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Is Bjerkandera adusta Important to Fungus-associated Chronic Cough as an Allergen? Eight Cases' Reports

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ORIGINAL ARTICLE

Is *Bjerkandera adusta* Important to Fungus-associated Chronic Cough as an Allergen? Eight Cases' Reports

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Background. Recently, we have reported a new clinical disease concept called fungus-associated chronic cough (FACC), which entailed the following manifestations: (1) chronic cough; (2) the presence of environmental fungi, particularly basidiomycetous (BM) fungi, in the sputum; and (3) good clinical response to antifungal drugs. To clarify the relationship between the exposure to environmental fungi and the development of cough attacks, we reviewed the clinical records of patients with FACC and performed a molecular biological analysis of the BM fungi. **Methods.** We successfully selected 8 patients with chronic cough, wherein a sputum culture yielded *B. adusta* beforehand; moreover, we conducted allergological tests such as the immediate-type skin test, a serological test, bronchoprovocation test, and lymphocyte stimulation test (LST), using the antigenic solution of *B. adusta*. The efficacy of individualized therapy and the clinical manifestations in the eight patients were examined. **Results.** All the eight patients were diagnosed with FACC. Although three patients who did not show a positive reaction to the bronchoprovocation test or LST showed excellent clinical response to anti-fungal drugs; other 5 patients who showed a positive reaction to the bronchoprovocation test and/or LST seemed to be more intractable because of taking a longer time for complete remission and more frequent recurrence of cough. **Conclusion.** It was suggested that so-called allergic fungal cough (AFC), which is characterized by sensitization to *B. adusta*, may be included in a part of chronic intractable cough.

Keywords allergic fungal cough (AFC), Basidiomycetous fungi, *Bjerkandera adusta*, chronic idiopathic cough (CIC), fungus-associated chronic cough (FACC)

INTRODUCTION

Despite extensive diagnostic evaluation and numerous treatment guidelines (1–4), a number of patients remain troubled by chronic and uncontrollable cough. From our series of clinical studies on fungus-associated atopic cough (5–8), we have focused on the possible role of basidiomycetous (BM) fungi as a fungal aeroallergen (9, 10), and, recently, we have reported a new clinical disease concept called fungus-associated chronic cough (FACC), which entailed the following manifestations: (1) chronic cough; (2) the presence of environmental fungi, particularly BM fungi, in the sputum; and (3) good clinical response to antifungal drugs (11). To examine the type of mechanism that underlies the association of the BM fungi with cough, at first, using 28S rDNA (D1/D2) sequencing and analysis, we identified the BM fungi obtained from the sputum of four patients with fungus-associated atopic cough as reported in a previous study (12, 13). And on the basis of the results, we concluded that *Bjerkandera adusta*, (*B. adusta*) was one of the important etiological fungi of FACC.

We successfully selected eight patients with chronic cough for whom an earlier sputum examination showed positive results for *B. adusta* and conducted allergological tests, using the antigenic solution of *B. adusta* (polysaccharide). Although this study was not conducted prospectively and is not beyond the scope of a case report, it will be helpful while considering the association between the exposure to environmental fungi and the incidence of cough attacks.

MATERIAL AND METHODS

Patients

Eight patients who were referred to our hospital for the diagnosis and treatment of chronic cough that was defined as isolated persistent cough lasting for 8 weeks or more during a 10-month period (from April 2006 to April 2007) and whose sputum examination yielded *B. adusta* were selected in this study. This group consisted of five men and three women with ages ranging from 20 to 68 years. Based on the history, the duration of cough was 2 to 12 months. None of the patients complained of shortness of breath or wheezing and had a post-nasal drip or sinobronchial syndrome (14) to attribute to the cough. All patients were life-time non-smokers. A carefully noted history, physical examination, complete blood count with differential, and chest and sinus roentgenograms were normal. This case study was approved

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by the institutional review boards and informed consent was obtained from the eight patients.

