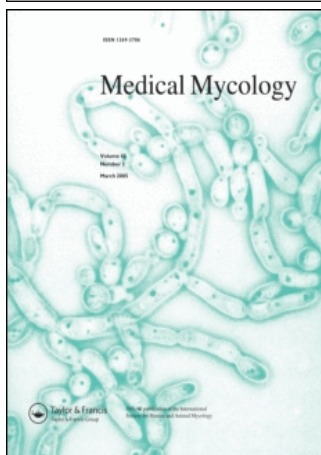


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Case Reports

Allergic fungal sinusitis caused by *Bipolaris spicifera* and *Schizophyllum commune*

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There have been very few reports in Japan of patients with allergic fungal sinusitis (AFS). We describe two cases caused by *Bipolaris spicifera* and *Schizophyllum commune*. The patients were a 70-year-old male (Case 1) and a 55-year-old female (Case 2). Both presented with nasal obstruction and purulent nasal discharge. CT scans revealed each to have a soft tissue mass extending from the ethmoid sinus to the sphenoid sinus. In addition, pathological studies on the contents of the paranasal sinuses of both patients revealed the presence of fungal elements in the allergic mucin. Microbiological studies resulted in the recovery of *Bipolaris spicifera* from Case 1 and *Schizophyllum commune* from Case 2. To date there have been no reports of AFS due to these two fungi in Japan. It is very important in the diagnosis of AFS to demonstrate the presence of fungal elements in the allergic mucin. Squash cytology of the paranasal sinus contents was especially useful for proving the presence of fungi.

Keywords allergic fungal sinusitis (AFS), *Bipolaris spicifera*, *Schizophyllum commune*, allergic mucin, cytology

Introduction

Allergic fungal sinusitis (AFS) was first reported by Millar *et al.* in 1981 [1], named by Katzenstein *et al.* in 1983 [2] and has subsequently been described throughout the world. Katzenstein *et al.* proposed the following three diagnostic criteria for AFS based on inspection of the mucinous material from the sinus; (1) eosinophilia, (2) the presence of Charcot-Leyden crystals and (3) the occurrence of *Aspergillus* hyphae. However, subsequent reports demonstrated that AFS could be caused by fungi other than aspergilli and this

in turn contributed to the wide adoption of the diagnostic criteria proposed by Bent *et al.* in 1994 [3]. Their five criteria are based on the premise that AFS is a disease that is similar to allergic bronchopulmonary aspergillosis (ABPA) and consists of: (1) CT findings of chronic paranasal sinusitis, (2) the presence of nasal polyps, (3) the occurrence of allergic mucin, in the absence of fungal invasion of the tissues, (4) demonstration of fungi in the paranasal sinus contents and (5) the presence of type I allergy on the basis of the past history, results of skin tests or serological studies.

There have been very few reports of AFS in Japan and our extensive search of the published literature indicates that only 11 cases have been described to date, including the two cases presented in this report [4–11]. However, since AFS has been reported to account for 5–10% of chronic paranasal sinusitis cases in Europe and the United States [12], it can be anticipated that

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additional cases of AFS will be reported in Japan as a result of increasing awareness of this disease. We described here two Japanese cases of AFS caused by *Bipolaris spicifera*, a black fungus, and *Schizophyllum commune*, a basidiomycetous fungus.

Case reports

Case 1

Beginning in February 2002, a 70-year-old Japanese male with a history of colon cancer (surgery and chemotherapy) and borderline diabetes developed bilateral nasal obstruction and purulent nasal discharge. Double vision developed in early July of that year causing the patient to visit the ENT department in our hospital. A CT scan showed a soft tissue mass extending from the right frontal sinus and the bilateral ethmoid sinuses to the sphenoid sinus. A bone defect was seen in the sphenoid sinus posterior wall and the clivus had been destroyed. Since a tumor or abscess was suspected, the patient was hospitalized for detailed examinations and therapy. Laboratory findings showed an eosinophil count of 1500/ μ l, a serum total IgE titer of 33 IU/ml (standard value: $\leq$ 250 IU/ml), a specific IgE titer for ticks of 0.84 IU/ml (standard value: $\leq$ 0.34 IU/ml), and cultures were negative for *Aspergillus*, *Candida* and *Penicillium*. Surgery was performed in September 2002 with the finding that the ethmoid and sphenoid sinus mucosae were edematous, with copious retention of green-brown

