Allergic Bronchopulmonary Mycosis Caused by the Basidiomycetous Fungus *Schizophyllum commune*

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We describe, to our knowledge, the first case of allergic bronchopulmonary mycosis (ABPM) caused by the basidiomycetous fungus *Schizophyllum commune* in an otherwise healthy woman. Bronchoscopic analysis repeatedly disclosed *S. commune* hyphae in the bronchi of the lingular lobe; these hyphae were originally misidentified as *Aspergillus* because the presence of clamp connections was overlooked. A lingular infiltrate with ectatic proximal bronchi, eosinophilia, an elevated serum level of IgE, and antibodies to *S. commune* supported the diagnosis. It is sometimes difficult to isolate and identify *S. commune* in clinical specimens, and hence only a limited number of cases of ABPM might have been correctly diagnosed in the past. We suspect, therefore, that some cases of ABPM caused by an allergic reaction to *S. commune* may be misdiagnosed as allergic bronchopulmonary aspergillosis or eosinophilic pneumonia of unknown origin. The significance of *S. commune* in allergic bronchopulmonary diseases is discussed.

Since Hinson and co-workers [1] proposed *Aspergillus fumigatus* as the cause of allergic bronchopulmonary aspergillosis (ABPA), many other fungi have also been reported as causative agents of allergic bronchopulmonary mycosis (ABPM); these fungi include *Candida albicans* [2, 3], *Torulopsis glabrata* [4], *Aspergillus ochraceus* [5], *Penicillium* [6], and dematiaceous fungi such as *Bipolaris* [7, 8], *Curvularium* [8, 9], and *Stemphylium* [10]. Yet, to our knowledge, no cases of ABPM caused by basidiomycetous fungi have been reported. *Schizophyllum commune*, a basidiomycetous fungus, has been regarded to be nonpathogenic, and studies of its clinical significance have been very limited. Herein, we report a case of ABPM caused by a *S. commune* agaric, which was at first misidentified as *Aspergillus* because of its similar microscopic appearance. This case illustrates the clinical significance of *S. commune*, especially when an allergic bronchopulmonary disease caused by fungi is suspected but no fungi known to be causative of this disease are identified.

Case Report

A 57-year-old Japanese housewife was referred to Chiba University Hospital (Chiba, Japan) in May 1989 for investigation of an abnormal shadow demonstrated by chest roentgenography during a routine check-up. She had been complaining of a recurrent common cold, and in 1988 she began to have a mild productive cough. Chest roentgenography in 1983 disclosed no abnormalities. She had never smoked, and her medical history was unremarkable except for a resected ovarian cyst and for sinusitis that was medically treated. She had no history of bronchial asthma or other allergic diseases. Her social history was also unremarkable.

On admission a physical examination did not reveal any abnormalities; she was in good general condition and complained of a mild productive cough. Laboratory examinations showed a white blood cell count of 4,100/mm^3, with 12% eosinophils. Results of blood chemistry analysis were normal, and the C-reactive protein level and erythrocyte sedimentation rate were unremarkable. A radioimmunosorbent test revealed an IgE concentration of 4,286 U/mL, and a radioallergosorbent test showed antibodies to *A. fumigatus* but no antibodies to *C. albicans*. Precipitins to *A. fumigatus* were undetectable by the Ouchterlony test. Skin prick tests revealed negative reactions to *A. fumigatus* and *C. albicans*. A tuberculin (purified protein derivative, single strength) skin test was negative. Serological tests for immunosuppressive viruses, such as human T cell lymphotrophic virus type I and human immunodeficiency virus, were negative. Results of urinalysis were normal. Immunological studies, including IgG, IgA, and IgM levels and the lymphocyte CD4/CD8 ratio, were all unremarkable. Tests of pulmonary function revealed that the vital capacity (101%) and the forced expiratory volume in 1 second (89%) were normal. Repeated analysis of the sputum, which disclosed eosinophils and Charcot-Leyden crystals, was negative for malignant cells, fungi, acid-fast organisms, and other pathogenic organisms. Chest roentgenography and computed tomography disclosed infiltrates and nodules in the right upper and left lingular lobes (figure 1).

