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Hypersensitivity Pneumonitis Resulting from *Aspergillus fumigatus* in a Greenhouse

KAZUKO YOSHIDA

The First Department of Internal Medicine
Kumamoto University School of Medicine
Kumamoto, Japan

ATSUSHI UEDA

Department of Environmental Medicine
Kagoshima University
Kagoshima, Japan

HISATO YAMASAKI

KEIZO SATO

The First Department of Internal Medicine
Kumamoto University School of Medicine
Kumamoto, Japan

KATSUHISA UCHIDA

Teikyo University Research Center
for Medical Mycology
Tokyo, Japan

MASAYUKI ANDO

The First Department of Internal Medicine
Kumamoto University School of Medicine
Kumamoto, Japan

ABSTRACT. A 57-y-old female who had cultivated vegetables in a plastic greenhouse developed a case of hypersensitivity pneumonitis from *Aspergillus fumigatus*. This report exemplifies a potential hazard caused by a thermotolerant fungus, *A. fumigatus*, in a poorly constructed greenhouse.

CASE REPORT

A 57-y-old healthy, nonsmoking female had been cultivating vegetables in plastic greenhouses in a subtropical zone in Japan. Such greenhouses were temporary structures, were small (3 m × 40 m × 2.5 m high), and were without ventilation systems (Figure 1). In July 1990, during the melon growing season, she developed cough, dyspnea, and high fever in the evening

after tractor tilling and mixing straw and manure into the greenhouse soil. The symptoms gradually disappeared by the time of harvest.

The next July, when she began preparing the seed bed with straw that had been kept in a closed greenhouse for several weeks, she developed the same symptoms again. When she first consulted on August 2, 1991, she complained of exertional dyspnea. She had no history of asthma. The results of a physical examina-