Strains and DNA Preparation

The sputum samples obtained from the patients with chronic intractable cough were cultured on Sabouraud's dextrose agar (SDA) containing chloramphenicol. A mycological specialist recorded the morphological features of the strains by using the slide culture method (30°C for 2 weeks) and lactophenol cotton blue staining. When white rot grew widely on SDA, the DNAs from the strains were prepared according to a previously described method (15). One microliter of the DNA solution was used as the template for the polymerase chain reaction (PCR).

PCR and Sequencing

PCR was performed with the primers for the 28 SrDNA partial sequence (D1/D2 region) (16), NL-1 (5'-GCATATCAATAAGCGGAGGAAAAG-3') and NL-4 (5'-GGTCCGTGTTTCAAGACGG-3'), under the following conditions: 25 cycles of 94°C, 1 minute; 60°C, 15 seconds; and 72°C, 15 seconds. The products were detected as a single band of 0.5 kbp by agarose gel analysis. The products were detected as a single band of 0.5 kbp by agarose gel analysis. Both strands of the PCR products were directly sequenced using a DNA sequencing kit (Applied Biosystems) and an automatic sequencer (Genetic Analyzer 310; Applied Biosystems) according to the manufacturer's instructions. The DDBJ/EMBL/GenBank nucleotide sequence databases were searched for matches of the confirmed sequences by using BLAST programs (17).

Diagnoses and Therapy for Chronic Cough

The cause of chronic cough in the eight patients was diagnosed according to the diagnostic criteria for causes of chronic cough recommended by both the Japanese Cough Research Society guidelines for diagnosis and treatment of chronic cough (2) and the Japanese Respiratory Society guidelines for management of cough (3); a questionnaire, blood examination (WBC (μ L), CRP (mg/dL), cell differential of peripheral blood, immunoglobulin E (IgE) (U/mL), specific IgE for house dust or mite), chest and sinus radiographs, induced sputum examination, pulmonary function tests (18), bronchial reversibility to inhaled β 2-agonists, cough reflex sensitivity to inhaled capsaicin (19), bronchial responsiveness to methacholine (20), and the efficacy of individual causes-specific treatments.

Capsaicin cough threshold was measured as an index of airway cough reflex sensitivity according to the method reported by the authors (19) at the first visit and after each therapy. The capsaicin cough threshold was defined as the lowest concentration of inhaled capsaicin eliciting five or more coughs. Positive bronchial reversibility was defined as percentage increase in forced expiratory volume (FEV₁) > 12% and absolute increase in FEV₁ > 200 mL. The non-specific bronchial responsiveness to methacholine was assessed according to the method described by Cockcroft et al. (20). The results were expressed as the provocation concentration (mg/mL) required to cause a 20% or more decrease from

the baseline FEV₁ (respiratory threshold of methacholine; RT-Meth).

Suspected cough-variant asthma (CVA) (21) was first treated with β 2-agonists (a combination of clenbuterol 40 μ g/day given orally and inhalation of salbutamol sulfate 200 μ g at bedtime and on demand). If this proved effective but insufficient, the treatment was stepped up in accordance with the guidelines. When the bronchodilator therapy failed to relieve the cough, atopic cough (AC) (22, 23)—bronchodilator-resistant non-productive cough associated with atopic constitution—was suspected and treatment with histamine H1 antagonists and inhaled corticosteroids (a combination of 10 mg/day cetirizine hydrochloride and 400 to 800 μ g/day fluticasone propionate) was induced. When cough was productive, sinobronchial syndrome (SBS) (14) was suspected and treatment with clarithromycin (200 mg/day orally) was initiated. Suspected gastroesophageal reflux disease (GERD) (4) was treated with a high dose of proton-pump inhibitors. The duration of each treatment was a minimum of 2 months.