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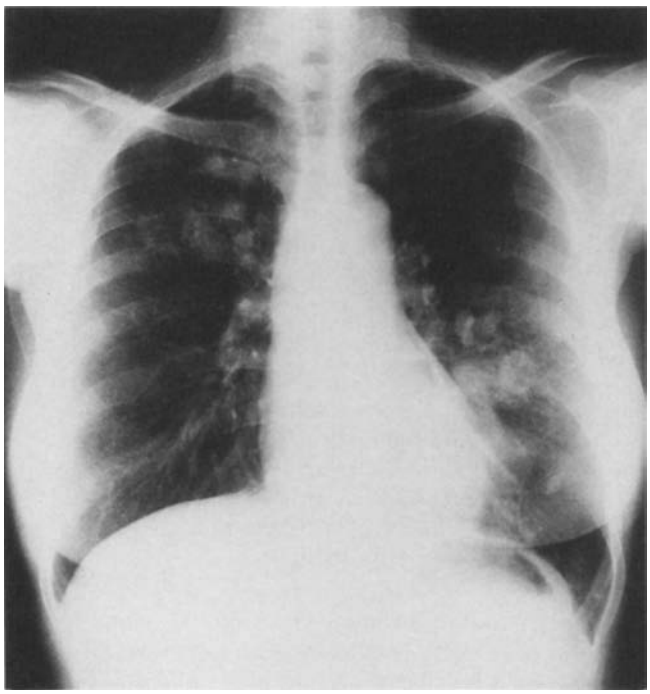

Figure 1. Chest roentgenogram on admission disclosing an infiltrate and nodular shadows in the right upper lobe and left lingular lobe.

At the first bronchoscopy, no endobronchial abnormalities were found, but analysis of specimens taken directly from the left B^4 and B^5 by transbronchial aspiration and by endobronchial brushing disclosed massive clumps of mycelia with some necrotic tissue. Culture of these specimens showed no growth of fungi. The mycelia were identified morphologically as *Aspergillus*. The patient was discharged with the diagnosis of pulmonary aspergillosis. Therapy with itraconazole (50 mg/d for 2 months and 100 mg/d for 8 months) was initiated, and no significant symptomatic abatement or roentgenographic improvement was noted.

In July 1990 she was admitted to the hospital again because of increased cough and abnormal shadows demonstrated by chest roentgenography. Bronchoscopic examination was performed for the second time, and growth of *S. commune* was seen (as described below) in both bronchoalveolar lavage fluid and lingular brushing specimens. We rechecked the specimens from the first bronchoscopic examination and confirmed that the mycelia, which were first identified as *Aspergillus*, were *S. commune*. Bronchography disclosed a proximal cylindrical bronchiectatic change mainly in the bronchi of the lingular lobe (figure 2). Serum antibody to *S. commune* at a titer of 1:320 was detected with use of ELISA (which is described below under Serological Study). The diagnosis of ABPM caused by *S. commune* was subsequently made.

In spite of the fact that her social history was carefully rechecked, no clue was found regarding the route of infection (e.g., a history of growing *S. commune* mushrooms). Because her symptoms abated spontaneously and her general condition was very good, a surgical procedure was not performed, and antifungal chemotherapy or steroid therapy was not initiated. Repeated spontaneous regression and mild deterioration of her symptoms (i.e., productive cough, an infiltrate, and nodules demonstrated by chest roentgenography) have occurred, both of which correlated well with the decrease and increase in serum IgE concentrations (between 3,434 and 5,076 U/mL).

Mycological Study

The bronchoalveolar lavage fluid and specimens of bronchial brushings recovered from the lingular lobe bronchi were centrifuged, and the pellets were cultured on plates of modified potato dextrose agar (Difco Laboratories, Detroit), i.e., 50% potato dextrose agar plus added agar for a final concentration of 1.5% agar. The plates were incubated at 25°C in the dark. Although several mycelial colonies that were white, felt-like, and somewhat fluffy at periphery appeared after 10 days of culture, no spores were observed. The plates were then exposed to regular cycles of daylight and darkness at room temperature (about 20°C). After 7 days, tiny masses that later turned out to be initial basidiocarps were noticed at the peripheral zone of the colonies; these

