JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 1995, p. 735–737 Vol. 33, No. 3
0095-1137/95/$04.00+0

Disseminated *Microascus cirrosus* Infection in Pediatric Bone Marrow Transplant Recipient

KAREN K. KRISHER,[1]* NINA B. HOLDRIDGE,[1] MAHMOUD M. MUSTAFA,[1]
MICHAEL G. RINALDI,[2,3] AND DEANNA A. MCGOUGH[2]

Clinical Microbiology, Children's Medical Center of Dallas, Dallas,[1] Fungus Testing Laboratory, Department of Pathology, University of Texas Health Science Center at San Antonio,[2] and Audie L. Murphy Memorial Veterans' Hospital,[3] San Antonio, Texas

Received 21 September 1994/Returned for modification 19 October 1994/Accepted 29 November 1994

Microascus cirrosus **Curzi and its associated anamorphic state, *Scopulariopsis*, were recovered from the cutaneous lesion of a 12-year-old male who had undergone an autologous bone marrow transplantation for acute myelogenous leukemia. Histopathology sections from the biopsied lesion demonstrated septate hyphae consistent with a fungal etiology. Radiographic studies of the lungs subsequent to progression of the lesion revealed a consolidation in the right upper lobe suggesting a primary focus of infection. While *M. cirrosus* is fairly abundant in nature and widely distributed in stored grains in North America, this is, to our knowledge, the first reported human infection by this species. Salient characteristics for the identification of this dematiaceous ascomycete, *M. cirrosus*, will be presented.**

Several case reports, while lacking convincing direct microscopy, have suggested the role of various *Scopulariopsis* species as etiologic agents in opportunistic human and animal disease (1, 6, 10). We report a case of invasive cutaneous and pulmonary fungal infection caused by a *Scopulariopsis* species and its teleomorph, *Microascus cirrosus*, in a pediatric bone marrow transplant recipient.

Case report. A 12-year-old male with secondary acute myelogenous leukemia received a purged autologous bone marrow transplant in March, 1992. His preparative regimen consisted of total body irradiation, etoposide, and cyclophosphamide. Antimicrobial prophylaxis included acyclovir, clotrimazole troches, and intravenous immunoglobulin administration. The patient's room was equipped with a high-efficiency particulate air filter. His early posttransplantation course was complicated by severe mucositis and prolonged fever and neutropenia. Empiric antibiotic therapy consisted of vancomycin, ceftazidime, and tobramycin. On day 12 posttransplantation, 1 mg of amphotericin B per kg of body weight per day was added to the antibiotic regimen because of persisting fever and neutropenia. No bacteria or viruses were isolated from the blood, urine, or tracheal aspirate specimens. Roentgenograms of both the chest and sinuses were normal. Absolute neutrophil counts ranged from 100 to 112/mm^3 throughout the patient's hospitalization.

On day 19 posttransplantation, a 1-cm erythematous skin macule was noted on the right leg below the knee. The lesion progressively became indurated, dusky, and tender. Sections were prepared from the biopsied specimen and stained with periodic acid-Schiff, hematoxylin-eosin, and Gomori-methenamine silver stains. Microscopic examination of all sections, as well as a homogenate of the biopsy specimen stained with KOH-Calcofluor, revealed branching, septate hyphae and moniliform fungal elements (Fig. 1). Additional slides cut from the same block to document the moniliaceous-dematiaceous nature of the hyphae with the Fontana-Masson stain failed to reveal any more fungal elements, and thus, melanin was not demonstrated. Because of these findings, a radiological examination of the brain sinuses, lungs, and abdomen was performed. The dosage of amphotericin B was increased to 1.2 mg/kg/day following the detection, by computerized tomography (CT), of a mass in the upper lobe of the right lung.

Although the patient tolerated therapy well, the fungal infection continued to progress. By day 34 posttransplantation, the skin lesion appeared larger and necrotic. A repeat CT scan of the lungs revealed an interval increase in the size of the consolidation involving the right upper lobe. Liposomal amphotericin B (Amphocil [amphotericin B collodial dispersion]; Liposome Tech. Inc., Menlo Park, Calif.) therapy was then initiated at 6 mg/kg/day. After 8 days of therapy, and despite persisting neutropenia, the skin lesion decreased in size and became dry and nontender. A resolving right upper lobe infiltrate was demonstrated by both CT scan and chest roentgenogram.

