Infection (2015) 43:371–375
DOI 10.1007/s15010-015-0724-7

CASE REPORT

Subcutaneous abscess due to the basidiomycete *Phellinus mori* in a patient with chronic granulomatous disease

T. Shigemura · Y. Nakazawa · Y. Amano · A. Sudo · M. Watanabe · M. Kobayashi · N. Kobayashi · K. Koike · K. Agematsu · K. Nishimura

Received: 24 October 2014 / Accepted: 7 January 2015 / Published online: 20 January 2015

Abstract Chronic granulomatous disease (CGD), a primary immunodeficiency caused by impaired phagocyte killing of intracellular pathogens, is characterized by recurrent, life-threatening, bacterial and fungal infections. As a result of improvements in microbiologic culture and identification techniques, a number of unique filamentous fungi have been reported as significant pathogens in patients with CGD. We report a case of subcutaneous basidiomycete *Phellinus mori* infection in a patient with CGD. To the best of our knowledge, this is the first reported case of human infection by this fungus. The causative fungus was identified on the basis of its morphological characteristics and nucleotide sequence on the internal transcribed spacer region of the ribosomal RNA gene. This is the fifth case report of filamentous basidiomycetes infecting a patient with CGD; all of these cases have been caused by *Phellinus* species. We highlight the importance of recognizing filamentous basidiomycetes *Phellinus* species as possible agents of non-*Aspergillus* fungal infections in patients with CGD.

Keywords Chronic granulomatous disease · *Phellinus mori* · Filamentous basidiomycete · Non-*Aspergillus* fungal infection

T. Shigemura · Y. Nakazawa (✉) · N. Kobayashi · K. Koike
Department of Pediatrics, Shinshu University School of Medicine, 3-1-1, Asahi, Matsumoto 390-8621, Japan
e-mail: yxnakaza@shinshu-u.ac.jp

Y. Amano · A. Sudo
Department of Pediatrics, Nagano Red Cross Hospital, Nagano, Japan

M. Watanabe
Department of Pathology, Nagano Red Cross Hospital, Nagano, Japan

M. Kobayashi
Department of Laboratory Medicine, Shinshu University Hospital, Matsumoto, Japan

K. Agematsu
Department of Infection and Host Defense, Graduate School of Medicine, Shinshu University, Matsumoto, Japan

K. Nishimura
Department of Microbiology, Dokkyo Medical University School of Medicine, Tochigi, Japan

Introduction

Chronic granulomatous disease (CGD) is a rare inherited primary immunodeficiency disorder. It is characterized by functional impairment of the phagocyte nicotinamide adenine dinucleotide phosphate (NADPH) oxidase complex and the consequent inability to generate reactive oxygen species (ROS) [1, 2]. Patients with CGD are susceptible to *Staphylococcus, Burkholderia, Serratia, Nocardia*, and fungi such as *Aspergillus* species because of impaired phagocyte killing of intracellular pathogens [2, 3]. Although the prognosis has markedly improved because of prophylaxis with trimethoprim–sulfamethoxazole, itraconazole (ITCZ), and interferon-γ in select cases, invasive fungal infections remain a critical issue for patients with CGD. *Aspergillus* species, especially *A. fumigatus* and *A. nidulans*, are the major pathogens responsible for one-third of all deaths among patients with CGD [2, 4]. Several novel and emerging filamentous fungi have recently been reported to infect patients with CGD. These filamentous and other opportunistic fungi have been demonstrated as significant pathogens as a result of recent improvements in microbiologic culture and identification techniques [4, 5].

We report a case of a patient with CGD who sustained a cutaneous infection caused by the basidiomycete *Phellinus mori*, a novel fungal pathogen.