In case the definite diagnoses could not be established because of the lack of complete relief of cough, according to our experience of successful treatment for atopic cough caused by BM antigens (12, 13), inhalation of amphotericin B (1 mL of 2.5 mg/mL solution, once or twice a week) (24) or oral administration of low-dose antifungal drug (ITZ 50 mg/day, 14 to 21 days) was given.

Assessment of the Efficacy of Treatment on Cough Symptom

The efficacy of cough treatment was assessed with a subjective cough symptom scale (SCSS) (11). It was assumed that the sum sensation regarding the intensity and frequency of coughs in the 1-week period before the first visit was recorded as 10 points, and the complete absence of cough was recorded as 0 points. The points on the cough scale were recorded for each patient during each visit. The efficacy of the treatment was evaluated on the basis of the change in the cough scale before and after the treatment (Δ cough scale): 10 to 8 points was regarded as excellent; 7 to 5 points as good; 4 to 3 points as fairly good; and 2 to 0 points as unsatisfactory.

Cough was considered recurrent when an isolated non-productive cough lasted for more than 2 weeks despite upper respiratory infection. The onset of typical asthma was defined as wheezing and/or a dyspneic attack responding to inhaled β 2-agonists.

Allergological Study

Induced Sputum. Sputum was induced by inhalation of 10 mL of 5% saline solution for 20 minutes through a jet nebulizer (MMN-20S, Millicon-S; Sin-Ei Industries, Inc., Tokyo, Japan). If spontaneous cough did not occur 10 minutes after the start of inhalation, the patient was asked to induce a deep cough. Patients who were not able to bring up sputum at the end of the 20-minute inhalation period were released. The specimens were considered adequate if alveolar macrophages were detected. A part of the sputum was used for fungal culture. Another part of the sputum was transferred to slides in small amounts and finely distributed over two microscopic slides. The smear was air-dried and stained by the May-Grunwald-Giemsa method. A total of

400 cells were counted from randomly selected fields under oil immersion objectives. The results were expressed in percentages and recorded. Sputum eosinophilia was defined when beyond 5%.

Preparation of the antigenic solution. One liter of Sabouraud's dextrose broth in 3-L flasks were sterilized by autoclaving at 121°C for 20 minutes. Five mL of *B. adusta* (NBRC 4983)'s spore suspension (10^5 spores/mL) in sterile physiological saline from 14-day-old Sabouraud's dextrose agar culture was used to inoculate the flask. The flask were shaken at 25°C at 150 rpm in a rotary shaker incubator. The flask was removed after 14 days. The mycelium was separated by filtration and centrifuged. The supernatant were dialyzed against 5 mM ammonium bicarbonate and lyophilized.

Immediate-Type Skin Test and Serological Test.

Reactions to the *Bjerkandera adusta* antigen. The antigenic solution (polysaccharide) was injected intradermally with a tuberculin syringe (0.02 mL, 1 mg/mL) to assess the skin response to the solution. The result was judged to be positive in a case of the longer axis of the flare beyond 10 mm at 15 minutes after the injection.

B. adusta-specific IgG was measured by a modified enzyme-linked immunosorbent assay (ELISA) inhibition technique described previously (25). For inhibition, 25 μ L of sera either from control patients or *B. adusta* cases were incubated with either *B. adusta* or irrelevant antigen and 200 μ L of ovalbumin diluent in polystyrene tubes overnight at 4°C. Microtiter plates were coated with 100 μ L of *B. adusta* regand and incubated for 12 hours at high humidity. Plates were then washed and blocked and 200 μ L of inhibited sera were added to each well. Alkaline-phosphatase-coupled developing antibody was added, followed by enzyme substrate amplifier solution. Plates were observed for color development, at which point absorbance was measured at 650 nm per 10 seconds in 5 minutes with an automated microtiter plate reader. Radioallergosorbent test (RAST) and ELISA mean values from the samples were calculated. The positive *B. adusta*-specific IgG was defined when greater than 0.41 mg/L.