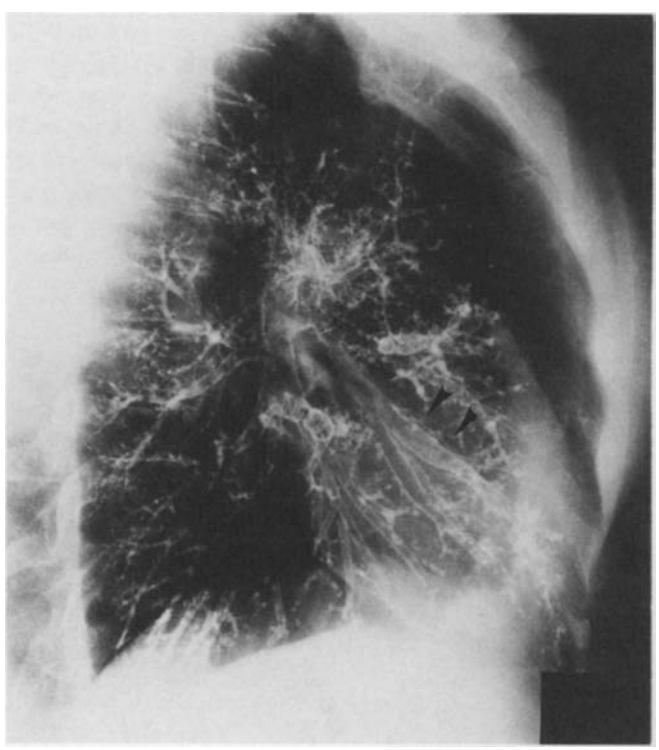

Figure 2. Bronchogram of the left bronchi. Proximal cylindrical ectatic changes are seen in the bronchi of the lingular lobe (*arrows*).

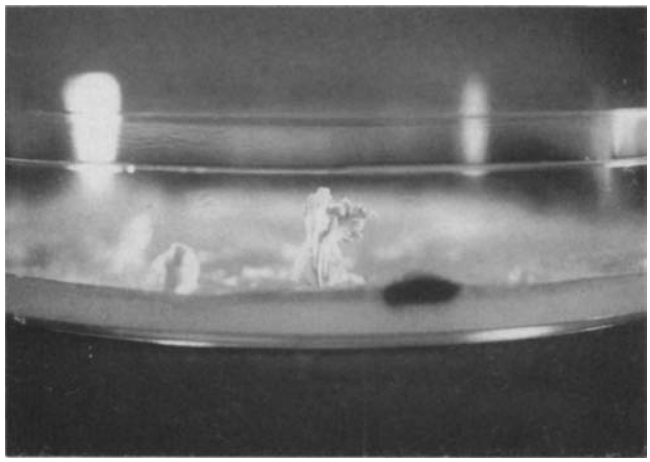

Figure 3. Basidiocarp of *S. commune* cultured from a specimen taken during the second bronchoscopy.

masses consisted of hyphae with clamps and spicules. After another 7 days, they produced fruiting bodies with a very short stipe with pilei that were yellow–grayish white, fan-shaped, split-gilled, and several millimeters in diameter (figure 3). Under microscopy, club-shaped cells were arranged closely in parallel in the hymenium, and some of the cells had four projections on the apical surface, which showed that they were basidia.

Subcultures from the mycelial growth phase yielded colonies with similar basidiocarps under the same culture conditions. Microscopic analysis of the mycelia disclosed hyphae with clamp connections and a series of spicules (figure 4). The fungus was identified as *S. commune*. Because of this identification, we rechecked the specimen obtained during the first bronchoscopic examination; this specimen had been stained with Papanicolaou's stain and preserved. Examination of this smear revealed hyphae that were branched non-dichotomously and had clamp-like structures at septal regions (figure 5), characteristics that are different from those of *Aspergillus* species. On the basis of these findings, we judged the hyphae as those of *S. commune*, which had been first regarded as *Aspergillus*. The living strain of *S. commune* was labeled as IFM 41942 and stored at the Research Center for Pathogenic Fungi and Microbial Toxicoses, Chiba University.

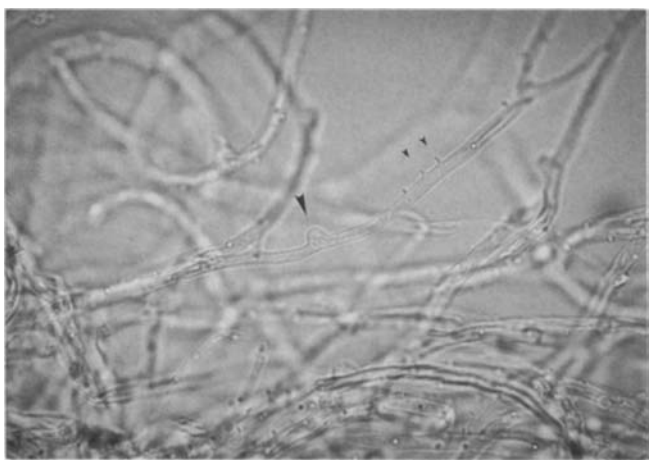

Figure 4. Spicular formations (*small arrows*) and clamp connections (*large arrow*) characteristic of *S. commune* seen on hyphae of a living strain (labeled as IFM 41942).