Case report

The patient was a 23-year-old man with X-linked CGD (gp91phox protein deficiency due to a 1369 T > C mutation in the *CYBB* gene). He had a past medical history of cervical lymphadenitis, a perirectal abscess, recurrent pneumonia, femoral osteomyelitis due to *Chadophialophora arxii* [6], and pulmonary nodules and generalized lymphadenopathy since the age of 7 years. He had been treated prophylactically with trimethoprim–sulfamethoxazole and ITCZ. Upon admission to our hospital, the patient had a 1-month history of dull pain in the anterior right chest and had developed subcutaneous swelling with reddish overlying skin at the painful site. There was no history of recent trauma. He had no other symptoms or signs. Laboratory findings included: leukocyte count, 3,490/μL; C-reactive protein, 3.0 mg/L; and erythrocyte sedimentation rate, 14 mm/h. Aspergillus galactomannan antigen and β-D-glucan (β-glucan Wako) were undetectable in his serum (cutoff values, <0.5 and <2.84 μg/mL, respectively). An ultrasound of the swollen reddish skin revealed a hypoechoic mass measuring 31 × 29 × 10 mm within the subcutaneous tissue, with no internal vascularity. Computed tomography of the mass showed no involvement of deep structures. Because a single fungal species was cultured from the pus obtained by needle aspiration, the disease was diagnosed as fungal subcutaneous abscess and oral voriconazole (200 mg twice daily) and intravenous micafungin (250 mg/day) were started. Nine days after starting antifungal therapy, the subcutaneous mass was surgically excised. The operative findings showed a large quantity of pus and surrounding granulomatous tissues, which were completely excised. A culture performed at the time of surgery, as well pus and tissue samples all produced fungal colonies similar to those of the initial aspirated sample. Culturing of these specimens yielded no bacteria on chocolate agar at 35 °C after 2 days.

Histopathology showed granulomas consisting of epithelioid cells and Langhans giant cells around the abscess. Staining with periodic acid–Schiff stain and Gomori methenamine silver stain revealed the presence of Y-shape form branching, septate fungal hyphae in the granulomas.

In vitro antifungal isolate susceptibility testing was not possible, because there was no mycelial growth in the growth control wells after 10 days of incubation at 35° for the minimal inhibitory concentration test using the broth microdilution technique. He was discharged after a total of 4 weeks of combined antifungal therapy with voriconazole and micafungin. Empiric therapy with voriconazole and interferon-γ (25 μg/m^2/week) was continued at the outpatient clinic with clinical resolution of symptoms.

Mycological examination

The obtained organisms in the three cultures were identical fungal species. The isolate (FL10-0016) grew well at 25 and 37 °C and scarcely at 42 °C. Colonies were light brown, undulate with peripherally immersed hyphae, irregular convexity from a side view with a floccose surface, and a hard basal mycelium mat that reached 50–58 mm in diameter after 28 days incubation on potato dextrose agar (PDA) at 25 °C. Colony reverse was yellowish with a brownish central part. In slide cultures on PDA at 25 °C, the mycelial structure consisted of septate, hyaline thin-walled and yellowish-brown, thick-walled hyphae (Fig. 1). No clamp connections were observed. It was noticed that there were darker yellowish-brown, thicker-walled, setae-like structures with spiny, falcate projections appearing intercalarily in the yellowish-brown mycelium (Fig. 2). The structure appeared to match setae described for *P. mori* [7]. No conidia were observed after two to three incubations, but a few dark brown chlamydospores appeared. Because the fungus could not be identified by its phenotypic characteristics, it was processed for molecular analysis at the Department of Microbiology, Dokkyo Medical University. From the mycelium grown on PDA, genomic DNA was extracted with a commercial kit (GenTLE™ High Recovery Kit, TAKARA Bio Inc., Shiga, Japan) and amplified by PCR with the fungus-specific universal primer sets of ITS5 (5′-GGAAGTAAAAGTCGTAACAAGG-3′) and ITS4 (5′-TCC TCC GCT TAT TGA TAT GC-3′) to analyze the internal transcribed spacer (ITS: ITS1-5.8S-ITS2) region of the ribosomal RNA gene (rDNA). The PCR product was purified with a QIAquick PCR Purification Kit (Qiagen, Valencia, CA, USA) and sequenced with Genetic Analyzer 3130xl (PE Applied Biosystems, Carlsbad, CA, USA), using a BigDye Terminator v1.1 Sequencing Standard Kit (PE Applied Biosystems, Foster City, CA, USA). The obtained 741 bp sequence was edited using ATGC Ver. 4 sequence assembly software (Genetyx Co., Tokyo, Japan) and referred to the Genbank of the National Center for Biotechnology Information (NCBI), NIH, USA for nucleotide Basic Local Alignment Search Tool (BLASTn) Search. The sequence showed 99 % similarity with *P. mori* (accession number: FJ627259). Two sequences of basidiomycota sp. showed 96 % similarity, but there were no species-specified basidiomycetes with more than 90 % similarity. On the basis of the microscopic findings and rDNA ITS region sequence, the isolate FL10-0016 was identified as *P. mori*. The sequence was registered to DNA Data Bank of Japan

