JOURNAL OF CLINICAL MICROBIOLOGY, Oct. 1995, p. 2774–2776 Vol. 33, No. 10
0095-1137/95/$04.00+0

Cutaneous Phaeohyphomycosis Caused by *Alternaria longipes* in an Immunosuppressed Patient

JOSEPA GENÉ,[1]* ANTONI AZÓN-MASOLIVER,[2] JOSEP GUARRO,[1] FREDERIC BALLESTER,[3] ISABEL PUJOL,[3] MONTSERRAT LLOVERA,[4] AND CARME FERRER[5]

Unitat de Microbiologia, Facultat de Medicina i Ciències de la Salut,[1] and Departaments de Dermatologia,[2] Microbiologia,[3] Oncologia,[4] Anatomia Patològica,[5] Hospital Universitari de Sant Joan, Universitat Rovira i Virgili, 43201 Reus, Tarragona, Spain

Received 10 April 1995/Returned for modification 3 June 1995/Accepted 20 July 1995

Alternaria longipes **was reported as the agent of a cutaneous infection in a patient with a neoplastic disease. The fungus has not been reported previously as causing disease in humans. It was distinguished by its rather small conidia with smooth or slightly verruculose walls and a pale brown beak which rarely extended into a secondary conidiophore. In vitro inhibitory activities of amphotericin B, ketoconazole, itraconazole, and miconazole were shown.**

Phaeohyphomycosis is a clinical entity which is diagnosed when dark-pigmented fungal elements (hyphae or yeast-like forms) are observed in animal or human tissue. Systemic phaeohyphomycosis is a rare infection; superficial, cutaneous, and subcutaneous are more common. In humans, this disease is considered to be an opportunistic infection which can be found in patients with induced or spontaneous immunosuppression and also among the healthy population (10). The etiological agents of phaeohyphomycosis can be numerous species belonging to different genera (6). The genus *Alternaria* Nees ex Fr. is one of them; some of its species occasionally cause subcutaneous or nasal granulomata, although cutaneous infections are relatively frequent, especially in European countries (1). Here we report the first known human infection caused by *Alternaria longipes* (Ellis & Everh.) Mason.

A 60-year-old patient was seen at the Dermatology Unit of our hospital in November 1993 because of granulomatous cutaneous lesions, which he had for 5 weeks, in his arms and legs. No evidence of the lesions was seen before. Past medical history revealed that he was a heavy smoker and drinker and had chronic hepatitis B. He lived in a rural area. In May 1992 he was diagnosed as having a moderately differentiated infiltrating squamous cell carcinoma of his left maxillary sinus with bone involvement (T4), without regional adenopathies (No) and without metastasis (Mo). He received three cycles of chemotherapy with cisplatin and 5-fluoracil and later six cycles of radiotherapy (total dose, 66 Gy). Disease entered only partial remission. In January 1993 he began a palliative therapy with dexamethasone (2 to 4 mg/day). On examination, he presented several ulceronecrotic granulomatous papules and plaques over the dorsa of his forearms (Fig. 1) and on the anterolateral aspects of his legs. On the dorsum of his right foot there was an extensive granulomatous plaque with several draining sinuses (Fig. 2). The rest of the physical examination showed a badly nourished man, bilateral ankle edemas, and signs of iatrogenic Cushing's syndrome. Routine laboratory data demonstrated normocromic normocytic anemia, hypoproteinemia, and slightly raised transaminases. Chest X ray was normal. Cultures of biopsy material from the lesions of the left forearm and right foot on Sabouraud's glucose agar (SGA) yielded fungal colonies which were practically identical. Routine bacteriological cultures and cultures for mycobacteria were negative. Histologic sections of biopsy material stained with periodic acid-Schiff revealed microabscesses and noncaseating granulomata with the presence of fungal elements in the dermis. These elements consisted of short, segmented hyphae and spheroidal bodies (Fig. 3). Brown pigment was present in the cell walls of only a few of the hyphal elements after hematoxylin and eosin staining. No vasculitis or blood vessel thrombosis was observed. The patient was first treated with oral ketoconazole (200 mg/12 h) for 21 days, with no response, and then with itraconazole (100 mg/12 h) for 2 months. New cultures and histologic sections were made from a control biopsy sample after 1 month of treatment, and both were positive. The histologic sections still showed spheroidal fungal bodies in the dermis. However, despite the lesions getting better and no new lesions developing, the patient died at home because of his neoplastic disease. No autopsy was performed.