B. adusta-specific IgE was measured by an AlaSTAT-MP technique that has been previously described (26). In brief, soluble *B. adusta* was first coupled to insoluble cyanogens-bromide-activated immunosorbent disks by incubation for 48 hours on a rotating device. Next, for inhibition, 50 μ L of serum from *B. adusta* cases or controls were incubated with 50 μ L of *B. adusta* antigen or irrelevant antigen (human serum albumin) for 6 hours at 4°C in polystyrene tubes. *B.*

adusta-coupled disks were then added to each tube for 12 to 16 hours. The disks were then washed four times with PBS-Tween and subsequently incubated with I^{125} -radiolabeled antihuman IgE for another 12 hours. Plates were observed for color development as same as *B. adusta*-specific IgG. The positive *B. adusta*-specific IgE was defined when greater than 0.35 U/mL.

Bronchoprovocation Test. When subjective cough symptom scale (SCSS) improved to be under four points after the standard therapy, patients were subjected to bronchoprovocation test using the antigenic solution (27); *B. adusta* and *Penicillium thomii* (as control) (2 mL of culture-filtrate antigen, 1 mg/mL) through a jet nebulizer. The responses were assessed to be positive when laboratory findings such as WBC and CRP elevated significantly and/or patients developed cough attacks with a significant increase in cough reflex sensitivity to inhaled capsaicin, which was measured before and 24 hours after the provocation test.

Lymphocyte Stimulation Test. The lymphocyte stimulation test using the antigenic solution was performed by using Lymphoprep system. The results were assessed to be positive when the magnitude of the response to *B. adusta* was beyond 200% when compared with these controls using PHA (28).

RESULTS

Estimation of Chronic Cough

Eight patients (five males and three females) reporting chronic cough, with a mean age of 50.4 ± 17.1 (SD) years, were examined (Table 1). A carefully noted questionnaire revealed that all patients complained of cough with "tickle and stuck" in their throat. Chest and sinus radiographs were normal in all the patients. Atopic tendency was recognized in four patients (patient 3, 4, 5, and 7) (Table 2).

Chronic airflow limitation was considered present when the ratio of FEV₁ to forced vital capacity (FVC) (FEV₁/FVC ratio) was < 0.7 FEV₁ of <80% of the predicted value, or positive bronchial reversibility was not observed in any of the 8 patients. RT-Meth was exaggerated in 3 patients (patient 3, 5, and 6). Cough reflex sensitivity as assessed by the estimation of the capsaicin cough threshold (19) was observed to be increased in 4 patients (patient 2, 6, 7, and 8) (Table 1).

Efficacy of Each Treatment and Diagnosis of 8 Patients

With the exception of two patients (patient 3 and 5) in whom the response was fairly good, bronchodilator therapy

TABLE 1.—Characteristics of patients.

Age	Gender	Pulmonary function tests					Bronchial reversibility (%)	RT-Meth mg/ml	Cough threshold (μ M)
		%FVC	FEV1	%FEV1	FEV1%				
1	20	M	135.6	5.24	123.9	88.8	2.2	20	31.3
2	46	M	126.9	4.08	120.7	83.3	2.0	20	0.98
3	58	F	113.2	1.86	109.4	72.1	8.6	5	1.95
4	66	F	94.7	2.33	103.1	76.9	-0.4	20	31.3
5	40	M	106.9	3.61	103.4	86.4	-1.4	1.25	31.3
6	68	M	135.5	2.41	120.5	72.6	7.8	5	3.91
7	39	M	135.6	5.24	123.9	88.8	3.2	20	3.91
8	66	F	128.7	2.38	126.6	77.0	4.7	20	0.49

TABLE 2.—Allergological findings (I).