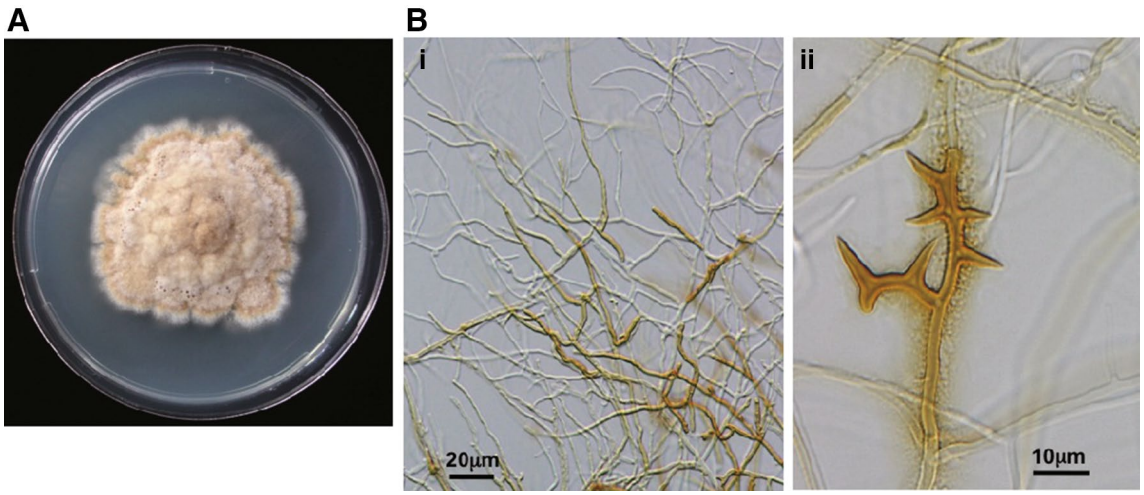

Fig. 1 **a** A colony incubated on potato dextrose agar at 25 °C for 28 days. **b** Mycelial structure on a slide culture incubated on potato dextrose agar at 25 °C for 14 days. Mycelium consists of hyaline thin-walled and *yellowish-brown*, thick-walled hyphae (*i*). Seta-like structures observed in generative hyphae are *darker brown* and thick-walled with spiny falcate projections. Note the *pale yellowish* exudate (*ii*)

(DDBJ), National Institute of Genetics as accession number LC002815. A living culture of FL10-0016 was deposited at Medical Mycology Research Center, Chiba University and maintained as IFM 59511.

Discussion

Phagocyte NADPH-oxidase is essential for killing catalase-positive bacteria and fungi by reducing molecular oxygen to superoxide, which subsequently reacts to form ROS. CGD, a primary immunodeficiency caused by a defect of NADPH oxidase [1, 2], confers susceptibility to fungal infections more so than other primary immunodeficiencies [3, 8]. Although the most common fungal pathogens associated with CGD infections are *Aspergillus* species, other rarer fungal pathogens have also been described [9, 10]. Dotis et al. [11] reviewed all 68 published cases of non-*Aspergillus* fungal infections in 65 CGD patients between 1984 and 2011. The majority of those infections were attributed to *Rhizopus*, *Scedosporium*, *Paecilomyces* or *Geosmithia* species. Infections with filamentous basidiomycetes were reported in two of the 68 cases (2.94 %). After 2011, there were two reports of infections associated with basidiomycetes in patients with CGD. This is the fifth case report of filamentous basidiomycetes causing infection in a patient with CGD.

Filamentous basidiomycetes are ubiquitous environmental fungi that are increasingly isolated from clinical specimens. However, their pathogenic role is often unclear, and many clinical microbiologists and clinicians may assume that these fungal isolates are environmental contaminants rather than pathogens [12, 13]. In our patient, isolated fungus were detected at two separate times, by needle aspiration and by surgical excision. In addition, fungal hyphae were confirmed in histopathology specimens. Although we did not check other fungal structures by PCR or quantitative PCR, the isolate is almost certainly the causative organism of this infection.

