CASE REPORT

Mushroom worker’s lung resulting from indoor cultivation of *Pleurotus ostreatus*

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Indoor cultivation of oyster mushroom *Pleurotus ostreatus* lead to an outbreak of extrinsic allergic alveolitis in two workers. High titer of indirect fluorescent antibody and positive precipitins against basidiospores of *P. ostreatus* were demonstrated in sera of the patients. Mushroom workers should protect themselves from the basidiospores, being aware of their pathogenicity.

Key words: Basidiospore; extrinsic allergic alveolitis; hypersensitivity pneumonitis; indirect fluorescent antibody; mushroom worker’s lung; *Pleurotus ostreatus*.

INTRODUCTION

Edible oyster mushrooms, *Pleurotus* species, are grown all over the world.1 A cultivation technique of *P. ostreatus* on artificial substrate in air-conditioned rooms rendered the production economical throughout the year. The indoor cultivation, however, regularly led to allergic symptoms in workers.2-5 We present outbreaks of mushroom worker’s lung (MWL) in a modern factory of *P. ostreatus* and discuss the diagnosis and prevention of the disease.

CASE REPORTS

Case 1

A 33-year-old man, who had been working in a factory of *P. ostreatus* for a month, developed a productive cough, low grade fever and exertional dyspnoea. He repeatedly had the symptoms on the evening of the day he worked in the growing room. He was a non-smoker and had no past history of respiratory diseases. The physical examination was normal. C-reactive protein (CRP) was 8.1 mg/dl and white blood cell count (WBC) was 10,700/mm³.

A chest radiograph and computed tomographic scan showed diffuse bilateral ground glass appearance and patchy infiltrates (Figure 1). Pulmonary function tests showed a reduced diffusion capacity to 68.9% of the prediction. Cultures of pathogenic microorganisms including mycobacterium from sputa and bronchoalveolar lavage (BAL) fluid were negative. The BAL was performed at 5 days after the last exposure. Of the 160 ml saline injected, 111 ml of bronchoalveolar fluid was recovered. Total cell yield was $23.8 \times 10^6$ (21.4 $\times 10^4$/ml), and the differential cell count was 32% lymphocytes, 66% pulmonary alveolar macrophages, 1% polymorphonuclear leukocytes and 1% eosinophils with a CD4/CD8 ratio of 1.36. A transbronchial lung biopsy specimen revealed granulomatous alveolitis.

On suspicion of MWL, he was subjected to a natural challenge in the growing room for 2 h. Cough was reproduced 3 h later and the maximum effect with a fever of 39.1°C was observed 10 h later. The shadows on chest radiographs reappeared, and CRP and WBC had also changed from 0 mg/dl to 11.3 mg/dl and 5000/mm³ to 13,400/mm³, respectively.

Case 2

A 65-year-old man, who had started working in the same factory with his son (Case 1) in August 1996. He was
a current smoker but had no symptoms or past history of respiratory diseases. In late September, he developed a productive cough, low grade fever and night sweat, followed one month later by exertional dyspnoea. The symptoms became worse after work and disappeared when he was away from the factory. He had fine crackles at both lung bases.

A chest radiograph and computed tomographic scan showed diffuse bilateral fine nodular shadows partially accompanied by bullae and fibrosis. His diffusion capacity was 71.6% of the prediction and arterial oxygen tension was 79 mmHg.

The BAL was performed the next day of the last exposure. Of 160 ml saline injected, 85 ml was recovered. Total cell yield was $15.3 \times 10^6$ ($18 \times 10^4$/ml), and the differential cell count was 24% lymphocytes, 73% pulmonary alveolar macrophages, 2% polymorphonuclear leukocytes, and 1% eosinophils with a CD4/CD8 ratio of 1:52.

