Phialophora repens, an Emerging Agent of Subcutaneous Phaeohyphomycosis in Humans

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A 63-year-old Japanese man had phaeohyphomycosis that occurred as a solitary subcutaneous nodule on the dorsal aspect of his left hand. In the nodule there were foci of mixed granulomatous and supplicative infiltrations circumscribed by thick fibrous tissue reaction. The foci contained short septate hyphae and occasionally small rounded aggregates of irregularly branched septate hyphae, both of which were nonpigmented or rarely weakly pale brown. Fungal culture from the nodule was positive for a dematiaceous mold. The mycologic features of the mold were typical of Phialophora repens. The infection was successfully treated by excision of the nodule. This is the second reported case of infection due to P. repens.

Phialophora repens is a dematiaceous mold commonly found in nature as a cause of bluish gray stain of logs and lumber (5, 6). The mold was not recognized as an opportunistic pathogen for humans until 1975, when Meyers et al. (15) first reported a case of mycotic granuloma caused by P. repens. The infection occurred as a subcutaneous granulomatous nodule in the scalp of an adult man with advanced lepromatous leprosy. Although the lesion did not resemble mycetoma grossly, it was suggestive microscopically by the presence in some granulomatous foci of small aggregates of hyphae similar to grains of eumycotic mycetoma. This P. repens infection was redescribed later by Emmons et al. (8) as being a case of phaeomyocytic cyst.

Recently we encountered a second case of subcutaneous phaeohyphomycosis caused by P. repens in an adult man. The purpose of this paper is to present the morphologic characteristics of this isolate, a clinical pathologic report of this case, and a review of the pertinent literature.

CASE REPORT

A 63-year-old Japanese man was admitted to the hospital on 3 October 1983. Physical examination revealed a 1.3- by 1.5-cm elevated, erythematous nodule on the dorsal aspect of his left hand. The patient was otherwise healthy. The nodule had begun, with no noticed antecedent trauma at the site, about two months earlier and gradually increased in size. There were no draining sinuses or grains. The routine laboratory examinations and immunologic tests in the patient disclosed no abnormality except for evidence of mild diabetes mellitus. The nodule was excised for examination immediately after the admission, but no microbial culture was made.

Examination of hematoxylin- and eosin-stained tissue sections of the excised nodule revealed a well-delineated granulomatous lesion extending from the deep dermis into the subcutaneous tissue. The subcutaneous tissue was focally fibrotic, and in the areas of fibrosis there were circumscribed small foci of mixed granulomatous and supplicative inflammations (Fig. 1). Palisading epithelioid cells, giant cells, and lymphocytes surrounded central microabcesses composed of polymorphonuclear leukocytes. The microabcesses contained a few, or rarely numerous, short septate hyphae and occasionally loose hyphal masses, both of which were nonpigmented or rarely weakly pale brown. The masses were rounded aggregates of irregularly branched septate hyphae of 2.5 to 5 μm width often with enlarged terminal cells, measuring 200 to 300 μm (Fig. 2). They looked like the grains of eumycotic mycetomas but did not manifest the Hoeplli-Sprendore phenomenon sometimes seen in the actinomycotic or eumycotic mycetomas. The free hyphae were 2.5 to 3 μm wide and were also seen among the epithelioid cells that bordered the granulomatous foci.

About 1 month after the surgery, a small similar nodule recurred within the subcutaneous scar of the last operation.

FIG. 1. Granulomatous foci circumscribed by thick fibrous tissue reaction and containing small rounded aggregates of hyphae. Bar, 200 μm. Periodic acid-Schiff stain.
so that skin biopsy and fungal culture were performed. Examination of the biopsy specimens revealed a few short hypheae in the nodule. Also, the biopsy was culture positive for a dematiaceous mold (SM 3531) identified as *P. repens*. The organism was identified on the basis of the morphologic characteristics described and discussed below.

