Rhizopus rhizopodiformis: Emerging Etiological Agent of Mucormycosis

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Mucormycosis is caused principally by members of the genus *Rhizopus*, especially *R. arrhizus* and *R. oryzae*. Infection attributable to *R. rhizopodiformis* has rarely been documented. Of 13 cases of mucormycosis diagnosed during a 4-year period (1974 to 1978) at The Mount Sinai Hospital, 6 cases, occurring within 9 months, were caused by *R. rhizopodiformis*. The six isolates were identified mainly by: growth at 50°C; production of short, sometimes branched, sporangio-phores arising from opposite rhizoids; elongated columellae; and small spherical-to-elliptical, smooth-to-finely striated sporangiospores. The possibility that this explosive occurrence of *R. rhizopodiformis* at our institution was because of nosocomial acquisition was strongly supported by the recovery of this same mycotic agent from adhesive bandages used in the cardiac intensive care unit, where a patient developed subcutaneous *R. rhizopodiformis* infection after cardiac surgery. The invasive potential of *R. rhizopodiformis* was manifested by the extensive subcutaneous and systemic infections in each of the six patients, three of whom developed antibody against this mucormycotic agent.

Mucormycosis, according to Baker (1), "nearly always has a predisposing factor of disturbed metabolism, blood dyscrasia, nutritional disturbance, or corticosteroid drug therapy." This disease is thus seen principally in patients with diabetes mellitus, acute and chronic leukemia, and lymphoma. Mucormycosis may also be encountered in patients with extensive burns (12).

Etiologically, mucormycosis has been caused chiefly by members of the genera *Rhizopus*, *Mucor*, and *Absidia*. Diagnosis is usually achieved postmortem by histological examination of tissue specimens. When material for culture has been obtained, the agents most frequently recovered have been *R. arrhizus* and *R. oryzae* (2).

Mucormycosis in humans that is attributable to *R. rhizopodiformis* has been very rare, documented on only one previous occasion (3). In contrast, we have recently recovered this species as the causative agent of mucormycosis in six patients, all diagnosed during life. This report presents the mycology and epidemiology associated with this emerging agent of mucormycosis.

MATERIALS AND METHODS

Six specimens subsequently revealing *R. rhizopodiformis* (Table 1) were obtained by biopsy of skin or nasal turbinates, percutaneous lung aspiration and biopsy, or by fiberoptic bronchoscopy. These were first

examined by phase-contrast microscopy (×400) and then inoculated to duplicate plates of 5% sheep blood (Baltimore Biological Laboratory) and to Sabouraud's dextrose agars (Difco Laboratories). Media were incubated at 22 and 37°C and examined after 24 and 48 h.

After initial isolation and characterization of the isolates as *Rhizopus* sp., subcultures were made onto potato dextrose agar and onto the yeast extract agar of Haynes et al. (7). These media were used to study the cultural and microscopic features of the isolate necessary for identification to species level. The maximum temperature of growth was determined by incubating inoculated media for 7 days at 25, 37, 45, and 50 to 51°C. Size of sporangia, sporangiospores, and sporangiophores was determined after 7 days of incubation of yeast extract agar at 37°C and after 7 to 10 days of incubation of potato dextrose agar at 25°C. Measurements and examination of sporangiospores for color, shape, and the presence of striations were made by suspending sporangiospores in water. All other microscopic characteristics were studied after the sporangiospores were mounted in lactophenol. The height of growth was determined after 7 days of incubation of inoculated butts of both potato dextrose and yeast extract agars.

Sera from five patients (no. 1, 3, 4, 5, and 6) were assayed for antibody to *Rhizopus*, *Mucor*, and *Absidia* through the courtesy of Morris A. Gordon of the New York State Department of Health, Albany, N.Y.

RESULTS

Direct phase-contrast microscopic examination of each of the six clinical specimens showed

numerous broad, nonseptate hyphal elements with nearly perpendicular branching. These observations were further enhanced by heating the specimens with 10% KOH before microscopic examination. Similar hyphal elements were demonstrable in histological sections of all six specimens.

