Pneumonia Caused by *Cunninghamella bertholletiae*
Complicating Chronic Lymphatic Leukemia

TIMOTHY E. KIEHN,1,2* FITZROY EDWARDS,1,2 DONALD ARMSTRONG,1,2 PAUL P. ROSEN,3 AND IRENE WEITZMAN

Diagnostic Microbiology Laboratory, Memorial Hospital,1 and Infectious Disease Service, Departments of Medicine2 and Pathology,3 Memorial Sloan-Kettering Cancer Center, New York, New York 10021; and Bureau of Laboratories, New York City Department of Health, New York, New York 10016

Received for publication 19 June 1979

A case of pneumonia caused by *Cunninghamella bertholletiae* is described in a patient with chronic lymphatic leukemia. The species of *Cunninghamella*, a genus in the order Mucorales, are characterized by the formation of conidia on the surface of an inflated conidiophore (vesicle). *C. bertholletiae*, not *C. elegans*, is the most appropriate name for this fungus. This zygomycete was resistant by in vitro sensitivity testing to amphotericin B. The source of the infection is not known.

There have been two reports of pulmonary infections due to *Cunninghamella elegans*: one in a patient with chronic myelogenous leukemia (4) and one infection, which became disseminated, in a patient with lymphosarcoma (3). This case of pneumonia is the first reported case of a *Cunninghamella* sp. infection in a patient with chronic lymphatic leukemia, and the second in this hospital, where we have documented an increase in infection with members of the order Mucorales (5). The mycological characteristics of this mold are described, and its identification to the species level is discussed.

**MATERIALS AND METHODS**

**Case report.** A 53-year-old black man with chronic lymphatic leukemia was admitted to Memorial Sloan-Kettering Cancer Center on 9 February 1978 for the seventh time because of generalized enlargement of lymph nodes. His present illness started 4 years and 1 month before admission, when he presented to a physician because of generalized lymphadenopathy. Evaluation revealed chronic lymphatic leukemia, stage III. He was given various chemotherapeutic agents, including 1,3-bis-(2-chloroethyl)-1-nitrosourea, cytoxan, alkeran, and prednisone.

His first Memorial Sloan-Kettering Cancer Center admission was in December 1975 because of fever and pneumonia. A specific microbial diagnosis was not made, but the patient responded to broad-spectrum antibiotics. His second admission was in October 1976 because of *Pseudomonas aeruginosa* pneumonia with empyema and bacteraemia. This responded to gentamicin and carbenicillin. He required further chemotherapy for his chronic lymphatic leukemia and was readmitted again in December 1976 and July 1977, the first time for fever of uncertain origin, which responded to broad-spectrum antibiotics, and the second time for *P. aeruginosa* bacteraemia, presumably from recurrent empyema, which responded to gentamicin and carbenicillin. In December 1977 he was admitted for cellulitis of the groin and a draining inguinal lymph node. Cultures of the drainage revealed enterococcus and *Staphylococcus epidermidis*, and blood cultures were negative. He was treated with a variety of antibiotics and radiotherapy to the inguinal lymph nodes. His seventh and last admission on 9 February 1978 was prompted by failure to respond to outpatient chemotherapy. Physical examination showed blood pressure of 90/70 mm Hg, pulse rate of 80/min, temperature of 38.5°C, and respiratory rate of 19/min. Lymph nodes in the neck, axilla, and groin were visibly enlarged and varied from 2.5 to 7 cm in diameter. The liver and spleen were enlarged to 6 cm below the costal margins. A testicular ulcer over an area of tender swelling was noted. The laboratory findings were: hemoglobin, 7.1 g/100 ml; hematocrit, 22.4 ml/100 ml; leukocytes, 50,000 per mm³; 100% lymphocytes; platelets, 167,000 per mm³. Urinalysis was unremarkable, and a culture was negative. Chest X-ray showed hilar and mediastinal adenopathy. Biochemical tests revealed an alkaline phosphatase of 126 IU. Gram stain of the testicular ulcer exudate revealed mononuclear cells, gram-negative rods, and gram-variable cocci. The culture yielded *P. aeruginosa* sensitive to gentamicin and resistant to carbenicillin. He was treated with gentamicin, carbenicillin, and clindamycin and showed prompt defervescence. The testicular ulcer became smaller, and the discharge decreased. On 20 February 1978, bleomycin (20 mg/m² per day) for 7 days was started. Fever to 39°C returned. A blood culture on 21 February 1978 yielded *Pseudomonas maltophilia* sensitive only to chloramphenicol. Colistin, sulfa-trimetoprim, and chloramphenicol were started. The fever resolved when bleomycin was stopped but recurred again and persisted throughout the remainder of his admission despite the addition of gentamicin, carbenicillin, oxacillin, and ethambutol. On 15 March 1978 a lumbar puncture was performed. Pressure was 160 mm Hg. There were no cells present, glucose was 98 mg/100 ml, and protein was 17 mg/100 ml. Microbiological studies were negative.