Since the patient remained asymptomatic during the next several weeks, with complete resolution of the skin infection, Amphocil was discontinued. The progress of the patient was monitored for 7 months following discharge, and no clinical evidence was found to indicate a recurring fungal infection. The patient eventually expired as a result of a recurrence of his leukemia. An autopsy was not performed.

Mycology. The biopsied specimen obtained from the cutaneous lesion was inoculated onto Sabouraud dextrose agar and brain heart infusion agar containing 5% sheep blood. Within 1 week of incubation at 30°C., small dark colonies, approximately 3 mm in diameter, were observed growing on the culture medium. Upon extended incubation, these colonies developed a dark, powdery surface which was marked by radial grooves and a slightly raised center. Microscopic examination of potato dextrose agar slide cultures revealed smooth, globose, catenulate annelloconidia, 3 to 4 μm in diameter, originating from single or penicillate flask-shaped conidiophores attached to dematiaceous, septate hyphae, which resembled those found in *Scopulariopsis* species. Within 4 to 5 weeks, small black fruiting structures were observed growing on the surface of a potato dextrose agar plate which had been inoculated from the primary culture. Examination under a dissecting microscope revealed globose perithecia, some of which

* Corresponding author. Mailing address: Clinical Microbiology, Children's Medical Center of Dallas, 1935 Motor Street, Dallas, TX 75235. Phone: (214) 640-2884.

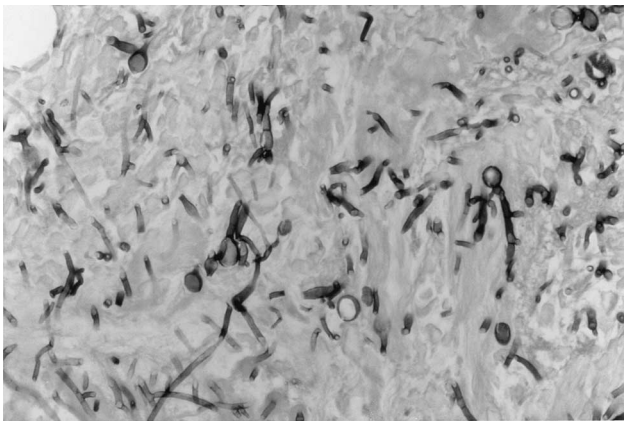

FIG. 1. Section of a biopsy specimen from the cutaneous lesion showing branching, septate hyphae, and moniliform fungal elements (Gomori methenamine silver stain; magnification, ×324).

appeared to be extruding ascospores. The isolate was then submitted to the Texas State Department of Health, Austin, Tex., and to the Fungus Testing Laboratory (FTL), Department of Pathology, University of Texas Health Science Center (UTHSC) at San Antonio, San Antonio, Tex., and it was identified as a *Microascus* sp. by the state laboratory and as *Scopulariopsis brumptii* by the FTL (UTHSC accession number, 92-780).

The isolate was subsequently resubmitted to the FTL in an attempt to clarify the discrepancy in the identification. The mould, which was received on Sabouraud dextrose agar and appeared reddish brown, was inoculated on potato flakes agar (PFA) (7) slide cultures and single plates and incubated at 25°C. Unlike examination of the initial isolate studied, reexamination of the same fungus resulted in observance of perithecia and ascospores in addition to the conidial-anamorphic structures of the *Scopulariopsis* species. Colonies on PFA grew rather slowly, reaching a diameter of 3 to 4 cm in 6 weeks at 25°C. They were initially olivaceous gray in color, later turning olivaceous brown and finally dark to reddish brown. Young cultures exhibited small droplets of a clear exudate at the periphery of the colony. Colonies had a mealy appearance due to the funiculose growth characteristics and were zonate with perithecia developing within these concentric rings. Numerous perithecia, which were often overgrown with the conidial anamorph, were produced. Perithecia were black, superficial, or

