Disseminated Beauveria bassiana Infection in a Patient with Acute Lymphoblastic Leukemia

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Received 22 December 2003/Returned for modification 12 February 2004/Accepted 12 March 2004

We describe a case of disseminated Beauveria bassiana infection in a patient with acute lymphoblastic leukemia. Her infection was successfully treated with amphotericin B and itraconazole. B. bassiana is rarely reported as a human pathogen. It is commonly found in soil and because of its pathogenicity to many insect species is incorporated into several pesticides.

CASE REPORT

A 44-year-old Caucasian woman was diagnosed with acute lymphoblastic leukemia. She had a history of recurrent sinusitis but was otherwise well. She lived in a rural area of the South Island of New Zealand and owned a garden center. At presentation she had a neutrophil count of 0.36 × 10⁹/liter but was clinically well with no signs of infection and commenced treatment with prophylactic ciprofloxacin and fluconazole. She was nursed in a positive-pressure single room and given a low-bacteria diet. At day 1 of induction chemotherapy (United Kingdom Acute Lymphoblastic Leukemia 12 protocol), ciprofloxacin and fluconazole were stopped, and co-trimoxazole and nystatin were started. At day 15 of treatment the patient became febrile, and Streptococcus viridans was isolated from blood cultures. She was treated with piperacillin and gentamicin, and her temperature stabilized. She remained neutropenic, and at day 20, small (<1-cm) purple macular “cigarette burn” lesions were noted on her left upper arm (Fig. 1A). An aspirate sent for bacteriology and skin scrapings sent for fungal culture yielded no microorganisms. Four days after the skin lesions developed, she complained of symptoms of sinusitis, headache, and facial pain and had percussion tenderness over her maxillary sinuses. Paranasal sinus disease was not identified by computed tomography scanning, and in view of her persisting neutropenia further invasive investigations were not performed. Serum transaminases were elevated on day 21, but an abdominal ultrasound scan was normal.

Fluconazole was restarted on day 28, and an excision biopsy of one of the skin lesions was performed the next day. Histopathological examination of the biopsy specimen revealed sharply demarcated areas of necrosis with lack of cellular reaction at the interface. The necrotic tissue was heavily permeated by fungal hyphae, which also invaded the local blood vessels (Fig. 2). Cultures of the tissue biopsy sample on blood agar and Sabouraud glucose agar plates at 30°C produced a pure growth of a white mould identified preliminarily as a Beauveria sp. No recovery of the mould was obtained on plates incubated at 35°C. The susceptibility of the isolate was assessed using Sensititre Yeast One susceptibility plates (Trek Diagnostic Systems, West Sussex, England). Testing was performed at 30°C, and the MIC was read after 72 h of incubation. The susceptibility results were as follows: the amphotericin B MIC was 2.0 mg/liter, the itraconazole MIC was 0.06 mg/liter, the fluconazole MIC was 8.0 mg/liter, the ketoconazole MIC was 0.125 mg/liter, and the 5-flucytosine MIC was >64 mg/liter.

Prednisone was stopped on day 37 of phase 1 induction, and conventional treatment with intravenous amphotericin B was commenced at 15 mg daily, escalating to a dose of 55 mg daily for 10 days. The skin lesions progressed, involving the patient’s arms, legs, buttocks, and face, and became necrotic and exudative (Fig. 1B). The patient developed a persistent hemorrhagic left-sided pleural effusion which was consistently sterile in microbial culture. A computed tomography scan of her chest was performed and was suggestive of lung necrosis. Therapy was then changed to liposomal amphotericin (AmBisome) at a dose escalating to 200 mg daily for 10 days before returning to conventional intravenous amphotericin in combination with itraconazole for a further 25 days. Antifungal therapy was continued throughout phase 2 induction and for the duration of her neutropenia. Her skin lesions continued to heal over several months with some scarring. She is now receiving maintenance chemotherapy and has had no recurrence of fungal infection.

The isolate (MR2097) was identified as Beauveria bassiana at the Mycology Reference Laboratory, Auckland Hospital, and referred for confirmation to the University of Alberta Microfungus Collection and Herbarium, Edmonton, Canada, where it was retained as UAMH 10179. Colonial and microscopic appearances were typical of B. bassiana. Colonies were fast growing, reaching diameters of 4.5 cm in 13 days at 30°C on potato dextrose agar (Difco Laboratories, Detroit, Mich.). They were densely cottony to flocculent, with droplets of exudate on the surface, yellowish white, raised, and dome shaped. Conidigenous cells occurred in sporodochial clusters and were slightly swollen at the base and narrowed at the tip to form a zigzag rachis. Conidia were oval to subglobose and apiculate and measured 2 to 3 μm long and 1.5 to 2 μm wide. Neither the patient isolate nor an environmental isolate (UAMH 7866) grew when stab inoculated onto PDA and in-

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cubated at 35°C (tested twice). Both isolates were confirmed as 
*B. bassiana* on the basis of their physiological profiles using the 
automated microtiter plate system BIOLOG (Biolog Inc., Hay-
ward, Calif.); however, a positive result was obtained after 96 h 
for the patient isolate compared with 72 h for the environmen-
tal isolate. In addition to morphological and physiological sim-
ilarities, a sequence of the nuclear ribosomal internal tran-
scribed spacer (ITS) region from the case isolate demonstrated 
the highest homology (98% identity) to the GenBank se-
quences of *B. bassiana* and its teleomorph *Cordyceps bassiana.*