All
antibiotics were stopped. Nausea and vomiting occurred, and the patient was described as tremulous. Penicillin, gentamicin, and sulfa-trimethoprim were started. There was no response. On 19 March 78 the patient died.

**Autopsy findings.** The autopsy revealed persistent leukemic infiltration of numerous lymph nodes, liver, and spleen. Leukemic involvement of the bone marrow was also apparent. However, normal hematopoietic elements were still identifiable in diminished numbers. Also of note were a 1-cm cortical mass of the right adrenal gland and a microscopic focus of well-differentiated prostatic adenocarcinoma.

Fibrinous and delicate fibrous adhesions were found in both thoracic cavities, but were more prominent on the left. The major pulmonary vessels were patent. In addition to diffuse congestion, discrete, dark-red, firm nodular areas were present predominantly in the lower lung fields. These measured up to 4 cm in greatest diameter. Some extended to the pleural surface and were associated with the pleural adhesions.

Histological examination of the nodular pulmonary lesions revealed hemorrhage and coagulation necrosis. Pulmonary vessels in these lesions were occluded by dense masses of tangled, broad, aseptate fungal hyphae, entrapped erythrocytes, fibrin, and numerous small lymphocytes (Fig. 1 and 2). The morphological features of the hyphae were more easily appreciated in sections stained with Gomori methenamine silver (Fig. 3). They proved to be nonseptate, wavy structures resembling ribbons, an appearance typical of the zygomycete group. Extension of hyphae to involve lung parenchyma surrounding the occluded vessels was also apparent. The fungal infection was limited to the areas of grossly identifiable infarction. Intervening areas of lung featured intrapulmonary hemorrhage. Tissue was sent to the Fluorescent Antibody Laboratory, Developmental Mycology Unit of the Mycology Division, Center for Disease Control, Atlanta, Ga., where the diagnosis of zygomycete infection was confirmed. No gross or microscopic abnormalities were found in the brain or spinal cord.

**Culture and antibiotic methods.** Lung tissue from autopsy was homogenized and inoculated onto Sabouraud dextrose agar and Sabouraud dextrose agar with chloramphenicol and cycloheximide. Sabouraud dextrose agar was incubated at 25 and 37°C; the agar with antibiotics was incubated at 25°C. After initial isolation, the fungus was observed and measured after incubation at 25°C for 5 to 7 days on modified MDA agar.
agar (2) and on potato dextrose agar (Difco). Material for microscopic examination was prepared in lactophenol cotton blue mounts. Observations and measurements of conidia were accomplished from water mounts. Temperature tolerance was determined at incubation temperatures of 25, 37, and 42°C. Our isolate was paired with cultures of C. elegans, C. bertholletiae, and C. polymorpha on potato dextrose agar at 25°C to induce formation of zygospores.

Susceptibility to amphotericin B was determined by the method of Shadomy and Espinel-Ingroff (7).

The isolate was sent to John J. Ellis, Northern Regional Research Laboratory, U.S. Department of Agriculture, Peoria, Ill., and Robert A. Samson, Centraalbureau voor Schimmelcultures, Baarn, The Netherlands, for confirmation of identification.

RESULTS

The mold grew on Sabouraud dextrose agar at 25 and 37°C after 24 h of incubation. There was no growth on the Sabouraud dextrose agar with chloramphenicol and cycloheximide. Colonies were light gray on modified MDA agar with good and rapid growth up to 2 cm high at 25°C, 1.3 cm at 37°C, and up to 1.0 cm at 42°C after 72 h. Figure 4 shows the growth on an MDA agar plate at 4 days. Hyphae were hyaline, granular, and filled with oil globules. Conidiophores were erect, were diversely and irregularly branched (Fig. 5), and exhibited dichotomous, trichotomous, verticillate, sympodial, and solitary branching. Lateral branches were up to 77 μm long but usually less than 50 μm. Terminal vesicles were globose to subglobose, smooth to verrucose, and up to 55 μm in diameter (Fig. 6). Lateral vesicles were globose to oval, smooth, and 18 to 30 μm in diameter. Conidia were globose, oval, lacrymoid to ellipsoidal (ellipsoidal conidia were found only on mature terminal vesicles), mostly smooth, some asperulate, hyaline to light tan, and brown in mass, often with granular contents, and they ranged in size from 4 to 15 μm in length to 3 to 11 μm in width (Fig. 7). Colonies on potato dextrose agar at 25°C grew up to 1.2 μm high and produced lateral branches up to 51 μm long, terminal vesicles up to 51 μm in diameter, and lateral vesicles 12 to