	WBC	E ₀ (%)	CRP	IgE (U/ml)	E ₀ in SP (%)	With <i>Bjerkandera adusta</i> antigen					
						Specific IgE		Skin test		Specific-IgE (U/ml)	Specific-IgG (mg/ml)
						House dust 1	Mite	Immediate	Late		
1	7100	4.6	0.12	228	NA	ND	ND	0 × 0/0 × 0	0 × 0/0 × 0	0.35>	0.92
2	5200	1.4	0.06	55	3	ND	ND	4 × 4/0 × 0	0 × 0/0 × 0	0.35>	<0.1
3	5000	11.3	0	628	NA	0.8(+)	0.47(+)	4 × 4/15 × 10	4 × 3/10 × 7	0.35>	4.35
4	5800	0.9	0.12	229	2	(−)	ND	7 × 7/30 × 30	0 × 0/0 × 0	0.35>	0.41
5	4800	1.0	0.12	238	0	(−)	2.35(+)	10 × 10/24 × 22	0 × 0/0 × 0	0.35>	16.76
6	6800	3.8	0.04	145	54	(−)	(−)	0 × 0/0 × 0	0 × 0/0 × 0	0.35>	16.38
7	5100	1.5	0.02	893	1	0.47(+)	2.28(+)	6 × 7/32 × 52	0 × 0/0 × 0	0.35>	6.93
8	4800	2.7	0.01	94.3	17	(−)	(−)	5 × 6/30 × 28	0 × 0/0 × 0	0.35>	1.48

NA; not available.
ND; not done.
Eo; eosinophil.

failed to produce any response. Further, histamine H1 antagonists and inhaled corticosteroids failed to produce any response in six patients while showing fairly good response in other two patients (patient 7 and 8) (Table 3).

Based on these results, the probable diagnoses for the causes of chronic cough in the eight patients were as follows: atopic cough (23) in two patients (patient 7 and 8) with cough reflex hypersensitivity; cough variant asthma (21) in two patients (patient 3 and 5) with heightened bronchial responsiveness to methacholine (20); however, even a probable diagnosis could not be achieved in the remaining four patients (patient 1, 2, 4, and 6).

Thus, we were unable to establish the definite diagnoses due to lack of complete relief of cough with individual standard therapies in all of the eight patients; however, it was remarkable that the addition of antifungal drugs; ITZ 50 mg/day 14 to 41 days and/or inhalation of amphotericin B (24) (1 mL of 2.5 mg/mL solution, once or twice a week, a total of two to seven times), or amphotericin B syrup (13) (2 mL/day for 12 days) proved efficacious with significant decrease of subjective cough symptom scale in all patients including the four patients given probable diagnosis (Table 4). On the basis of these results, all of the eight patients were diagnosed with FACC.

Allergological Tests for 8 Patients with FACC

Peripheral blood eosinophilia was noticed in patient 3. The total serum IgE levels were found to be elevated in four patients (patient 3, 4, 5, and 7). Positive AlaSTAT for house dust and mite was observed in two patients (pa-

tient 3 and 7) and three patients (patient 3, 5, and 7), respectively.

Out of the six patients who underwent the induced sputum examination, sputum eosinophilia was observed in two patients (patient 6 and 8).

Five of the eight patients demonstrated immediate cutaneous response to the *B. adusta* antigenic solution. The positive *B. adusta*-specific IgG was observed in six patients, whereas *B. adusta*-specific IgE was not observed in any one (Table 2).

The Bronchoprovocation Test and the Lymphocyte Stimulation Test

Five of seven patients (patient 2, 4, 5, 7, and 8) demonstrated positive reaction to the bronchoprovocation test with appearance of some cough attacks. Four of the five patients (patient 2, 4, 5, and 8) revealed a positive reaction to the lymphocyte stimulation test (Table 4). All patients who demonstrated positive reaction to LST showed positive results for bronchoprovocation tests.

Clinical Course of 8 Patients with FACC

The eradication of BM from the sputum was confirmed only in two patients because the character of the cough became non-productive in cough with success of the itraconazole therapy in most patients.