mucus in the sinuses. Intraoperative rapid pathological specimens (frozen sections) revealed clumps of eosinophils and fragments of filamentous fungal-like elements. Because AFS was suspected, the surgical procedure involved the opening of the ethmoid and sphenoid sinuses. Squash cytology specimens of the paranasal sinus contents were stained with Papanicolaou stain. Microscopic observation revealed that the mucus contained numerous aggregations of eosinophils, which showed degenerative changes of the nuclei and cytoplasm, as well as partial degranulation and Charcot-Leyden crystals. The mucus also contained elongated fungal-like hyphae, about 5 μ m in width and primrose in color. Some of the hyphae were observed to have branches and the branching angle ranged from 30° to 90°, while other filaments showed irregular swelling. Observation of Grocott-stained specimens revealed septation at irregular intervals, and measurement at the sites of septation showed the hyphae to have a width of about 2–7 μ m, with a mean of 3.3 μ m. In addition, markedly swollen hyphae up to a width of 15 μ m were observed at some non-septated sites (Fig. 1). Histopathological specimens consisted of sphenoid and ethmoid sinus mucosae, and the semi-solid contents of the paranasal sinuses. For each of the mucosal specimens, the epithelial basement membrane was slightly thickened, and the stroma showed inflammatory cell infiltration including numerous eosinophils and small numbers of lymphocytes, plasma cells, etc. Hematoxylin and eosin staining of the paranasal sinus

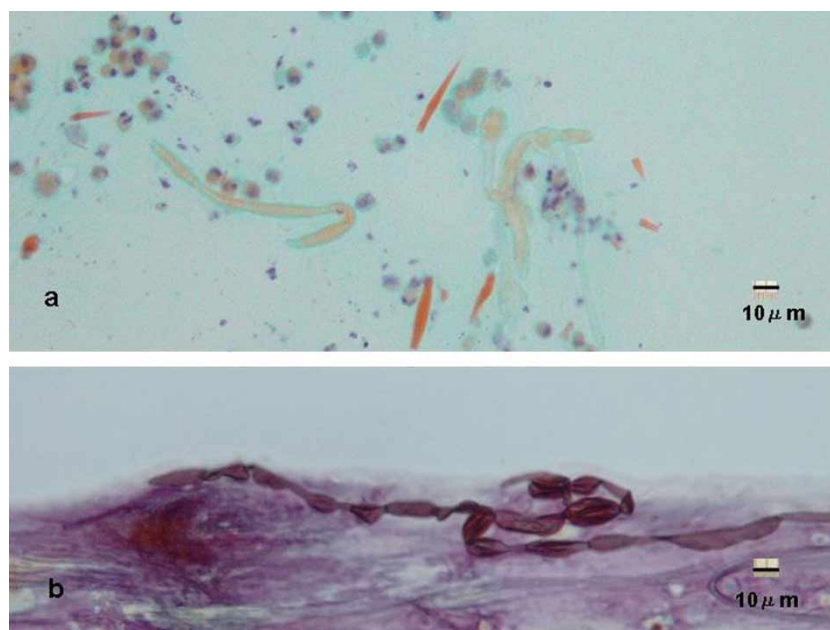


Fig. 1 (a) Squash cytology of the contents of the paranasal sinuses of Patient 1 showed numerous aggregates of degenerative eosinophils, numerous Charcot-Leyden crystals and scattered fungal elements with hyphal extension (Papanicolaou stain, $\times$ 400). (b) Fungal hyphae showing irregular swelling (Grocott stain, $\times$ 400) (Please see colour online).

contents revealed thick mucus that contained clumps of degenerated eosinophils and Charcot-Leyden crystals which are consistent with the finding of so-called allergic mucin. Eosin-stained sinus contents confirmed the presence of a small number of hyphal-like structures. Microscopic examination of Grocott-stained specimens revealed that the mucin contained fragmented fungal filaments, some of which appeared swollen and degenerated. However, none of the biopsied sites showed invasion of the mucosal tissue by fungi.

Mycological studies: The contents of the paranasal sinuses were cultured on Sheep Blood agar (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) at 35°C. After 4 days, a number of small black dots, less than 1 mm in diameter, were observed on the agar surface and by the 6th day restricted filamentous growth was detected. Therefore, a slide culture was prepared with Czapek-Dox agar (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) and microscopic observation revealed a filamentous fungus forming poroconidia that consisted of only thin transsepta. This fungus was identified as *Bipolaris spicifera*, a black fungus, by the Research Center for Pathogenic Fungi and Microbial Toxicoses, Chiba University, Japan.