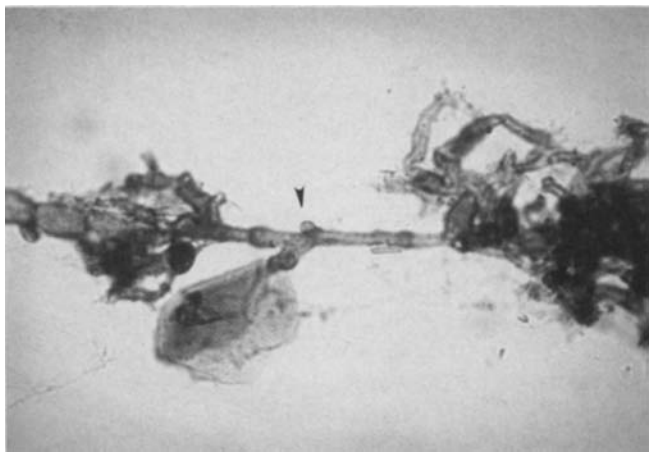

Figure 5. Clear demonstration of clamp connections characteristic of *S. commune* (*arrow*) in the specimen taken during the first bronchoscopy (Papanicolaou's stain; original magnification, × 400).

Serological Study

Preparation of S. commune antigen. Two kinds of antigen of *S. commune*, i.e., cytosol antigen and extracellular culture filtrate antigen, were prepared. For the extraction of cytosol, *S. commune* was cultured in yeast nitrogen base (Difco Laboratories) with 1% glucose and 0.1% asparagine for 28 days at 37°C in an incubator with 5% CO_2 and 95% air. After washing in PBS (Gibco Laboratories, Grand Island, NY) and then in buffer A (0.01 *M* Tris–HCl buffer, pH 7.4, containing 1 m*M* phenylmethylsulfonyl fluoride), the pellet was disrupted by vortex mixing with glass beads (0.45 mm in diameter; B. Braun, Glasperlen, Germany). The disrupted pellet was centrifuged at 500*g* for 10 minutes, and the supernatant was collected and used as cytosol antigen. For the extracellular culture filtrate antigen, *S. commune* was cultured in yeast nitrogen base for 90 days at room temperature (about 20°C), and the supernatant was sterilized with vacuum filtration (0.45-μm filter; Disposable Sterile Filter System, Corning, Corning, NY). The protein concentrations of the cytosol and extracellular culture filtrate antigens were determined with use of the method of Lowry et al. [11].

ELISA. Antibody to *S. commune* in the patient's serum

was determined with an ELISA modified from previously described methods [12, 13]. In brief, 96-well polystyrene plates (Immulon Microwells, Danatech Laboratories, Alexandria, VA) were coated with 0.2 mL of *S. commune* antigen (1,000 μg of protein/mL for the extracellular culture filtrate antigen and 700 μg of protein/mL for the cytosol antigen) per well, which was diluted with carbonate buffer (pH 9.6) and incubated at 4°C overnight. The optimal dilution of the antigen was determined by block titration. After washing with PBS–Tween 20 (0.05%) and blocking with 4% bovine serum albumin at room temperature (about 20°C) for 1 hour, 200 μL of the patient's serum was serially diluted across the plate, and the plates were incubated at 37°C for 30 minutes. After washing, horseradish peroxidase–conjugated goat antibody to human immunoglobulins (IgG, IgA, and IgM) (Cappel Laboratories, Cochranville, PA) was added to each well, and the plates were incubated at 37°C for 1 hour. After another washing, the substrate solution containing 0.2 m*M* 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid and 2 m*M* hydrogen peroxide in 0.05 *M* citrate buffer (pH 4.0) was added. The plates were incubated for 30 minutes at room temperature (about 20°C). The enzymatic reaction was stopped with 50 μL of 5 *M* hydrofluoric acid (pH 3.3) per well, and the optical densities of each well were read at 410 nm using an Ortho Microwell Reader (Ortho Diagnostic Systems, Raritan, NJ). Sera from healthy volunteers were used as negative controls. Results showed that the patient's sera, which were obtained at two separate times, were positive for both cytosol and extracellular culture filtrate antigens at a titer of 1:320 and 1:80, respectively.