Classically recognized basidiomycetes human pathogens are *Schizophyllum commune*, *Coprinopsis* species, and *Phanerochaete chrysosporium*. Lesser known members from this class of molds have lately been incriminated as agents of human disease [14]. *P. mori* was isolated from *Morus* (mulberries) in the Heilongjiang Province of northern China as a new basidiomycete species in 2008 [7, 15]. Nagano prefecture, where our patient lived, is a wooded mountainous area located in the center of the main island of the Japanese archipelago and is rich in basidiomycetous flora. The region has more of an inland climate compared to many areas of Japan and is somewhat similar to that of northern China. Although *P. mori* has been not recognized in Japan yet, the species may inhabit mountainous areas of Japan.

The *Phellinus* genus belongs to the family Hymenochaetaceae, as does the closely related genus *Inonotus*, one of the largest basidiomycetous genera of wood-destroying fungi (220 named species worldwide) [16]. There have been only five documented infections with *Phellinus* species in human disease: three cases of *P. tropicalis*, one case of *P. undulates*, and a recent case of a new agent closely related to *P. umbrinellus* [16–20]. Four of the five reports associated with *Phellinus* species were in patients with CGD [17–20]. Therefore, all reported filamentous

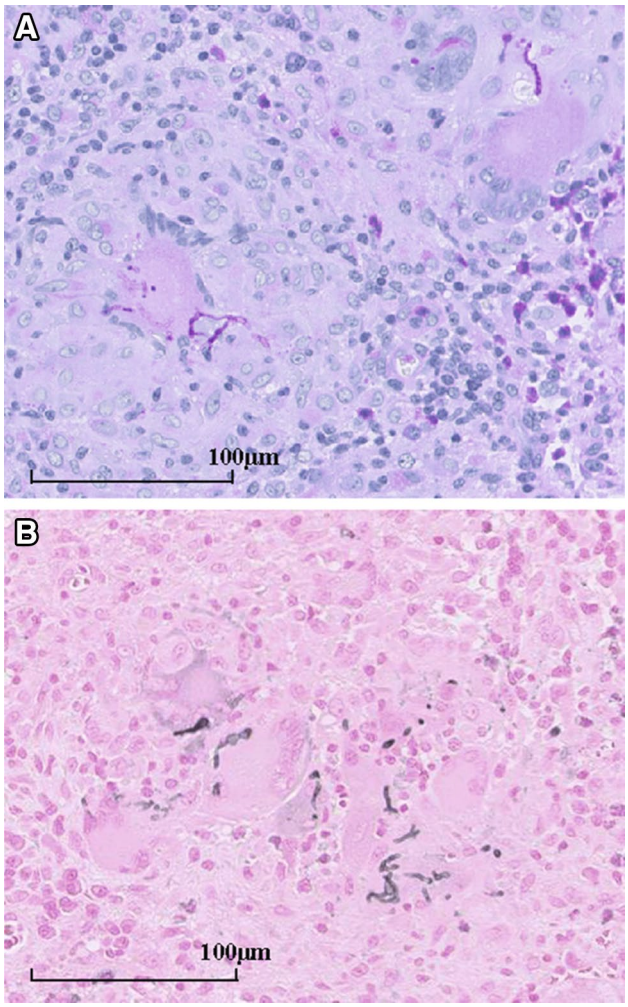

Fig. 2 Photomicrographs of granulomatous specimens from the cutaneous abscess show Y-shape form branching, septate fungal hyphae (**a** periodic acid–Schiff stain, **b** Gomori methenamine silver stain)

basidiomycetes infections in patients with CGD, including our patient, resulted from *Phellinus* species. Taken together, these cases highlight the importance of recognizing filamentous basidiomycetes *Phellinus* species as possible agents of non-*Aspergillus* fungal infections in patients with CGD.

In vitro antifungal susceptibilities provide useful guidance for choice of antifungal drugs. Unfortunately, antifungal susceptibilities were immeasurable in this case. In general, filamentous basidiomycetes are sensitive to ITCZ, but all reported filamentous basidiomycetes *Phellinus* species infections in patients with CGD have occurred during ITCZ prophylaxis [17–20]. Alternative therapy could be considered if the causative agents remain susceptible to ITCZ. Apart from administration of appropriate antifungal drugs, successful management for invasive fungal infections often requires surgical intervention such as drainage, debridement, and excision. Surgical interventions generally play an important role, especially for patients with CGD [2]. Infection with this fungal species is characterized by an insidious course with extensive tissue involvement [17–20]. In our case, early surgical excision of the lesion may have been critical for limiting the infection. Surgical intervention is likely necessary as soon as a diagnosis is made.