**Environmental study.** A pilot indoor cultivation of *P. ostéatus* was started in 1991, and the new factory was built in August 1996. Every worker takes part in the whole process, that is to place sawdust in plastic bottles, to sterilize the substrate, to inoculate the fungus into bottles, to keep them at 20°C and 90% of relative air humidity for 20 days and 17-18°C and 95% for 4 days in spawning rooms and then grow mushrooms at 16-18°C and 95% for 4 days in a growing room. Workers are exposed to basidiospores for 4 hours per day when they work in the growing room, and when they pick and pack the mushrooms in a shipping room. Most dominant particles of dust on a mask worn in the room were basidiospores of *P. ostéatus*, 3–4 × 7.5–11 μm in size. A few species of bacteria and fungi were cultured from the dust but they were not significant.

**Indirect fluorescent antibody (IFA) test.** The method of Vogel, slightly modified, was performed as reported previously. Basidiospore of *P. ostéatus* or the dust was smeared on a glass slide and fixed by drying, then incubated with serum of the patients, eight co-workers or 10 healthy non-exposed controls at 37°C for 30 min. The slide was stained with fluorescent-labelled antiserum to human IgG, IgA and IgM and examined under an ultraviolet microscope. The antibody titer was determined by the end point of serum dilution for the positive staining. The basidiospores alone reacted to the patients' sera at the titer of 1:512, while it did not react to control sera (<1:8).

**Gel double diffusion test.** The method of Gerber and Jones was performed with a slight modification, using 1% agar in veronal buffer (pH 8.6) containing 1% sodium citrate and 0.1% NaN₃. This is well known as a standard method in the detection of specific antibody activities in EAA. Antigen was extracted from basidiospores of *P. ostéatus* with NaHCO₃-buffered saline by a modified version of Santilli's method. The sera of the patients showed precipitins against the extracted antigen (10 mg of dry weight/ml) but not against *Thermoactinomyces vulgaris*, *Micropolyspora faeni* or *Aspergillus*-related antigens (Hollister-Steir, Spokane, Washington).

**Symptoms and antibodies of co-workers.** We interviewed all eight co-workers in the factory and examined their serum antibodies to *P. ostéatus*. The results are given in Table 1.

We advised patients and co-workers to wear a mechanical filter respirator that filtrates 97% or more particles larger than 0.44 μm in size. All workers continue working in the factory with minimum complaints since using the mask.

**DISCUSSION**

The diagnosis of extrinsic allergic alveolitis (EAA)/hypersensitivity pneumonitis (HP) involves documenting symptoms related to the antigen exposure, and clinical, radiological and pathological findings compatible with EAA. The establishment of antigen exposure through a careful history-taking is a cornerstone of the diagnosis. Serum antibody determination to relevant antigens may serve to confirm the exposure, though it must be evaluated in light of a patient's clinical feature. Provocation testing of the suspected environment or antigen may
confirm the diagnosis. In Japan, criteria for EAA proposed by the research committee on diffuse pulmonary diseases organized by the Japanese Ministry of Welfare are standard. The present cases were definite MWL, a well-known type of EAA, according to the criteria.

Although BAL is not essential for the diagnosis of EAA, it has the advantage of sensitive detection of alveolitis in EAA and distinguishing it from other diseases. For clinicians, a positive BAL finding (i.e., the characteristic profile) in a patient with interstitial lung disease of unknown origin should direct them towards the probable diagnosis of EAA. A careful re-examination of the occupational environmental history and the screening of serum antibody might then reveal previously unknown sources of relevant antigen exposure and confirm the diagnosis. For researchers, information on phenotypes of BAL lymphocytes is necessary to investigate immunological etiology of EAA; this is because the phenotypes of BAL lymphocytes vary with the type of EAA, probably depending on factors such as causative agent, smoking or staging of the diseases. This is, to our best knowledge, the first report of BAL findings in EAA caused by P. ostetatus.

Basidiomycetes consist of approximately 25,000 species and include mushrooms, puffballs, smuts, rusts and bracket fungi. Recent studies have demonstrated that basidiospores are important aeroallergens and P. ostetatus is one of the most reactive species among patients with atopic respiratory diseases in the USA and Europe. In spite of the significance in type I and III/IV allergy, basidiospore antigens for diagnostic or therapeutic use are not available commercially. A few groups have extracted and purified the antigens from P. ostetatus or other mushrooms and detected the specific IgE or IgG antibodies in sera from patients. However, the preparation of spore extracts requires many mushrooms and the procedure is too complicated and time-consuming to follow in clinical laboratories.