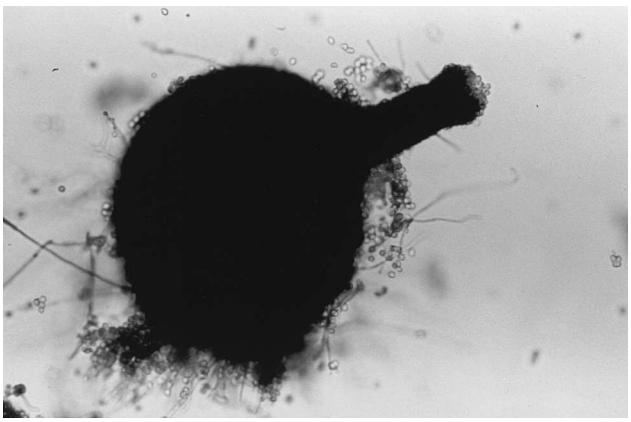

FIG. 2. Perithecium of *M. cirrosus* UTHSC 92-780 showing perithecial neck measuring approximately 40 μm (magnification, ×216).

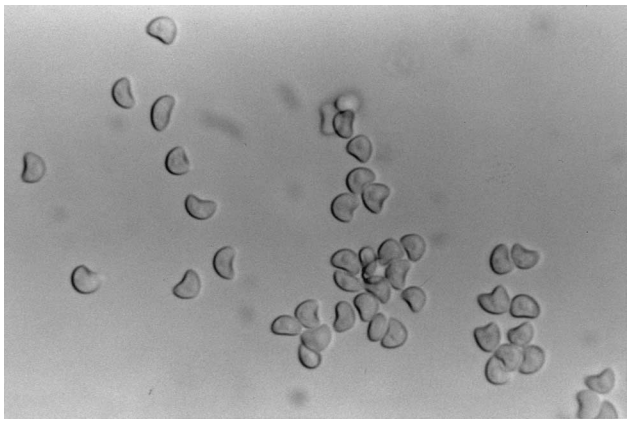

FIG. 3. Straw-colored plano-convex and concavo-convex ascospores of *M. cirrosus* UTHSC 92-780 (Nomarski optics; magnification, ×900).

partially immersed; they were primarily spherical and approximately 175 to 214 μm in diameter, with short, cylindrical necks measuring less than 100 μm in length (most were approximately 40 μm; Fig. 2). At maturity, ascospores were extruded from the perithecia in the form of a long cirrus. Asci were ovate (9 to 14 by 7 to 11 μm) and evanescent. Ascospores appeared light brown, measured 4.5 to 6.5 by 3 to 5 μm, and were variably shaped; i.e., some were plano-convex or slightly concavo-convex, while others appeared almost heart shaped (Fig. 3). Frozen stock cultures of the original isolate, UTHSC 92-780, subcultured onto PFA, also produced characteristic perithecia and ascospores. The dematiaceous *Scopulariopsis* anamorph-conidial phase was also present on PFA. Dark vegetative hyphae bore conidiophores along its length. Annellophores were broader in the center, narrowing toward both ends. Annelloconidia were globose to subglobose in shape, rounded, sometimes slightly pointed at the ends, smooth to delicately roughened, measured 3.5 to 4.5 by 3 to 4 μm, were borne in tangled chains 20 to 50 μm in length, and were pale, gray brown in color. The truncate character of other *Scopulariopsis* spp. is not as well seen in this species (Fig. 4). On the basis of these criteria, according to the key of Morton and Smith (5), the isolate was tentatively identified by the FTL as *Microascus cinereus* (Emile-Weil et Gaudin, Curzi) with its anamorphic form being *Scopulariopsis cinerea* (Emile-Weil et Gaudin) (4), confirming the identification by the Texas State

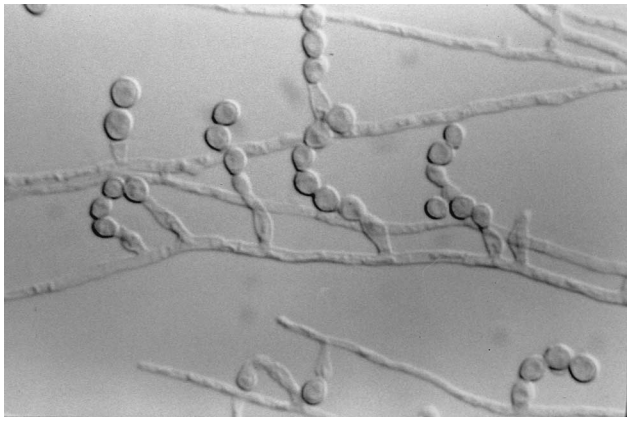

FIG. 4. Vegetative hyphae, annellides, and catenulate annelloconidia of the dematiaceous *Scopulariopsis* anamorph UTHSC 92-780 (Nomarski optics; magnification, ×900).