For identification, fungal colonies from biopsy material were inoculated into SGA and other routine mycological media such as potato dextrose agar, cornmeal agar, and malt extract agar and were incubated at room temperature. All the media displayed grown colonies with similar characteristics but always without sporulation. The colonies on SGA measured 55 to 57 mm in diameter after 7 days and were cottony and white, becoming pale gray in time. Growth was also observed at 37°C. The identification of *A. longipes* was made by transferring small blocks of SGA with mycelium to tap water agar with small pieces of sterile filter paper on its surface and incubating them at room temperature. After a minimum of 14 days, the new cultures obtained showed long and sparsely branched conidial chains. This procedure was repeated various times with the same result. The conidia measured 28 to 65 by 9 to 15 μm, and they were obclavate and pale brown, with a smooth or slightly verruculose wall, tapering gradually into a pale brown beak, often slightly swollen at the tip, with three to seven transverse septa and one or two longitudinal or oblique septa (Fig. 4).

Antifungal susceptibility testing of the isolate was accomplished by the broth microdilution method (2) performed mainly according to the National Committee for Clinical Laboratory Standards guidelines for yeasts (8) by using RPMI 1640 medium buffered to pH 7.0 with 0.165 M morpholinepropane-

* Corresponding author. Mailing address: Unitat de Microbiologia, Departament de Ciències Mèdiques Bàsiques, Facultat de Medicina i Ciències de la Salut, Universitat Rovira i Virgili, Carrer Sant Llorenç, 21, 43201-Reus, Tarragona, Spain. Phone: 34 77 75 93 59. Fax: 34 77 75 93 22. Electronic mail address: umb@ee.urv.es.

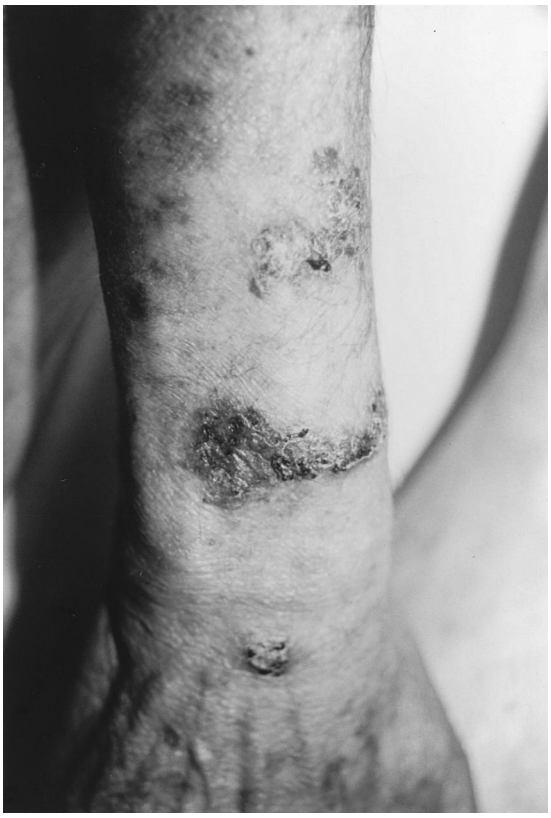

FIG. 1. Several ulceronecrotic granulomatous papules and plaques over the dorsum of the patient's left forearm.

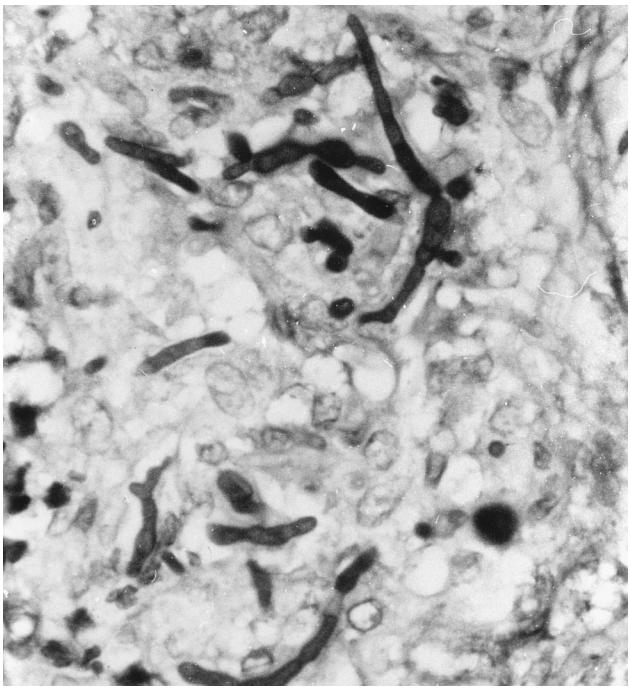

FIG. 3. Periodic acid-Schiff stain of biopsy specimen from the left forearm showing segmented hyphae and spheroidal bodies. Magnification, ×440.

sulfonic acid (MOPS), an inoculum of 10^3 CFU/ml, a temperature of incubation of 30°C, a second-day reading (48 h), and an additive drug dilution procedure. MICs were 0.29 to 0.58 μg of amphotericin B per ml, 40 μg of fluconazole per ml, >322.75 μg of 5-fluorocytosine per ml, 0.24 μg of itraconazole per ml, 1.6 μg of ketoconazole per ml, and 2.5 μg of miconazole per ml; the isolate showed resistance only to fluconazole and 5-fluorocytosine.