Case 2

A 55-year-old Japanese female had a history of allergic rhinitis. Beginning in October 2003, the patient complained of left nasal obstruction and an increase in purulent nasal discharge which caused her to visit a family doctor. Plain X-ray films showed a diffuse mass

in the left paranasal sinus. Antimicrobial therapy reduced the purulent discharge, but the nasal mucosa swelling remained. Thereafter, low-dose antimicrobial therapy was administered for 3 months, but there was no striking change in the patient's condition. A CT scan performed in January 2004 showed the residual presence of a diffuse mass stretching from the left maxillary sinus to the ethmoid and sphenoid sinuses. The patient was referred to our hospital for detailed examinations and treatment. Laboratory findings showed an eosinophil count of 443/ μ l, a serum total IgE titer of 302 IU/ml, a specific IgE titer for ticks of 15.20 IU/ml, and negative for *Aspergillus*, *Candida* and *Penicillium*. Surgery was performed in March 2004, which revealed that the ethmoid and sphenoid sinus mucosae to be edematous, with retention of a brown, highly viscous, peanut-butter-like substance in the sinuses. The findings for intraoperative rapid pathological specimens (frozen sections) were the same as for Case 1, and because AFS was suspected, surgery, squash cytology and microscopic observation were performed as for Case 1. The microscopic findings were the same as for Case 1 and characteristic of so-called allergic mucin. The mucus also contained elongated fungal hyphae, primrose in color that were disseminated and did not form clumps. In Grocott-stained specimens, those hyphae were observed to have a nearly uniform width of 2–3 μ m, with a mean width of 2.1 μ m at sites of septation, and some of the hyphae showed bifurcation. In addition, short branches were frequently observed at sites of septation and extending from some hyphae (Fig. 2). The histo-

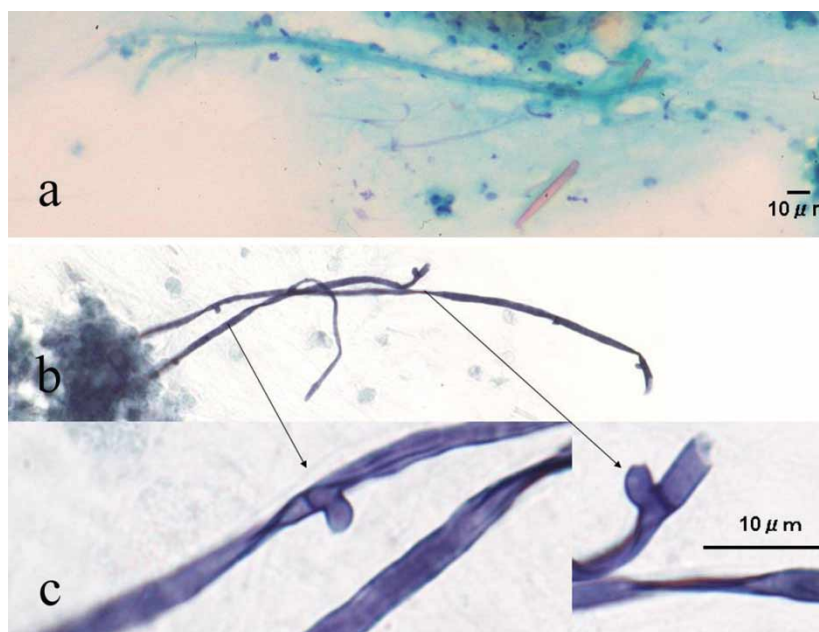


Fig. 2 (a and b) Squash cytology of the paranasal sinus contents of Patient 2 contained numerous aggregates of eosinophils showing degeneration, Charcot-Leyden crystals and elongated fungal hyphae (a) Papanicolaou stain, $\times 400$, (b) Grocott stain, $\times 400$). (c) Short branches were frequently observed at sites of septation and extending from hyphae, but clamp connections were not detected (Grocott stain $\times 1000$) (Please see colour online).

pathological specimens consisted of mucosa taken from the left middle nasal concha and the highly viscous contents of the paranasal sinuses. The mucosal tissue was edematous, and the stroma showed moderate chronic inflammatory cell infiltration, with numerous eosinophils mixed in. The surface layer was covered with ciliated cylindrical epithelial cells. In addition, no fungal elements were observed inside the mucosa. Observation of the paranasal sinus contents revealed mucus that contained various-sized clumps made up of numerous eosinophils. The mucus also contained numerous Charcot-Leyden crystals together with fragments of filamentous fungi having degenerating septa.

Mycological studies: The contents of the paranasal sinuses were cultured on Sheep Blood agar (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) at 35°C. After 2 days, a number of fungal colonies showing hypha-like growth were observed. Slide culture of the organism on Czapek-Dox agar (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) revealed white, felt-like colonies but identification was not possible due to the lack of conidial formation. Since the isolate was suspected to be *S. commune* based on its odor and spicular formation on the hyphal wall, it was mated at the Research Center for Pathogenic Fungi and Microbial Toxicoses, Chiba University, Japan, against 9 monokaryotic and 5 dikaryotic strains of *S. commune* on potato dextrose agar plates. All cultures were incubated first in total darkness for 7 days at 25°C and then under fluorescent and natural light at ambient temperature for 52 days. The fungus was dikaryotized by all tester strains and produced clamped hyphae. Small basidiokarps were observed in three pairs, i.e., with one monokaryotic and two dikaryotic strains (Fig. 3). Based on these microscopic findings and mating behaviors, this fungus was identified as a primary (monokaryotic) mycelium of *S. commune*.