Department of Health. This isolate was then referred to Lynne Sigler, University of Alberta, Edmonton, Alberta, Canada, for confirmation.

Examination of the isolate there, according to the key for *Microascus* species of Barron et al. (3) and a revision of the genus by von Arx (9), revealed the fungus to be *Microascus cirrosus* Curzi (*Microascus desmosporus* sensu Morton & Smith).

Discussion. The development of cutaneous invasive fungal infections in transplant recipients has been associated with a variety of filamentous fungi including dematiaceous moulds, hyaline hyphomycetes, and dimorphic fungi, as well as yeasts such as cryptococci and *Candida* species. Infections result from either direct skin inoculation or hematogenous spread from a systemic focus. Since *Microascus* spp. are common in the environment, one must be cautious when differentiating true invasive infection from contamination or colonization. A lack of antecedent skin trauma in this patient, however, coupled with observance of fungal elements in the histologic section of the biopsy and isolation of the fungus, suggests that the cutaneous lesions resulted from dissemination of the fungus from the pulmonary site. Radiographic evidence of consolidation in the upper lobe of the right lung, which resolved in response to antifungal therapy, also would lend credence to the hypothesis of a pulmonary focus.

The infection, which persisted despite treatment with amphotericin B, resolved after initiation of therapy with a colloidal dispersion formulation of amphotericin B, Amphocil. Since in vitro resistance to amphotericin B is documented to occur among *Scopulariopsis* spp., one could expect a similar situation with its teleomorph, *Microascus*. Therefore, the use of a liposomal preparation of amphotericin B, which permits escalated dosages because of reduced toxicity, may be more efficacious against this etiologic agent.

The genus *Microascus* was described by Zukal in 1885 (11) with *Microascus longirostris* as the type species. The genus contains 14 described species which are often common soil fungi. They are also quite often isolated from plant seeds and various kinds of animal dung. Many of the species in this genus possess anamorphs (asexual forms) which have been implicated as pathogens of humans and animals. The species described in this case report, *Microascus cirrosus* Curzi (3a), represents the teleomorph (sexual, perfect form), while the dematiaceous *Scopulariopsis* sp. is its associated anamorph. This fungus is of interest not only because of its newly observed status as an agent of invasive mycosis, but also because it is dematiaceous in both of its forms. Most laboratorians equate *Scopulariopsis* species with tan-colored colonies rather than dematiaceous ones. There are other species of *Scopulariopsis* which are darkly pigmented and some of which have been associated with human disease, e.g., *S. brumptii* Salvanet-Duvalin; however, this species has no associated teleomorph. Thus, one could misidentify a dematiaceous *Scopulariopsis* sp. if the teleomorph was not expressed.

Microascus spp. produce ascocarps termed perithecia, which contain asci and evanescent ascospores. The perithecia produce a neck structure which, in combination with the size and shape of the ascospores, is an important criterion for ascertaining the species identity.

Culturally, *M. cirrosus* is very close to *M. cinereus* but differs in the darker color and less-stable habit. Microscopically, differences between these two species are seen in the size and shape of the ascospores, the length of perithecial necks, and the diameter of ripe perithecia. The ascospores of *M. cinereus* are plano-convex or slightly concavo-convex, shaped like segments of an orange and appearing oval in end view. Those of *M. cirrosus* are concavo-convex or almost heart shaped. This species, however, shows a wide variability in ascospore shape and size within and between isolates, making a determination of the predominant size and shape somewhat difficult. In *M. cinereus*, perithecial necks are lacking or very short, and ripe perithecia are larger than those seen in *M. cirrosus* (300 to 350 versus 130 to 220 μm in diameter) (8). While *M. cirrosus* grows at 37°C, it may not produce characteristic perithecia at this temperature.