Culturally, the first suggestion of fungal

TABLE 1. Source of clinical isolates of *R. rhizopodiformis*^a

Patient no.	Age (yr)	Associated disease ^b	Site of involvement	Source of specimen
1	49	ALL	Perinasal orbit	Biopsy of nasal turbinates
2	54	AML	Skin	Scrapings from necrotic lesion
3	67	Cardiac surgery	Skin	Biopsy
4	30	Renal transplant	Skin	Biopsy of subcutaneous tissue
5	45	Renal transplant	Lung	Bronchoscopic aspirate, biopsy
6	49	AML	Lung	Percutaneous lung biopsy

^a All patients tested were males.

^b ALL, Acute lymphocytic leukemia; AML, acute myelogenous leukemia.

growth usually occurred with 24 h. Growth was noticeable on blood and Sabouraud media as finely radiating filaments from a more dense central core of growth. Microscopically, this surface growth was composed of broad, ribbon-like, coenocytic hyphae with right-angled branching and pronounced cytoplasmic streaming.

The fungus grew more rapidly at 37°C than at room temperature and, after only 48 to 72 h at 37°C, the entire agar surface was enveloped in cottony, black-speckled aerial hyphae which impinged against the underside of the petri dish cover. Microscopic examination of teased aerial growth revealed numerous sporangia-bearing sporangiophores, some branched and arising directly opposite the rhizoids (Fig. 1) or occasionally from arching filaments (stolons) (Fig. 2). These morphological features observed in all six isolates characterized them as a *Rhizopus* species.

On subculture, all six isolates grew profusely at 25, 37, 45, and 50–51°C. Growth and sporulation at 50°C, however, were not as luxuriant as those observed at 25 to 45°C. The height of colonies on the butt cultures measured only 1 cm or less. Growth was floccose and charcoal gray to almost black. Sporangia were brown to

FIG. 1. Sporangiophores of *R. rhizopodiformis* arising opposite rudimentary rhizoids. Note branched sporangiophore.

FIG. 2. Phase-contrast photomicrograph of *R. rhizopodiformis*, showing sporangiophores arising directly from a stolon.

black and spherical, and ranged in size from 66.6 to 140 μm (average 92 μm). Columellae were hyaline or brown, often elongated (cylindrical or pyriform) (Fig. 3). Sporangiophores were hyaline or brown-colored, sometimes branched, and arose from stolons or, more often, opposite rhizoids either singly (Fig. 4) or in clusters of two or three (Fig. 1). Sporangiophores ranged from

120 to 1,026 μm , but most were less than 500 μm in length. Rhizoids were hyaline or brown, finger-shaped, and ranged from rudimentary to densely ramified. Sporangiospores, which were hyaline singly, but black in mass, ranged from predominantly spherical and nonstriate (Fig. 5) to very finely striated, and ranged in length from 4.5 to 6.7 μm (average, 5 to 6 μm). Cylindrical,

FIG. 3. Single sporangiophore of *R. rhizopodiformis* arising from densely ramified rhizoids, showing elongated columella and angulated apophysis (arrow).

spherical, or oval chlamydospores were observed, but zygospores were absent. Key features of *R. rhizopodiformis* differentiating this species from *R. oryzae*, another frequently encountered etiological agent of mucormycosis, are noted in Table 2.

Precipitin antibody to *Rhizopus* sp., but not

to *Absidia* or *Mucor* sp., was demonstrated in three of five sera assayed (patients no. 1, 3, and 4).

DISCUSSION

In 1962, Baker et al. (3) reported a case of subcutaneous mucormycosis in a diabetic pa-

FIG. 4. Single short sporangiophore of *R. rhizopodiformis* bearing sporangia arising directly from finger-like rhizoids.

tient caused by *R. rhizopodiformis*. Since this report, to our knowledge, *R. rhizopodiformis* had not been documented as an agent of mucormycosis until the recent report of the nosocomial acquisition of this species from contaminated adhesive bandages (4).

Initially, Keys et al. (10) reported in the Center for Disease Control's *Morbidity and Mortality Weekly Reports* a nosocomial outbreak of subcutaneous *Rhizopus* sp. infections associated with Elastoplast adhesive wound dressings in which the *Rhizopus* isolate was characterized as *R. oryzae*. Subsequent isolates recovered from Elastoplast at 3 of 4 hospitals and from 8 of 17 patients infected were identified as *R. rhizopodiformis* (4). Unfortunately, the hallmark isolate

of Keys was no longer available for reevaluation. Among the 11 isolates submitted to the Center for Disease Control, 2 were forwarded from our laboratory; 1 was recovered from patient no. 3, who developed subcutaneous infection after cardiac surgery, and the second was isolated from an Elastoplast bandage retrieved from the cardiac intensive care unit housing this patient. A previous patient also treated in the same cardiac intensive care unit expired 12 days after cardiac surgery with fulminating cutaneous and subcutaneous mucormycosis diagnosed only after death (5).