*Beauveria* species are rarely verified as agents of human 
infection. *B. bassiana* has been reported as the cause of mycotic 
keratitis in three cases (4, 5, 6), but other reports concerning 
this species have not been substantiated (2, 3, 9, 10). Our case 
of disseminated *Beauveria* infection is the second involving an 
immunosuppressed leukemic patient. In the first report, a pa-
tient undergoing therapy for acute myeloid leukemia pre-
sented with a dry cough and fever and was found by lung biopsy 
to have allergic alveolitis (3). Ultrasonography revealed mul-
tiple liver and splenic lesions, and the fungus was isolated from 
a liver biopsy specimen. No skin lesions were reported. Our 
patient’s first indication of infection was the appearance of skin 
lesions and respiratory symptoms developed much later. The 
skin lesions were widespread, appearing and resolving in con-
cert. Tissue sections revealed deep tissue invasion and blood 
vessel involvement by fungal hyphae, strongly suggesting that 
the lesions were caused by hematogenous spread of the fungus 
from a primary site in the lung rather than by extrinsic con-
tamination, direct inoculation, or secondary opportunistic col-
onization. While necrosis was observed in the lesions of both 
patients, little cellular reaction to the presence of fungal hy-
phae was noted.

The isolates from these leukemia patients were similar in 
their inability to grow at 37°C. In our case and that of Henke 
et al. (3), primary isolation on Sabouraud glucose agar recov-
ered several colonies of the mould on plates incubated at 26 to 
30°C, but no growth was found on plates incubated at 35 or 
37°C. Henke et al. (3) reported a 6.5% conidial germination 
rate after 72 h at 35°C for their patient isolate, but the isolate 
did not grow at 37°C. An insect-associated control strain failed 
to germinate. The two isolates that we tested, one clinical and 
one environmental, did not grow at 35°C. Ability to grow at 35 
to 37°C is considered a requirement for human pathogenicity, 
but these two cases suggest that *Beauveria* species may have the 
potential to grow in tissues of the profoundly immunocompro-
mised host. In contrast, animal studies have demonstrated that 
*B. bassiana* can survive in animal organs without becoming 
invasive. In a survey of the mycobiota of small mammals, *B.
*bassiana* was one of the commonest fungi cultured from lungs 
without evidence of tissue invasion (1). Similarly, *B. bassiana* 
was noninvasive when inoculated intramuscularly into normal 
mice (7).

Our isolate was identified as *B. bassiana* by the characteristic 
clusters of basally swollen conidiogenous cells and the shape of 
the conidia, by its profile in the BIOLOG system, and by a high 
ITS similarity to published sequences of *B. bassiana* and its 
teleomorph *C. bassiana.* Henke et al. (3) suggested that ITS 
region sequences of their case isolate were closest to those of 
isolates deposited under the name *Botrytis tenella,* which has 
long been considered a synonym of *B. bassiana.* Their analysis 
uncovered greater variation than expected among the isolates 
of *Beauveria* examined, leading to uncertainty as to whether 
some species formerly considered as synonyms may need re-
evaluation. However, the sequences from their case isolate 
(AJ457169 and AJ457170) are deposited under the name *B.
bassiana.*

*B. bassiana* is a well known and widely dispersed insect 
pathogen. Some strains have been developed for use as bio-
logical insecticides, and these products have been approved 
since 1999 by the U.S. Environmental Protection Agency for 
use on all food and feed crops. At work in her garden center,
our patient used a combined insecticide and fungicide that did not contain *B. bassiana*. However, it is possible that she was exposed to the fungus, either from the soil or from organic pesticides, while living in an agricultural area. *B. bassiana* is a cosmopolitan fungus found in soil and many other substrates. The fungus is occasionally detected in air sampling (8), and Henke et al. (3) suggested that their patient was initially infected by airborne conidia. In an experimental study, pneumonitis was induced in rodents exposed to airborne *Beauveria* spores (11).

Although the evidence concerning the pathogenicity of *B. bassiana* in the present case is weakened by the isolate’s failure to grow at 35°C and recovery of the fungus from only one site, it is strengthened by the histopathology revealing extensive soft tissue infiltration and invasion of blood vessels and by similarities with the case reported by Henke et al. (3) of invasive *Beauveria* infection in a leukemia patient.

**Nucleotide sequence accession number.** The sequence of the nuclear ribosomal ITS region from the case isolate was deposited in GenBank with the accession number AY513236.

We thank Maria Johnston, Department of Microbiology, Dunedin Public Hospital, New Zealand, and Ben Wilson, Department of Biological Sciences, University of Alberta, Edmonton, Canada. Special appreciation is expressed to Connie Fe C. Gibas, Microfungus Collection, University of Alberta, for assistance with sequencing the case isolate.

Financial assistance to L. Sigler from the Natural Sciences and Engineering Research Council of Canada is gratefully acknowledged.

**REFERENCES**