Oral administration of 7.5 g of flucytosine daily was started in the patient but discontinued 3 months later because only a slight clinical improvement had been noted. The MIC of flucytosine for the isolated organism was 60 μg/ml when tested later by the method of Shadomy and Espinel-Ingroff (20). Ultimate cure was achieved with additional surgical excision of the reccured nodule.

The isolate of *P. repens* was subcultured under various conditions and maintained in our mycology laboratory at the Department of Dermatology, Shiga University of Medical Science, as no. SM 3531. The living culture of this isolate has been deposited in the American Type Culture Collection, Rockville, Md., with the accession number ATCC 58115.

**MATERIALS AND METHODS**

Reference strains were supplied by Michael R. McGinnis at the University of Texas Medical Branch, Galveston. The strains and their sources were as follows: *P. repens* CBS 294.39 (UTMB [University of Texas Medical Branch] 227), isolated from pine lumber (used to prepare nomenclatural type) (6); *P. repens* CBS 423.73 (UTMB 184), from mycotic granuloma (15).

The organisms were studied after 1 to 4 weeks of incubation on potato glucose agar (Difco Laboratories) at 25°C or, if necessary, at 37°C. Slide culture preparations were examined by bright-field and Nomarsky differential interference contrast microscopies.

Scanning electron microscopy (SEM) was performed as follows. Briefly, small squares of sporulating thallus were gently cut out of the colony of the organism examined, including the supporting agar block, after incubation at 25°C. The specimens were fixed in 5% glutaraldehyde at 3°C for 12 h and further in 1% osmium tetroxide at 3°C for 3 h, dehydrated in a graded ethanol series, and then washed in increasing concentrations of amyl acetate in absolute ethanol and finally in absolute amyl acetate. The materials were quickly dried with a critical point dryer (JEE-4C), coated with gold by using an ion IB-3 (Eiko Engineering Ltd.), and examined with a scanning electron microscope S-500S (Hitachi) generator operated at 10 kV.

**RESULTS**

Colony morphology. The new isolate SM 3531 (ATCC 58115) grew moderately well at 25°C on potato glucose agar; the colony attained a diameter of 2.8 cm after 1 week, 4.2 cm after 2 weeks, and 7.2 cm after 4 weeks. The colony was flat, moist, and whitish at first and became brown, dark brown to olivaceous brown, and velvety to more or less cottony later, with tufts of grayish white aerial hypheae (Fig. 3). The reverse side was brown, dark olivaceous brown to nearly black with age. The isolate was able to grow at 37°C, but the growth rate was reduced by about one-half that at 25°C.

SM 3531 was closely similar to the reference strain CBS 423.73 in the color and texture of colonies, although the...
growth rate was slightly higher in the former. However, the type strain CBS 294.39, as presently examined, was no longer typical and formed a rapidly growing, pale brown to grayish brown colony with some farinose aerial hyphae and similarly colored reverse.

**Microscopic morphology.** The new isolate SM 3531 produced septate hyphae that were hyaline at first and pale brown later, smooth, 2 to 4 \( \mu \text{m} \) wide, and often fasciculated (Fig. 4). The conidiophores were hyaline to pale brown, smooth, and undifferentiated. The conidiogenous cells were intercalary, hyaline to pale brown, smooth, 6 to 30 \( \mu \text{m} \) long, and 1.2 to 3.2 \( \mu \text{m} \) wide just above the base; they were monophialidic or sometimes polyphialidic and lageniform to elongate and apically had short inconspicuous or slightly flared collarettes of 1 to 1.8 \( \mu \text{m} \) width (Fig. 4 through 8). Coiled hyphae with phialides were typically formed. SEM of the conidiogenous cells revealed an enteroblastic phialidic manner of conidial development in this isolate. The lageniform or cylindrical phialides ended in short tubular or slightly flared collarettes without constriction at the neck; the secondary conidial initials appeared to emerge in basic