Nosocomial acquisition of *R. rhizopodiformis* may be inferred for patient no. 3 and possibly patient no. 4 of this series in whom subcutaneous

FIG. 5. Spherical-to-oval, nonstriate sporangiospores of *R. rhizopodiformis*.

infection ensued along the needle tract after a kidney biopsy. The epidemiology of infections in patients no. 1, 2, 5, and 6, however, is less well defined. Patient no. 1 had nasal and orbital involvement, whereas patients no. 5 and 6 had pulmonary infection caused by *R. rhizopodiformis*. These associations have not heretofore been attributable to this species.

Patient no. 2 had skin lesions clinically diagnosed as ecthyma gangrenosum which yielded *R. rhizopodiformis* on culture. This patient, who expired shortly after diagnosis, had pulmonary infiltrates which could have been of mycotic origin. However, because a postmortem examination was not performed, the nature of the pulmonary lesions remains unknown. We can only speculate that he did have a pulmonary focus with metastatic spread to the skin.

The temporal relationship between the occurrence of our first documented case of *R. rhizopodiformis* infection and that reported by the Center for Disease Control (4) is noteworthy. In our series, the index patient was diagnosed in January 1977, 3 months before the first patient with the Elastoplast-associated cutaneous infec-

tion (4). The next five patients encountered at The Mount Sinai Hospital were all diagnosed within a 9-month period (September 1977 through June 1978) which overlapped with cases being reported to the Center for Disease Control. It is conceivable, despite our failure to recover *R. rhizopodiformis* on numerous settling plates placed throughout our institution during this period (5), that spores of this species were introduced into our hospital through the contaminated adhesive bandages and that the nasal and pulmonary infections noted in three immunocompromised patients (no. 1, 5, and 6) arose as a result of inhalation of *R. rhizopodiformis* spores. Indeed, this concept is particularly possible in view of the etiology of the preceding seven culturally proven cases of mucormycosis diagnosed during life at The Mount Sinai Hospital (Table 3). Beginning in January 1974, four patients had mucormycosis due to *R. arrhizus*, and in one it was due to *A. corymbifera*. Unfortunately, two *Rhizopus* isolates were not identified to species level. One of these, however, was recovered from a 14-year-old patient with acute lymphocytic leukemia who also had nasal and

TABLE 2. Key differential features of *R. rhizopodiformis* and *R. oryzae*^a

Organism	Maximum growth temp (°C)	Sporangiospores	Columellae	Diam of sporangia (μm)	Length of sporangio-phores (μm)
<i>R. rhizopodiformis</i> ^b	50–52	Mostly spherical, smooth, or very finely striated	Mostly elongated (cylindrical or pyriform)	90–140	500–1,000 ^c
<i>R. oryzae</i>	42–44	"Lemon-shaped," distinct striations	Subspherical	160–240	1,000–2,500 ^d

^a *R. arrhizus* was omitted from this chart because we are in agreement with H. J. Schöler and E. Muller in considering *R. arrhizus* and *R. oryzae* conspecific (Abstr. Annu. Meet. Br. Soc. Mycopathol. 1971, 7th, Edinburgh, Scotland). Recent antigenic analysis supports this contention (9). Inui et al. (8) list them as separate species, although they state that "differences between these species are not always clear and there are intermediate species or varieties." Hesseltine in his key to the species of the genus *Rhizopus* separates them on the basis of size of sporangiospores (Hesseltine, Ph.D. thesis). Larger sporangiospores, averaging 7 to 9 μm in length and up to 12.4 μm, are *R. oryzae*, whereas smaller sporangiospores 5 to 7 μm in length and never over 8 μm are *R. arrhizus*. Also, *R. oryzae* have sporangio-phores 2 to 4 mm high, whereas *R. arrhizus* have sporangio-phores up to 1 mm high (Schöler and Muller, Abstr. Annu. Meet. Br. Soc., Mycopathol. 1971).