![Fig. 2. Higher magnification of contents of vessel from Fig. 1. The hyphae are broad, nonseptate wavy structures. Hematoxylin and eosin, x140.](http://jcm.asm.org/.../May%2020,2021)
27 μm in diameter. Conidia ranged in size from 4 to 16 μm long by 4 to 12 μm wide. Chlamydospores were not observed.

The fungus is heterothallic. Zygospores were produced when the mold was crossed with two strains of C. bertholletiae, NRRL 1375+ and NRRL 1379+, and with C. polymorpha strain NRRL 6428, which corresponds to NRRL

Fig. 3. Hyphae stained with Gomori methenamine silver reaction; ×140.

Fig. 4. Three-day-old colony of the Cunninghamella isolate on MDA agar medium.

Fig. 5. Conidiophores of the Cunninghamella species produced on MDA agar; ×100.
A69721 and Centraalbureau voor Schimmelcultures (CBS) 779.68 (6). Zygospores were not observed in crosses with tester strains of C. elegans NRRL 1392+, NRRL 1393−, NRRL 1388+ and NRRL 1389−. Most of the strains of C. bertholletiae and the two isolates of C. polymorpha also produced zygospores in the intra-specific crosses.

In vitro sensitivity tests with amphotericin B indicated that 100 μg of the antibiotic was needed to inhibit growth of the organism.

**DISCUSSION**

*Cunninghamella* species are members of the order *Mucorales* in the class *Zygomycetes*. The class is characterized by sexual reproduction in which a zygospore is produced from gametangial copulation. Asexual reproduction occurs by the production of nonmotile sporangiiospores or conidia. These fungi are saprophytes living primarily on decaying plant and animal matter and are commonly found in soil.

The genera *Mucor*, *Rhizopus*, and *Absidia*, which are in the family *Mucoraceae* (1), have been responsible for the majority of infections caused by the members of the order *Mucorales*. The members of this family, which is the most primitive of the order, form asexual sporangiiospores within a globose sporangium, at the tip of a sporangiophore. The sporangium may contain hundreds of spores. The asexual spores of the genus *Cunninghamella*, in the family *Cunninghamellaceae*, are conidia borne on the surface of inflated conidiophores.

Our isolate possessed the characteristics of the genus *Cunninghamella*. Dr. Ellis identified our isolate as *C. elegans* (*C. bertholletiae*, if one considers, as we do, that this is a distinct species and not a variant of *C. elegans*). Dr. Samson called it *C. polymorpha*, since the matings with *C. elegans* did not result in zygospores. We feel that the appropriate name is *C. bertholletiae*. Our isolate did not mate with tester strains of *C. elegans*, but did mate with two cultures of *C. bertholletiae*, mating type (+), and with a culture of *C. polymorpha*. *C. bertholletiae* does not mate with *C. elegans* (2), but one isolate produced zygospores in a cross with the neotype strain of *C. polymorpha*. In addition, *C. elegans* does not grow at 40°C (2), whereas our isolate and some isolates of *C. bertholletiae* and *C. polymorpha* grow well at 40°C.

This taxonomic uncertainty was resolved in studies reported in a recent paper by Weitzman and Crist (Mycologia, in press). Clinical isolates of *Cunninghamella* species were identified as *C. bertholletiae* based on mating reactions and temperature tolerance. Weitzman and Crist concluded that *C. bertholletiae* is a distinct species and not a synonym of *C. elegans* and that *C. polymorpha*, as redescribed by Samson and represented by his designated neotype strain (6), is not a valid species.

This patient was similar to those previously
described at this hospital who were infected with members of the order Mucorales (5). The molds, when isolated in those cases, proved to be among the more commonly identified species of Rhizopus, Mucor, or Absidia. This patient was on intensive immunosuppressive therapy, was extremely neutropenic, and had been receiving broad-spectrum antibiotic therapy over a prolonged period of time. It is not clear whether the infection was contracted in the hospital, although the patient had been hospitalized for more than 4 weeks before his death. It is certainly possible that the patient was colonized before entering the hospital and then became invasively infected.

There have been no patients successfully treated for infection with this fungus. In vitro studies of this isolate suggest that amphotericin B would be ineffective.

LITERATURE CITED