The IFA assay is simple, semi-quantitative and applicable to a small amount of particles collected from the environment directly, without preparation of antigen. In previous studies, we demonstrated the high specificity and sensitivity of the assay in 262 patients with summer-type HP, and the practicality in cases of EAA caused by new antigens. In the present study, the IFA titer against basidiospores of P. ostetatus was significantly higher in patients than controls. The higher IFA titer tended to correlate to positive precipitins, but did not reflect the duration of engagement or symptoms.

Mushroom worker’s lung was originally reported 40 years ago. The original and common type of MWL is caused by the inhalation of large numbers of thermophilic actinomycetes from compost used for the cultivation of Agaricus bisporus. The white button mushroom A. bisporus (Lange) Imbach (= A. brunnescens Peck) has been the most popular mushroom cultivated worldwide. Recently, however, alternative mushrooms such as P. ostetatus and Shiitake Lentinus edodes are becoming important crops for a developing market. Consequently, MWL in P. ostetatus or L. edodes farms is emerging and the cause has proved to be basidiospores of the mushroom itself.

The pathogenicity of P. ostetatus and L. edodes should be recognized as distinct from A. bisporus. It is because the former has no veil and releases a tremendous amount of basidiospores during cap growth before attaining a marketable size, while the latter is harvested before the veil is broken and thus minimum spores are released. A cross-sectional study in a farm of A. bisporus revealed that approximately 20% of the more heavily exposed workers reported symptoms consistent with MWL and 10% had impaired pulmonary function. Seven cases (2–3% of total workers) of MWL were diagnosed but no specific antigens were identified as the cause. In the present study, two cases of MWL were diagnosed and 70% of participants were symptomatic. It has also been reported that all workers of a Shiitake farm developed respiratory symptoms consistent with MWL caused by basidiospores of L. edodes. The high incidence of MWL or respiratory symptoms may result from not only the high antigenicity but also the high concentration of the basidiospores in indoor cultivation of these mushrooms.

Mushroom workers should become aware that basidiospores themselves are pathogenic. Instructions for the indoor cultivation of P. ostetatus need to contain sufficient information of health risks and the protective measures. Occupational physicians are responsible

<table>
<thead>
<tr>
<th>Subject</th>
<th>Duration of engagement</th>
<th>Symptoms</th>
<th>Antibodies</th>
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<tbody>
<tr>
<td>1 33 yr, male (case 1)</td>
<td>1 month</td>
<td>Cough, fever, dyspnoea</td>
<td>1:512</td>
</tr>
<tr>
<td>2 65 yr, male (case 2)</td>
<td>3 months</td>
<td>Cough, fever, dyspnoea</td>
<td>1:512</td>
</tr>
<tr>
<td>3 33 yr, female</td>
<td>4 months</td>
<td>Cough</td>
<td>1:512</td>
</tr>
<tr>
<td>4 65 yr, male</td>
<td>5 years</td>
<td>Cough</td>
<td>1:512</td>
</tr>
<tr>
<td>5 67 yr, female</td>
<td>4 months</td>
<td>Cough, sputum</td>
<td>1:128</td>
</tr>
<tr>
<td>6 61 yr, female</td>
<td>4 months</td>
<td>Cough, sputum</td>
<td>1:256</td>
</tr>
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<td>7 51 yr, male</td>
<td>4 months</td>
<td>Cough, sputum</td>
<td>1:128</td>
</tr>
<tr>
<td>8 57 yr, female</td>
<td>5 years</td>
<td>Nil</td>
<td>1:512</td>
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<tr>
<td>9 46 yr, male</td>
<td>4 months</td>
<td>Nil</td>
<td>1:256</td>
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<tr>
<td>10 29 yr, male</td>
<td>4 months</td>
<td>Nil</td>
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</tr>
</tbody>
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IFA = indirect fluorescent antibody.
for advising workers on effective mechanical filter respirators and to monitor workers health via questionnaires, pulmonary function tests and specific antibodies, since an old problem is recurring in a new work environment.

ACKNOWLEDGEMENT

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REFERENCES