**DISCUSSION**

*P. repens* was described as a new species of the genus *Cadophora* Lagerberg by Davidson in 1935 (6). The fungus was later placed in the genus *Phialophora* Medlar by Conant (5) and subsequently discussed in reviews written by various workers (3, 4, 7, 12, 18). Typically penicillate bushes of phialides were originally described in the type strain (CBS 294.39) (6) and have been considered characteristic of *P.
repens (4, 7, 18). However, we could find no penicillate conidiophores with phialides in our new isolate SM 3531 (ATCC 58115) or in the type strain. As pointed out by de Hoog (7), whether this branching pattern has taxonomic significance still needs examination of additional isolates. The microscopic morphology of SM 3531 was otherwise identical with that of the type strain and of the isolate of Meyers et al. (CBS 423.73) (15).

Some species of Phialophora Medlar or Lecythophora Nannfeldt (9) microscopically mimic P. repens in some degree, so that identifying isolates of these species is rather difficult (3, 4, 12, 21). The difficulty is also due to the fact that the phialides formed by these species are often variable in shape and size in different strains of each species or even with the ages of cultures.

The closest species to P. repens is Phialophora parasitica, although as a rule P. repens produces more delicate conidiophores and phialides than the latter. P. parasitica is characterized microscopically by the encrustations of hyphae and conidiophores, the collarettes with a somewhat constricted neck, and the sometimes proliferating phialides; in contrast, P. repens lacks all of these characteristics (9, 12, 21). Since our new isolate SM 3531 (ATCC 58115) also lacks all of such microscopic characteristics, it does not appear to be conspecific with P. parasitica.

Lecythophora hoffmannii and L. mutabilis are also somewhat close to P. repens but can be distinguished by the
slimy, pink to olivaceous brown colonies and by the predominantly forming adelphialides with reduced collarettes. *L. mutabilis* is also distinct by the centrally brown colonies that are due to chlamydospores (7, 9, 12, 18). Our isolate SM 3531 is also not compatible with these two because of the differences in colony and microscopic morphology.

Meyers et al. (15) were the first to report *P. repens* infection in humans. In the report, they clearly noted that there were hyphae and occasionally microcolonies of a nonpigmented fungus in tissue sections. Nevertheless, Emmons et al. (8) described later that the fungal elements were often nonpigmented and listed *P. repens* as an agent of phaeomycotic cyst. Likewise, most medical mycologists have regarded *P. repens* as an agent of phaeohyphomycosis (1, 12, 17). Our histologic observations described herein also afford evidence that the hyphal walls of *P. repens* are rarely or partially dematiaceous in the human subcutaneous tissue.

In some cases of phaeohyphomycosis, the dematiaceous nature of the fungal elements is not apparent in tissue sections and is revealed only later in culture (17). As in our case, however, careful examination of hematoxylin- and eosin-stained tissue sections or unstained sections often reveals a few hyphae that are dematiaceous (1). Moreover, the pigment of hyphal walls of a fungus may be variable in tissue of different organs as well as even in its different strains. In fact, *Bipolaris spicifera* was seen as brown-pigmented hyphae in the skin or the cornea (13, 23) but formed nonpigmented hyphae when it invaded the brain (22).

The inflammatory reaction elicited by *P. repens* is a mixed granulomatous and suppurative one and does not differ from that in cases of phaeohyphomycosis caused by *P. parasitica* (2), *Phialophora richardsiae* (19), *Exophiala jeanselmei* (10), *E. monilae* (11, 14), *E. spinifera* (16), or other dematiaceous molds (17). *P. repens* infection, however, can be differentiated histologically by the unique aggregates of hyphae in tissue sections. Phaeohyphomycosis caused by *P. repens* thus appears somewhat atypical and intermediate between phaeohyphomycosis and eumycotic mycetoma.

Our patient had *P. repens* infection despite the absence of predisposing factor except for mild diabetes mellitus. The role of the diabetes mellitus for establishing the fungus infection in the patient seems minor. In general, phaeohyphomycosis due to *P. repens*, whether in a normal host or in an immunologically compromised host (15), is relatively benign and good in prognosis.

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**LITERATURE CITED**