Acknowledgments This work was supported by JSPS KAKENHI Grand Numbers 26860791.

Conflict of interest We declare no conflicts of interest.

References

1. Segal BH, Leto TL, Gallin JI, Malech HL, Holland SM. Genetic, biochemical, and clinical features of chronic granulomatous disease. Medicine (Baltimore). 2000;79:170–200.
2. Seger RA. Modern management of chronic granulomatous disease. Br J Haematol. 2008;140:255–66.
3. Winkelstein JA, Marino MC, Johnston RB Jr, et al. Chronic granulomatous disease. Report on a national registry of 368 patients. Medicine (Baltimore). 2000;79:155–69.
4. Holland SM. Chronic granulomatous disease. Clin Rev Allergy Immunol. 2010;38(1):3–10.
5. Beaute J, Obenga G, Le Mignot L, et al. Epidemiology and outcome of invasive fungal diseases in patients with chronic granulomatous disease: a multicenter study in France. Pediatr Infect Dis J. 2011;30:57–62.
6. Shigemura T, Agematsu K, Yamazaki T, et al. Femoral osteomyelitis due to *Cladophialophora arxii* in a patient with chronic granulomatous disease. Infection. 2009;37:469–73.
7. Dai YC, Cui BK, Tao WQ. *Phellinus mori* sp. nov. (Basidiomycota, Hymenochaetales) from northern China. Mycotaxon. 2008;105:53–8.
8. Martire B, Rondelli R, Soresina A, et al. Clinical features, long-term follow-up and outcome of a large cohort of patients with chronic granulomatous disease: an Italian multicenter study. Clin Immunol. 2008;126:155–64.
9. van den Berg JM, van Koppen E, Ahlin A, et al. Chronic granulomatous disease: the European experience. PLoS ONE. 2009;4:e5234.
10. Kobayashi S, Murayama S, Takanashi S, et al. Clinical features and prognoses of 23 patients with chronic granulomatous disease followed for 21 years by a single hospital in Japan. Eur J Pediatr. 2008;167:1389–94.
11. Dotis J, Pana ZD, Roilides E. Non-*Aspergillus* fungal infections in chronic granulomatous disease. Mycoses. 2013;56:449–62.
12. Sigler L, de la Maza LM, Tan G, Egger KN, Sherburne RK. Diagnostic difficulties caused by a nonclamped *Schizophyllum commune* isolate in a case of fungus ball of the lung. J Clin Microbiol. 1995;33:1979–83.
13. Pounder JI, Simmon KE, Barton CA, Hohmann SL, Brandt ME, Petti CA. Discovering potential pathogens among fungi identified as nonsporulating molds. J Clin Microbiol. 2007;45:568–71.
14. Chowdhary A, Agarwal K, Kathuria S, et al. Clinical significance of filamentous basidiomycetes illustrated by isolates of the novel opportunist *Ceriporia lacerata* from the human respiratory tract. J Clin Microbiol. 2013;51:585–90.
15. Cao C, Peng F, Cui BK. Chemical characterization and structure of exopolysaccharides from submerged culture of new medicinal

mushroom from China, *Phellinus mori* (higher Basidiomycetes). Int J Med Mushrooms. 2013;15:57–69.
16. Williamson D, Pandey S, Taylor S, et al. A case of infection caused by the basidiomycete *Phellinus undulatus*. J Med Microbiol. 2011;60:256–8.
17. Sutton DA, Thompson EH, Rinaldi MG, et al. Identification and first report of *Inonotus (Phellinus) tropicalis* as an etiologic agent in a patient with chronic granulomatous disease. J Clin Microbiol. 2005;43:982–7.
18. Davis CM, Noroski LM, Dishop MK, et al. Basidiomycetous fungal *Inonotus tropicalis* sacral osteomyelitis in X-linked chronic granulomatous disease. Pediatr Infect Dis J. 2007;26:655–6.
19. Ramesh M, Resnick E, Hui Y, et al. *Phellinus tropicalis* abscesses in a patient with chronic granulomatous disease. J Clin Immunol. 2014;34:130–3.
20. De Ravin SS, Parta M, Sutton DA, et al. Paravertebral mushroom: identification of a novel species of *Phellinus* as a human pathogen in chronic granulomatous disease. J Clin Microbiol. 2014;52:2726–9.