Discussion

Our patient was otherwise healthy and had no signs of immunosuppression or systemic fungal infection. An elevated serum level of IgE, eosinophilia, and spontaneous symptomatic and radiographic fluctuations, which correlated well with decreases and increases in serum levels of IgE, strongly suggested that our patient had an allergic disease. Moreover, proximal bronchiectasis (one of the important features of ABPA), repeated detection of *S. commune* in the bronchi, and the presence of antibodies to *S. commune* in the serum indicated the diagnosis of ABPM caused by *S. commune*. The absence of asthmatic episodes is compatible with ABPA or ABPM [14].

Cases of ABPM caused by fungi other than *Aspergillus* and *Candida* have been rarely reported, and most of these cases have been due to dematiaceous fungi, e.g., *Bipolaris* [7, 8], *Curvularium* [8, 9], and *Stemphylium* [10]. Cases of ABPM due to *Helminthosporium* [15, 16] have also been reported, but no precise mycological data were documented. Although basidiomycetous fungi are known to cause bronchial asthma [17–20], to our knowledge no cases of ABPM due to these fungi, including *S. commune*, have been reported, and this is apparently the first report of ABPM caused by a basidiomycetous fungus.

Causative agents of human basidiomycosis are mostly limited to basidiomycetous yeasts such as *Cryptococcus neoformans* and *Trichosporon*. The basidiomycetous fungi that produce macroscopic basidiocarps, i.e., hymenomycetes, cause human infectious diseases in extremely limited cases. For instance, mycoses due to *Coprinus* [21, 22] and *S. commune* [23–29] have been reported, although some criticisms have been made [30] about the reports by Kligman [24] and Bastina et al. [25]. The sites of infection with *S. commune* were the nails [24], the oral cavity of a dehydrated child [26], the meninges [25], and the maxillary sinuses of healthy [23, 27, 28] and diabetic patients [28] and a patient with AIDS [29]. The only report related to the lower airways in which *S. commune* was repeatedly detected in sputa was made by Ciferri et al. [31]. No samples were taken directly from the lung, and, therefore, neither microbiological nor histopathologic study of the lower respiratory tract was done. Thus, the clinical significance of *S. commune* isolated in this case was undetermined [30].

The antifungal activities of itraconazole against *S. commune* are not known. The MIC of amphotericin B for *S. commune* was shown to be low (<0.025 μg/mL) in one case [29]. The administration of amphotericin B was reported to be effective [26, 29]. In our case, steroid treatment could have been tried if the patient's condition had deteriorated. Concomitant administration of amphotericin B, intravenously or by aerosol inhalation, could have also been considered.

S. commune is ubiquitous in the environment, and hence its presence in medical specimens may be considered as simple contamination [24]. *S. commune* is somewhat morphologically similar to mycelium of *Aspergillus* and some other fungi microscopically, and this fact may lead to misidentification as *Aspergillus*. The characteristic basidiocarps of *S. commune* do not grow in the dark, which is a common environment in incubators of clinical microbiology laboratories, and this circumstance may cause falsely negative growth. *S. commune* has been regarded as nonpathogenic, and its clinical significance has been ignored even when it is isolated. Moreover, its identification is feasible, like other basidiomycetous fungi, only when it is in dikaryotic hyphal forms with basidiocarps. Many cases of basidiomycosis, therefore, may still be undiagnosed.

As *S. commune* was misidentified in our case, we suspect that some cases of ABPM due to *S. commune* could have been misdiagnosed as ABPA or ABPM caused by other fungi; this misdiagnosis is particularly likely to occur when routine examination has failed to demonstrate either a significant organism or antibodies to causative agents like *Aspergillus*. *S. commune* may also cause other allergic diseases such as bronchial asthma or eosinophilic pneumonia, in which case no causative agents are determined. Actually, our case was a

good example of false aspergillosis. Use of new technologies, e.g., serological diagnosis, DNA hybridization with polymerase chain reaction, or chromatographic analysis of genomic traits, may disclose a higher incidence of *S. commune*–related diseases. Therefore, we suggest that more attention should be paid to *S. commune* and other basidiomycetous fungi in the diagnosis of allergy-related lung diseases.

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