Histopathologic examination of a transbronchial biopsy specimen revealed interstitial alveolitis with lymphocyte-macrophage infiltrate (Fig. 3).

Gel electrophoresis of esparto extract (concentrated 100×) and *A. fumigatus* (1% wt/vol) was carried out in 12% polyacrylamide gel in reducing conditions. Separated proteins were electrotransferred to Immobilon-P membranes (Millipore Corp., Bedford, Mass.). Unreacted membrane sites were blocked with phosphate-buffered saline (PBS) containing 5% skimmed milk and 0.05% Tween 20 (PBS-T milk). The membranes were then incubated overnight with the patient's serum diluted 1:25 in PBS-T milk. After three washings with PBS-T, the membranes were incubated for 60 minutes at room temperature with polyclonal anti-human IgG peroxidase-conjugate (Tago, Burlingame, Calif.) diluted 1:2000 in PBS-T milk, washed, and developed with ECL system as substrate (Amersham Int., Buckinghamshire, U.K.). Immunoblots showed IgG binding to bands of apparent molecular weights of 45 and 70 kd in *A. fumigatus* extract. IgG binding to bands with an apparent molecular weight of 47 and 55 kd and a more diffuse band between 30 to 35 kd was observed in esparto extract (Fig. 4).

DISCUSSION

Esparto dust contains a complex mixture of organic components, including vegetal proteins and several microorganisms that contaminate esparto fibers. Manipulation of these fibers readily disperses a fine dust that can easily reach the distal airway spaces and cause HP in a sensitized individual.

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FIG. 1. Bilateral micronodular pattern.

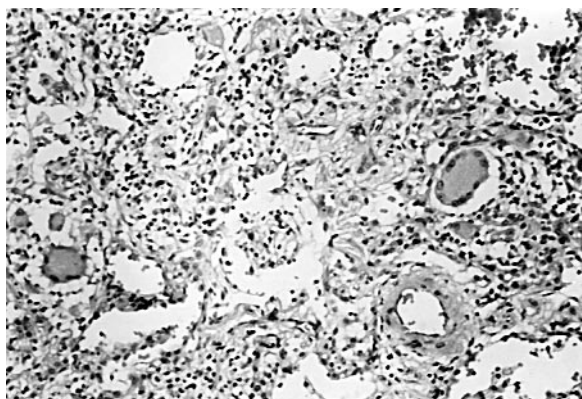


FIG. 3. Lung specimen obtained by transbronchial biopsy showing an interstitial lymphocyte-macrophage infiltrate (hematoxylin-eosin stain; magnification $\times 100$).

However, the causative antigen of this form of HP has not yet been identified.¹⁻⁴ In a previous study *A. fumigatus* was isolated from esparto fibers, and IgG antibodies against this organism were detected in sera from five patients with HP caused by esparto dust.⁴

IgG antibodies against *Rhizopus* and *Aspergillus* spp. were demonstrated in our patient's serum, suggesting that these fungi could be the responsible antigens of HP. Immunoblot experiments showed IgG-binding proteins in both esparto and *A. fumigatus*. The results of specific IgG determinations and ELISA inhibition assays may reflect partial cross-reactivity between esparto fibers and *A. fumigatus* or cosensitization to antigens from both extracts. Positive serologic responses, however, may represent only an indication of exposure and a resulting immune response to the antigens without a definite association with the disease.⁵ To confirm that *A. fumigatus* was the causative antigen, an immune response to this organism was demonstrated, and the symptoms and laboratory abnormalities were reproduced by PIPT to this fungus alone.

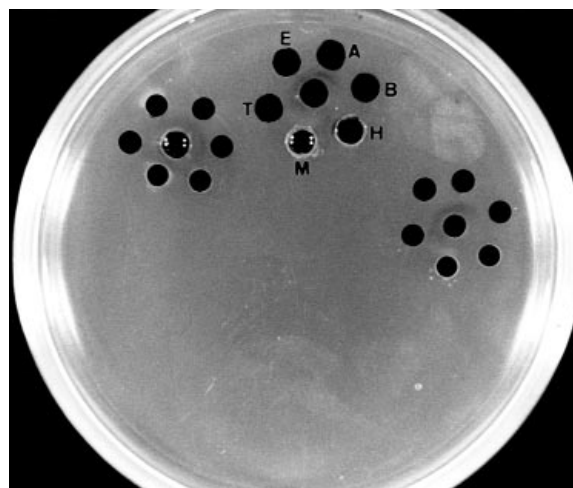


FIG. 2. Precipitation bands between patient's serum and both esparto extract (E) and *A. fumigatus* (A) were detected by Ouchterlony's method. No bands against pigeon serum (P), hay (H), *Micropolyspora faeni* (M), and *Thermoactinomyces vulgaris* (T) were observed.

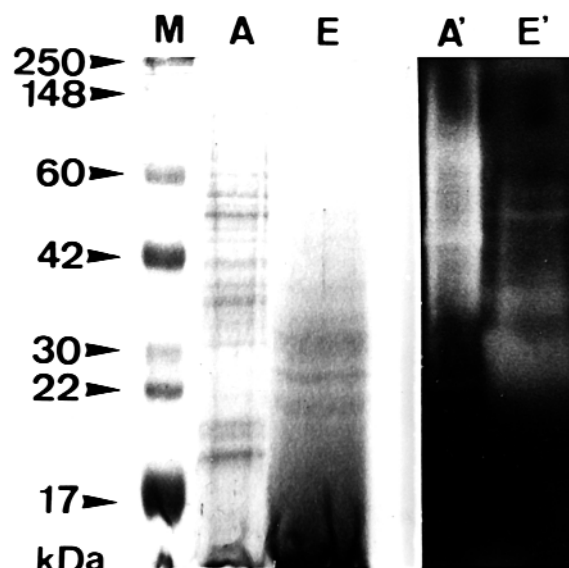


FIG. 4. Gel electrophoresis of *A. fumigatus* (A) and esparto extracts (E) and corresponding IgG immunoblots (A', E'). M, Molecular weight markers.

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