^b Synonyms for *R. rhizopodiformis* (Cohn, Zopf, 1890) (Schöler and Muller, Abstr. Annu. Meet. Br. Soc. Mycopathol. 1971) include: *Mucor rhizopodiformis* Cohn and Lichtheim, 1884 (11); *R. cohnii* (Cohn) Berlese and de Toni, 1888; *R. equinus* Constantin and Lucet, 1903; and *R. chinensis* Saito, 1904.

^c Based on findings of Schöler and Muller (Abstr. Annu. Meet. Br. Soc. Mycopathol. 1971). Maximum length, 800 μm; mostly <500 μm (Hesseltine, Ph.D. thesis.)

^d Average length, 500 to 1,000 μm.

TABLE 3. Distribution of 14 cases of mucormycosis diagnosed during life at The Mount Sinai Hospital from 1974 through 1978

Date mo/yr	Patient age (yr)/sex	Source	Associated disease ^a	Species
1/74	53/M	Ethmoid sinus	Renal transplant	<i>R. arrhizus</i>
4/74	71/F	Nasal biopsy	Diabetes mellitus	<i>R. arrhizus</i>
5/74	48/F	Ethmoid sinus	Diabetes mellitus	<i>R. arrhizus</i>
6/74	58/F	Palatal biopsy	Diabetes mellitus	<i>R. arrhizus</i>
9/74	49/M	Nasal biopsy	AML	<i>Rhizopus</i> sp.
3/75	55/F	Lip lesion	ALL	<i>A. corymbifera</i>
6/75	25/F	Ethmoid sinus	Diabetes mellitus	Failed to grow
1/77	14/M	Palatal scraping	ALL	<i>Rhizopus</i> sp.
1/77	49/M	Nasal biopsy	ALL	<i>R. rhizopodiformis</i>
9/77	54/M	Necrotic skin lesion	AML	<i>R. rhizopodiformis</i>
12/77	30/M	Biopsy tract	Renal transplant	<i>R. rhizopodiformis</i>
2/78	67/M	Skin biopsy	Cardiac surgery	<i>R. rhizopodiformis</i>
2/78	45/M	Bronchoscopic aspirate	Renal transplant	<i>R. rhizopodiformis</i>
6/78	49/M	Lung biopsy	AML	<i>R. rhizopodiformis</i>

^a AML, Acute myelogenous leukemia; ALL, acute lymphocytic leukemia.

orbital involvement. Interestingly, he was hospitalized in January 1977 concomitant with patient no. 1, who developed nasal and orbital *R. rhizopodiformis* infection. Although these two patients were housed in geographically distinct units, the possibility exists that even this *Rhizopus* isolate not identified to species level was indeed *R. rhizopodiformis*. The fact that *R. rhizopodiformis* was recovered from a patient with orbital disease at a neighboring institution (M. Corrado, Kings County Hospital, Brooklyn, N.Y., personal communication) during the very same month supports the introduction of this

unusual mucormycotic agent into the hospital environment in January 1977.

This explosive rise of *R. rhizopodiformis* at our institution, as well as nationally, strongly indicates nosocomial acquisition of this heretofore rare agent of mucormycosis. Epidemiologically, the scarcity of previous cases may be related to the infrequent occurrence of *R. rhizopodiformis* as a saprophytic contaminant (S. W. Hesseltine, Ph.D. thesis, University of Wisconsin, Madison, 1950).

The exact incidence of mucormycotic infection caused by *R. rhizopodiformis* may be diffi-

cult to establish because most diagnoses of mucormycosis are made histologically after death. In our 4-year experience with the 13 culturally proven cases of mucormycosis, *R. rhizopodiformis* comprised 46% of the isolates. Whether the present emergence of *R. rhizopodiformis* as an etiological agent of mucormycosis represents a transitory phenomenon linked to the contaminated adhesive bandages or results from the increased prevalence of this microorganism in the environment, and its adaption to humans, remains to be elucidated through continued isolation and specific identification of mucoraceous agents from human infections. Nevertheless, *R. rhizopodiformis* must be regarded as a human pathogen. This otherwise harmless saprophyte of moss and *Crataegus* sp. leaves (Hesseltine, Ph.D. thesis) has produced extensive cutaneous, nasal, and pulmonary lesions in humans to which an antibody response was elicited at least in some of them (patients no. 1, 3, and 4 reported herein).

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ADDENDUM

Since the acceptance of this manuscript, the occurrence of *R. rhizopodiformis* skin infections under post-operative wound dressings in two healthy orthopedic patients has been reported (13).

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