Discussion

AFS is a refractory form of chronic sinusitis that was first reported as allergic aspergillosis by Millar *et al.* in 1981 [1]. Later, there were reports of similar diseases caused by fungi other than aspergilli, and it has now been elucidated that AFS due to aspergilli does not account for more than 15% of all AFS cases [13]. For this reason this disease is now generally referred to as allergic fungal sinusitis.

AFS was first discussed in the Japanese literature by Ogawa *et al.* in 1995 [4], and in 1997 Kurosaki *et al.* described the first Japanese AFS case [5].



Fig. 3 A basidiocarp formed between the fungus obtained from Case 2 and a dikaryotic strain of *S. commune* isolated from a patient with mucoid impaction (Please see colour online).

Reported cases of AFS in Japan remain very small in number even today, and our extensive search of the published literature indicates that only 11 have been described to date. One of the main reasons for this has been an inadequate level of awareness of this disease entity on the part of the medical profession in Japan. Accordingly, when treating this disease by surgical elimination of the allergic mucin or washing out the paranasal sinuses, mucosal tissues are collected and subjected to histopathological studies. However, not enough study has been devoted to the paranasal sinus contents, which carry important information for diagnosis of AFS. In addition, although various fungal genera have been implicated as causative fungi of AFS, i.e., *Bipolaris*, *Curvularia*, *Drechslera*, *Aspergillus*, *Exserohilum*, *Alternaria*, etc. [14], diagnosis has been hampered in Japan because for many of the fungi it is difficult to obtain antigens that can be used in skin tests and serologic diagnosis.

B. spicifera was first reported as a cause of AFS by Gourley *et al.* in 1989 [15], and since then many cases have been described in Europe and the United States. This fungus is considered the most common causative organism of AFS. On the other hand, there have been reports of more than 10 patients from whom *S. commune* was recovered, in most instances from sinusitis cases. However, our search of the literature found only one case that was diagnosed as AFS, reported by Clark *et al.* [16]. In Japan, Kamei *et al.* were the first to report in 1993 a patient from whom *S. commune* was isolated, and the diagnosis was allergic bronchopulmonary mycosis (ABPM) [17].

Since then, more than 10 patients with *S. commune* infections have been documented in Japan, with all being ABPM or mucoid impaction of bronchi (MIB) in which the fungus had colonized the lung or bronchi [18,19].

S. commune is a basidiomycetous fungus that colonizes rotting wood, and it is distributed throughout the world. Identification of *S. commune* is difficult because it does not form conidia or sporangiospores in culture. Therefore, it can be surmised that there are many patients who are left untreated even when *S. commune* is cultured from samples because it is not identified. In addition, although detection of clamp connections that are characteristic of basidiomycetous fungi might point to *S. commune*, it is said that in Japan the hyphae isolates of this fungus collected from most patients lack clamp connections [18].

In our Case 2, we were also unable to identify the isolated fungus. Therefore, we sent it to the Research Center for Pathogenic Fungi and Microbial Toxicoses, Chiba University, Japan. They suspected primary hyphae might represent *S. commune* because some of the hyphae showed spicule formation. When they performed a mating test, the results showed formation of secondary hyphae and basidiocarps. On that basis, they identified our isolate as *S. commune*. AFS is a disease that resembles ABPM, and we expect that if a reliable method for identification of *S. commune* becomes generally established, there will be an increase in the number of patients diagnosed as having AFS due to this fungus.

Demonstration of fungi in the paranasal sinus contents is important for diagnosing AFS. However, since the fungi are in the highly viscous mucin, it is often difficult to isolate them by simply inoculating a portion of the paranasal sinus contents on agar and incubating. Ponikau *et al.* reported that it became easy to detect fungi if an enzyme, dithiothreitol, was first employed to degrade the mucin [20]. However, for cytodiagnosis, squash cytology is a very simple technique that makes it possible to observe even overlapping cells. Moreover, the basic approach is to carry out screening using Papanicolaou-stained specimens, but cytodiagnosis is superior to observing the kinds of inflammatory cells and the background. In addition, after destaining of Papanicolaou-stained specimens, it is easy to perform another special staining. For the two patients reported here, Papanicolaou-stained specimens that had been destained and then stained with Grocott's stain were very good for detailed morphological observation of hyphae.

We conclude that when AFS is suspected, performance of cytodiagnosis in parallel with microbiological studies on the paranasal sinus contents will be very useful for improving the rate of achieving a definitive diagnosis of AFS.

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