The mean duration for apparent relief of cough symptoms that showed significant decrease of subjective cough symptom scale after initiation of anti-fungal drugs was 2.8 ± 1.0 weeks (median 2.0, ranged from 2 to 4 weeks); however, the

TABLE 3.—Clinical manifestations of 8 patients (I).

	Subjective cough symptom scale on each therapy			Total use of anti-fungal drugs for complete remission of cough symptoms
	β ₂ [−] stimulant	Histamine H1 antagonists and inhaled corticosteroids	Anti-fungal drugs	
1	10	10	1	ITZ 50 mg × 14d
2	9	8	3	ITZ 50 mg × 28d, APTB 7 times
3	7	ND	4	ITZ 50 mg × 14d, APTB 1 time
4	9	8	0	ITZ 50 mg × 2 1 d, APTB 5 times
5	7	6	0	APTБ 5 times
6	10	8	3	APTБ syrup 2 ml × 12d
7	10	7	1	ITZ 50 mg × 41 d, APTB 6 times
8	10	7	4	ITZ 50 mg × 25d, APTB 2 times

ITZ; itraconazole.
APTБ; inhalation of amphotericin B.

TABLE 4.—Allergological findings (II).

Bronchoprovocation test						Lymphocyte stimulation test	
Cough threshold (μ M/I)							
	(before)	(after)	Cough attack	Result	S.I.	Result	
1	15.6	31.3	no	Negative		ND	
2	1.95	1.95	8 hr	Positive	702	Positive	
3	ND	ND	ND	ND		ND	
4	125	62.5	5 hr	Positive	205	Positive	
5	1.95	1.95	8 hr	Positive	312	Positive	
6	31.3	125	no	Negative		ND	
7	125	62.5	9 and 10 hr	Positive	132	Negative	
8	15.6	1.95	7 and 11 hr	Positive	888	Positive	

ND; not done.

mean duration required for the first complete remission after the initiation of anti-fungal drugs was 12.4 ± 7.4 weeks (median 11.5, ranged from 4 to 24 weeks). The duration required for the complete remission with the antifungal drugs was significantly longer in the sensitized patients (median 17 weeks, patient 2, 4, 5, 7, and 8) than the non-sensitized patients (median 6 weeks, patient 1, 3, and 6) ($p = 0.0339$ by Mann-Whitney U test).

The recurrence of chronic cough was noticed in five patients and in three patients fungal culture of sputum revealed BM fungi again. The onset of typical asthma was recognized in three patients (Table 5).

DISCUSSION

Despite extensive diagnostic evaluation and numerous treatment guidelines (1–4), a number of patients remain troubled by chronic and uncontrollable cough.

Haque et al. have described a subgroup of chronic idiopathic cough (CIC) patients for whom systematic investigation did not yield a specific diagnosis and in whom specific trial therapies did not result in an improvement of symptoms (29, 30). CIC is not rare but a serious problem worldwide; therefore, it is important to investigate how to address the problem of CIC and minimize the diagnosis of CIC.

We have previously reported cases of atopic cough (AC) caused by a hypersensitivity to *Trichosporon asahii* (5), *Pichia guilliermondii* (6), *Streptomyces albus* (7), and also reported the first case of nonasthmatic sputum eosinophilia caused by an allergic reaction to a BM fungus (originally mislabeled as *Humicola fuscoatra*) in which the increase of eosinophils in the induced sputum was established by repeated environmental surveys to be closely related to the ap-

pearance of BM fungi in the patient's house (8). Furthermore, we have reported several cases of AC that were sensitized to BM fungi and successfully eradicated and treated with low-dose itraconazole (12) or oral cleansing with amphotericin B (13). From our series of clinical studies on FACC (11), we have focused on the possible role of BM as a fungal aeroallergen (9).