The genus *Petriella* Curzi is very similar to *Microascus* in the following characteristics: (i) ascocarps are perithecium-like with a well-marked ostiole (opening), (ii) the asci are distributed at all levels within the centrum and are evanescent (released from the ascus before being liberated from the perithecium), (iii) the ascospores are asymmetric in most species, and (iv) at maturity, the ascospores are extruded either in the form of a long cirrus or as a gelatinous ball at the mouth of the ostiole, depending upon environmental conditions. The genus *Petriella* was originally described as distinct from *Microascus* on the basis of the hairy condition of the perithecium in *Petriella* spp. (2). This distinction was not justified, however, as a number of *Microascus* spp. show development of setae (hairs) on the perithecial body or neck. *Petriella* spp. may be distinguished, however, by other criteria: ascospores are much larger and bulkier, averaging 9 to 10 μm long by 4 to 6 μm wide, and are usually red brown in color, while those of *Microascus* are smaller and straw colored; *Petriella* spp. possess *Graphium* and *Sporotrichum* anamorphs while *Scopulariopsis* is the anamorphic stage of *Microascus*, with the single exception of *Microascus stysanophorus*, which has both *Stysanus* and *Sporotrichum* asexual forms.

Conclusion. There is a paucity of information available concerning clinical infections caused by *Microascus* species. Two reports have documented three cases of onychomycosis caused by *M. cinereus* (1, 10). To our knowledge, isolation of *M. cirrosus* has not been previously reported as an etiologic agent of phaeohyphomycosis in an immunocompromised patient. The case presented here reflects yet another example of the ever-escalating number and diversity of fungi capable of inciting disease in the host with an abrogated immune system.

The authors gratefully acknowledge the expertise of Lynne Sigler, Curator, University of Alberta Microfungus Collection and Herbarium, Devonian Botanic Garden, Edmonton, Alberta, Canada, who provided the final identification of this isolate.

REFERENCES

1. **Agarwal, G. P., and S. M. Singh.** 1980. *Microascus cinereus* infection of human nail. Indian J. Med. Sci. **34:**261–265.
2. **Barron, G. L., R. F. Cain, and J. C. Gilman.** 1961. A revision of the genus *Petriella*. Can. J. Bot. **39:**837–845.
3. **Barron, G. L., R. F. Cain, and J. C. Gilman.** 1961. The genus *Microascus*. Can. J. Bot. **39:**1609–1631.

3a. **Curzi.** 1930. Boll. Stn. Patol. Veg. Roma **10:**302–310.

4. **de Vries, G. A.** 1983. Ascomycetes: Eurotiales, Sphaeriales, and Dothideales, p. 81–111. *In* D. Howard (ed.), Fungi pathogenic for humans and animals, part A. Biology. Marcel Dekker, Inc., New York.
5. **Morton, F. J., and G. Smith.** 1963. The genera *Scopulariopsis* Bainer, *Microascus* Zukal, and *Doratomyces* Corda. Commonw. Mycol. Inst. Mycol. Pap. **86:**1–96.
6. **Neglia, J. P., D. D. Hurd, P. Ferrieri, and D. C. Snover.** 1987. Invasive *Scopulariopsis* in the immunocompromised host. Am. J. Med. **83:**1163–1166.
7. **Rinaldi, M. G.** 1982. Use of potato flakes agar in clinical mycology. J. Clin. Microbiol. **15:**1159–1160.
8. **Udagawa, S. I.** 1962. *Microascus* species new to the mycoflora of Japan. J. Gen. Appl. Microbiol. **8:**39–51.
9. **von Arx, J. A.** 1975. Revision of *Microascus* with the description of a new species. Persoonia **8:**191–197.
10. **Wheat, L. J., M. Bartlett, M. Ciccarelli, and J. W. Smith.** 1984. Opportunistic *Scopulariopsis* pneumonia in an immunocompromised host. South. Med. J. **77:**1608–1609.
11. **Zukal, H.** 1885. Ueben einige neue Pilze, Myxomyceten, und Bakerien. Verhandl. Zoolbotan. Ges. Wein. **35:**333–342.