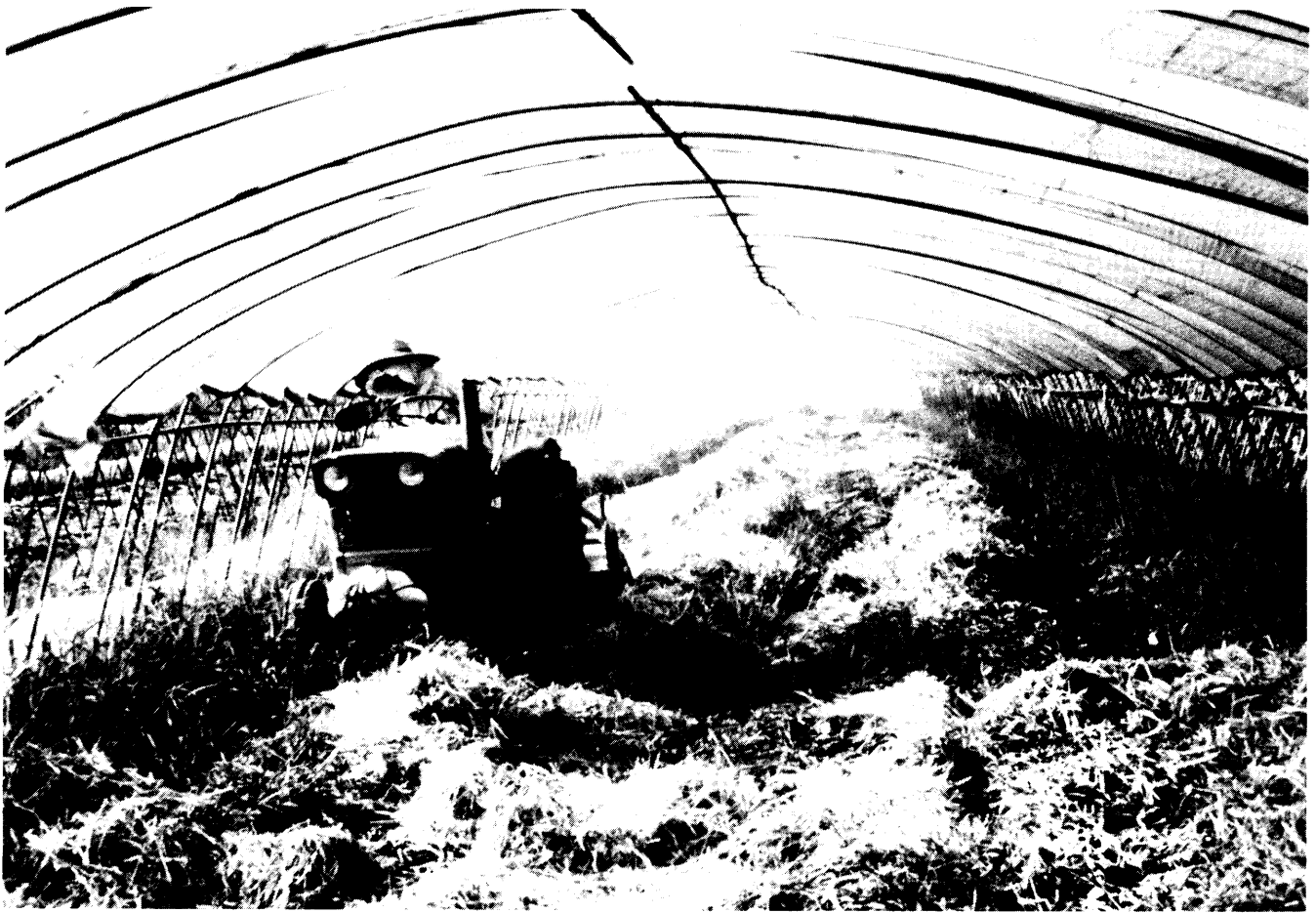


Fig. 1. Tractor tillage in plastic greenhouse in which subject worked.

tion and routine laboratory studies were normal, except for a rise of erythrocyte sedimentation rate and a positive CRP. A chest radiograph showed diffuse bilateral fine-nodular shadows, and high-resolution computed tomographic scan revealed a pattern of interstitial pneumonitis. There was no evidence of cavitory lung disease or aspergilloma. Vital capacity was 1.57 l (66.8%), and forced expiratory volume in 1 s (FEV_{1.0}) was normal. Sputum culture produced a normal flora absent any fungi. A transbronchial lung biopsy revealed lymphocytic interstitial infiltration. The cell populations in the bronchoalveolar lavage fluid (BALF) were 59×10^6 (89.4×10^4 /ml BALF) of the total cell yield, 57.9% lymphocytes, and a CD4/CD8 ratio of 6.71.

Given the suspicion of hypersensitivity pneumonitis (HP), she was subjected to a natural provocation test, i.e., tractor tilling in her greenhouse for 2 h. Approximately 7 h later, headache, cough, dyspnea, sputum, and a fever of 38°C were reproduced, and the laboratory findings worsened.

Several procedures, as described previously,¹⁻³ were completed during the investigation of the causative antigen.

Environmental mycological study. Mycological sampling was taken by open-plate culture and swab culture on Sabouraud and Trypto-Soya agar plates. Fifteen species of microorganisms were isolated from the

working environment. Although we focused on thermoactinomycetes, we did not isolate them. Tilling for 30 min increased the count of colonies on 10-min-exposed Sabouraud plates from 41.3 ± 3.8 colonies/plate (comparable to 36.2 ± 2.6 colonies/plate outside) to 500 colonies/plate. Seventy-five percent of the colonies on open plates were those of *A. fumigatus*, which was the predominant isolate from the straw manure by swab culture.

Indirect fluorescent antibody (IFA) test. This test was performed on all strains of isolates. The patient's serum was reactive to *A. fumigatus* at 1:512 in IFA titer, and the three other species showed at 1:128 or more. These four strains were identified at Teikyo University Research Center for Medical Mycology as *A. fumigatus*, *A. niger*, *Mucor sp.*, and *Bacillus sp.* Further studies—including culture at 50°C, cross-absorption test, and tests described below—were conducted to determine which strain was causative.

Gel double-diffusion test. This was performed on the prepared culture-filtrate antigens from the four isolates and commercially available 10 HP-related antigens and 7 *Aspergillus*-related antigens (Hollister-Steir, Spokane, Washington).

Antigen-induced lymphocyte proliferative response test for peripheral blood cells. The results of this test are expressed as a net stimulation (NS), which is equal

to the geometric mean counts per minutes of cultures with antigen minus the geometric mean counts per minutes of unstimulated control cultures. The results of this test can also be expressed as a stimulation index (SI), which is the geometric mean counts per minute of cultures with antigen divided by the geometric mean counts per minute of unstimulated control cultures. Results are arbitrarily considered positive if SI is greater than 3.0 and if NS is greater than 1 000. Lymphocytes of a healthy control were also used to rule out the nonspecific responses.

A. fumigatus grew well at 50°C. Its antibodies were absorbed only partially by the other isolates. The precipitins were positive only to the isolated *A. fumigatus* antigen. Also, the antigen induced cell proliferation in a dose-dependent manner, and the values of NS and SI were 5 648 and 71.5, respectively, at 20 µg/ml in concentration. The other three antigens, however, did not react at the same concentration.