Initially we performed pharyngeal swab cultures for the detection of fungi in 141 patients with chronic nonproductive cough and identified *Candida* and BM in 10.6% and 6.4% of all the examined patients, respectively (10). BM fungi are rarely detected in the culture of pharyngeal swabs taken from non-coughers (9). Furthermore, because the positive ratio in the immediate subcutaneous reaction to BM fungi in allergic airway diseases, such as AC (18.8%), cough variant asthma (26.9%), and cough predominant asthma (22.4%), are significantly higher than that in physically unimpaired persons (3.4%) (Sequentially, $p = 0.049$, $p = 0.014$, $p = 0.021$) (Unpublished data), we suspect that the colonization was not caused by an environmental fungal contamination. Therefore, we have hypothesized that BM fungi colonizing in the pharynx or lower respiratory tract play an important role in allergic airway disorders (10).

As matters that require attention in the expectoration eumycetes culture, we mainly used Sabouraud's dextrose agar (SDA), but PDA is also useful. It should be noted that although it requires only about 2 days to detect *Aspergillus*, which is well known as a cause of the disease eumycetes, about 10 days is required to culture BM. During the annual meeting of the 10th Japan Cough Research Society, it was found that several laboratories store the nutrient medium only for approximately 2 days. This may have made the detection of BM difficult in many institutions.

Although only a limited number of papers have documented infectious diseases caused by BM fungi, such as *Schizophyllum commune* (31) and *Coprinus* species (32), much of the literature reports the possible role of basidiospores as airborne allergens (33–35). The phylum of Basidiomycetes (mushrooms, puffballs, rusts, smuts, and bracket fungi) deserves special attention because of the high atmospheric concentration of basidiospores during the fall season. Such spores are generally small (3 to 7 μ m in diameter); this size enables easy deposition within the airway (36).

According to our previous examination using molecular biological analyses described in the introduction, we assumed that *B. adusta* (Yakeirotake in Japanese, 100% homology to DDBJ/EMBL/GenBank DNA database accession number

TABLE 5.—Clinical manifestation of 8 patients (II).

	Duration for apparent relief of cough after initiation of anti-fungal drugs (weeks)	Duration required for first complete remission after initiation of anti-fungal drugs (weeks)	Duration required for first complete remission after first visit (weeks)	Eradication of BM in sputum	Relapse of cough	Fungal culture of sputum on relapse	onset of BA
1	2	4	6	Not obtained	(–)	ND	(–)
2	4	21	26	Not obtained	(+)	BM	(–)
3	2	8	36	ND	(–)	ND	(+)
4	2	15	20	Not obtained	(+)	Peni	(–)
5	2	13	17	Not obtained	(+)	ND	(+)
6	4	4	4	Not obtained	(–)	ND	(–)
7	2	10	11	Successful	(+)	BM	(–)
8	4	24	25	Successful	(+)	BM	(+)

AB096738) represents one of the important potential etiological agents of chronic cough among species of BM phylum, consisting of approximately 30,000 species.

B. adusta is well-known in the field of agricultural science because it secretes multiple lignin peroxidase isozymes (37, 38). Gloria et al. (39) studied a total of 44 clinical isolates of BM fungi, namely, *S. commune* (n = 5), the *Coprinus* species (n = 8), *B. adusta* (n = 14), and sterile, uncharacterized Basidiomycetes (n = 17). In their report, the white-rot Basidiomycetes – *B. adusta* – were detected from bronchial washes (n = 8), sputum (n = 3), skin, pleural fluid, and lung tissue (n = 1 for each); however, the clinical significance of each isolate was not clarified.

Consideration of the use of antifungal drugs in cough treatment strategy based on the basis of the understanding of FACC will be useful and the clinical application has far-reaching possibilities. However, it is difficult to ascertain whether such an exposure to environmental fungi causes a true allergy (34), an infection, or a combination of both an allergy and an infection.