We diagnosed this case as HP resulting from *A. fumigatus* in straw manure in the greenhouse, and the patients was directed to eliminate the antigen. The straw and the old vinyl plastic were removed, the field was left exposed throughout the winter and was tilled prior to recovering with new vinyl plastic, ventilation was improved, and an artificial fertilizer was used. She was able to harvest melons the following summer without experiencing any symptoms.

Discussion

According to the 1989 report of the Ministry of Agriculture, Forestry, and Fisheries, total floor space of greenhouses in Japan had increased from 5×10^7 m² in 1965 to 5×10^8 m² in 1989. Greenhouses are used for vegetable cultivation throughout the year in all parts of the country, but most are as temporary, small, and poorly equipped as that of this case. For example, 95% are made of vinyl plastic and 85% are without a ventilation system. The inside temperature exceeds 50°C in summer, accompanied by a humidity over 70%.⁴ Even worse, tractors used for soil tillage create severe dust conditions, and pesticides are also used in such a closed space. Recently, the so-called "vinyl greenhouse syndrome" that affects farmers who work in these hazardous conditions has become a focus of medical attention.⁴

To our knowledge, this is the first reported case of HP caused by *A. fumigatus* in a greenhouse. The reported cases of HP due to *A. fumigatus* are few, but the medical importance of *A. fumigatus* as an antigen that

causes several forms of pulmonary hypersensitivity reactions is well investigated.⁵⁻¹⁰ Although *A. fumigatus* is fairly ubiquitous, its association with compost heaps and stacks of hay or baled straw—in which high levels of moisture are contained and in which self-heating occurs—has long been recognized.^{10,11} Whereas it is a thermotolerant fungus that can survive at temperatures as high as 53°C and will grow successfully because of lack of competition by other organisms, a poorly constructed and controlled greenhouse can be a beneficial incubator for *A. fumigatus*. This case exemplifies a potential hazard caused by a thermotolerant fungus, and it should compel much more consideration of the plastic greenhouse environment.

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Submitted for publication July 22, 1992; revised; accepted for publication October 2, 1992.

Requests for reprints should be sent to Kazuko Yoshida, M.D., The First Department of Internal Medicine, Kumamoto University School of Medicine, 1-1-1 Honjo, Kumamoto 860, Japan.

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References

1. Yoshida K, Ando M, Sakata T, Araki S. Environmental mycological studies on the causative agent of summer-type hypersensitivity pneumonitis. *J Allergy Clin Immunol* 1988; 81:475-83.
2. Yoshida K, Ando M, Sakata T, Araki S. Prevention of summer-type hypersensitivity pneumonitis: effect of elimination of *Trichosporon cutaneum* from the patients' homes. *Arch Environ Health* 1989; 44:317-22.
3. Ando M, Sakata T, Yoshida K, et al. Serotype-related antigen of *Trichosporon cutaneum* in the induction of summer-type hypersensitivity pneumonitis: correlation between serotype of inhalation challenge-positive antigen and that of the isolates from patient's homes. *J Allergy Clin Immunol* 1990; 85:36-44.
4. Usutani S, Kimura H, Nishidono Y, Mori K. Labor and health of the farmers growing vegetables in vinyl greenhouses. *JJRM* 1970 19:50-85.
5. Yocum MW, Saltzman AR, Strong DM, et al. Extrinsic allergic alveolitis after *Aspergillus fumigatus* inhalation. Evidence of a type IV immunologic pathogenesis. *Am J Med* 1976; 61:939-45.
6. Anish SA. Acute allergic alveolitis. *J Med Soc NJ* 1979; 76:31-33.
7. Vincken W, Roles P. Hypersensitivity pneumonitis due to *Aspergillus fumigatus* in compost. *Thorax* 1984; 39:74-75.
8. Kryda MMJ, Emanuel MA, Marx JJ Jr, Treuhaft MW. Hypersensitivity pneumonitis due to *Aspergillus fumigatus* in papermill workers. In: Dosman JA and Cockcroft DM, Eds. *Principals of health and safety in agriculture*. Florida: CRC Press, Inc., 1989; p. 80.
9. Bardana EJ Jr. The clinical spectrum of aspergillosis. *CRC Crit Rev Clin Lab Sci* 1980; 13:21-159.
10. Kramer MN, Kurup VP, and Fink JN. Allergic bronchopulmonary aspergillosis from a contaminated dump site. *Am Rev Respir Dis* 1989; 140:1086-88.
11. Mullins J, Harvey R, Seaton A. Sources and incidence of airborne *Aspergillus fumigatus* (Fres). *Clin Allergy* 1976; 6:209-17.

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