In this study, we selected eight patients with chronic cough, wherein a sputum culture yielded *B. adusta* beforehand; moreover, we conducted allergological tests using the antigenic solution of *B. adusta* (polysaccharide). We described the clinical manifestations of the eight patients on the basis of the medical reports.

We were only able to establish a probable diagnosis of the causes of chronic cough in four patients, and we were unable to establish a definite diagnosis in any of the eight patients, because none of them experienced complete relief of cough with individual standard therapies. However, in line with our experiences in successful treatment for atopic cough caused by BM antigens (12, 13), inhaled amphotericin B [24] or oral low-dose antifungal drug therapy successfully eliminated the cough symptoms in the eight patients. Thus, all eight patients recruited in this study were all diagnosed with FACC.

From the results of allergological tests including skin tests, bronchoprovocation test, and lymphocyte stimulation test, among the eight patients with FACC, we found that a subgroup of the five patients was sensitized to *B. adusta*. The clinical features of the so-called allergic fungal cough (AFC) (patients 2, 4, 5, 7, and 8) are (1) chronic intractable cough with a sensation of tickling and something being stuck in their throat, which lasts for more than 8 weeks; (2) absence of wheezing, dyspnea, hemoptysis, or pleurisy, and no adventitious lung sounds on physical examination; (3) the presence of environmental fungi such as BM fungi, particularly, *B. adusta*; (4) at least one positive reaction to immediate cutaneous reactivity and/or inhalation bronchoprovocation test and/or lymphocyte stimulation test to the fungus; (5) good clinical response to antifungal drugs for which a considerable period of time (more than 10 weeks approximately) is required for the complete remission of the cough symptoms; (6) frequent recurrence of cough; (7) peripheral blood eosinophilia, elevated total IgE level in the serum, and eosinophilia in the induced sputum, which are not necessarily observable; (8) normal limits of FEV₁, FVC, and FEV₁/FVC ratio; (9) normal to slightly increased airway cough reflex sensitivity; (10) bronchial responsiveness within normal limits.

Interestingly, the three other patients who were not sensitized to *B. adusta* showed excellent clinical response to antifungal drugs and no recurrence of cough. Despite the remarkable fact that antifungal drug therapy was so efficacious that it eliminated both the cough symptoms and the sense of something being stuck in the throat and successfully eliminated BM fungi from the specimen of the respiratory tract in the three patients without sensitization to *B. adusta*, the other five patients sensitized to *B. adusta* were apparently intractable even after having undergone treatment and management for their symptoms. Furthermore, although the onset of typical asthma was recognized in three patients with AFC, it is a future problem whether AFC is a precursor of asthma.

According to these findings, it seems that chronic cough related to *B. adusta* has different clinical manifestations, depending on whether the patient with FACC is sensitized to fungus (allergic fungal cough: AFC). Therefore, AFC may be included in a comprehensive disease concept such as FACC.

Because the major species of BM fungi associated with FACC may change their profiles or proliferate on account of natural environmental factors such as global warming or other geographical characteristics, an epidemiological investigation of BM fungi is required.

Thus, it is suggested that in the future, the so-called AFC which is observed in patients sensitized to various kinds of BM fungi as well as *B. adusta*, may be included in chronic intractable cough as a part of FACC. Further studies are required to determine whether crossing-over reactivity in BM fungi except *B. adusta* is observed and whether such a reaction is peculiar to *B. adusta*.

As intractable allergic diseases associated with environmental fungi have been well known in the area of otolaryngology (40) and dermatology (41); such association between intractable allergic disorders and environmental fungi is expected in the field of respiratory medicine. Therefore, an allergological study of *B. adusta* with respect to chronic intractable cough patients should be planned prospectively at many institutions. We hope that the recognition of the concept of AFC will benefit the study of chronic intractable cough.

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DECLARATION OF INTEREST

The authors declare that they have no competing interests that might be perceived to influence the results and discussion reported in the present manuscript.

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