Alternaria is a large genus with species usually found as soil saprophytes and plant pathogens, some of which can occasionally affect humans and animals. The most common pathogenic *Alternaria* species are *A. alternata* (Fr.) Keissler and *A. tenuissima* (Kunze ex Pers.) Wiltshire. In a recent review of human cutaneous infections produced by *Alternaria* species, 48 cases were reported (1). The majority of these cases were caused by the two above-mentioned species. However, infections caused by other *Alternaria* species, e.g., *A. dianthicola* Neergaard (7),

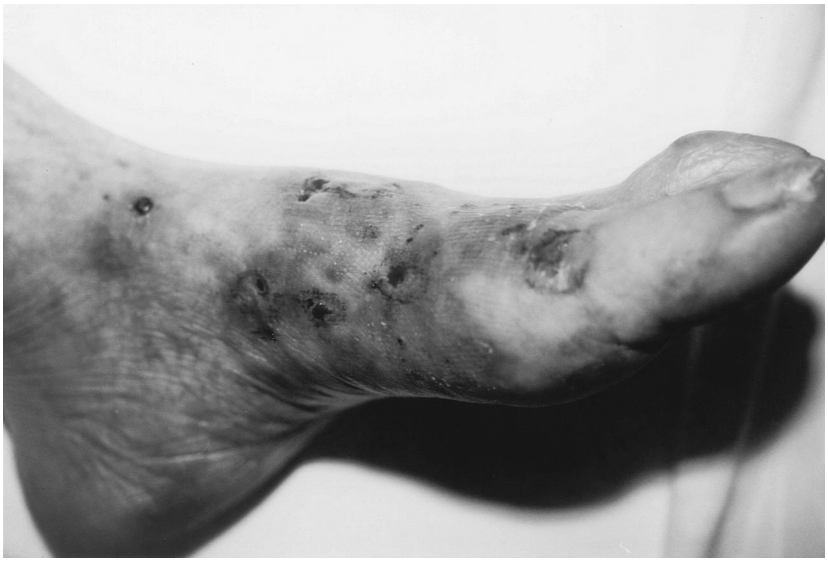

FIG. 2. Extensive granulomatous plaque and several draining sinuses on the dorsum of the patient's right foot.

FIG. 4. *A. longipes* FMR 4985 conidia. Magnification, ×1,280.

A. chlamydospora Mouchacca (12), and *A. infectoria* E. G. Simmons (10), have also been reported. Descriptions, illustrations, and a key for the identification of the pathogenic species of *Alternaria* have been recently published (6). Thus far, *A. longipes* has not been reported as a cause of mycoses. It is a cosmopolitan species, reported from many countries (5) and often reported as a causal agent of brown spot lesions of tobacco (11). A closely related species is *A. infectoria*, which differs in having conidia in strongly branched chains. These conidia usually have only transverse septa and a very short apical beak which extends into a geniculate secondary conidiophore. Our isolate shows conidia slightly smaller than those described in the original description of *A. longipes*. This difference is probably caused by the influence of the artificial media (3, 11).

Cutaneous infection due to *Alternaria* species has been reported mainly from Mediterranean countries, the majority of infections being from France and Spain (1). These infections usually originate by traumatic implantation of fungal spores, being relatively common in farmers. However, a risk factor is treatment with immunosuppressive agents and corticoids or the presence of underlying diseases such as hemopathy, neoplastic illnesses, nephrotic syndrome, Cushing's syndrome, AIDS, and diabetes, etc., or organ transplantation. These infections may respond to surgical resection, especially when antifungal treatment is not effective; however, they may recur (1). Ketoconazole has been until now the drug of choice, but its efficacy has been very irregular. Injections of amphotericin B or miconazole into the lesions are sometimes also effective (1). Recently, several cases have been resolved with the use of oral itraconazole (100 to 200 mg/day) with no significant side effects (4, 9). Our patient was initially treated by ketoconazole, which demonstrated an inhibitory effect in vitro, but because of the lack of improvement of lesions and the appearance of new ones, this drug was replaced by itraconazole. After 2 months of therapy, an improvement of the old lesions and no appearance of new lesions were noticed, but a follow-up was not possible because the patient died of his underlying disease.

We thank J. Mena-Portales from the Instituto de Ecologiá y Sistemática, Academia de Ciencias of Cuba, for confirming the identification of *A. longipes.*

REFERENCES

1. **Badillet, G.** 1991. Les alternarioses cutanées. Revue de la littérature. J. Mycol. Med. **118:**59–71.
2. **Barchiesi, F., A. L. Colombo, D. A. McGough, and M. G. Rinaldi.** 1994. Comparative study of broth macrodilution and microdilution techniques for in vitro antifungal susceptibility testing of yeasts by using the National Committee for Clinical Laboratory Standards' proposed standard. J. Clin. Microbiol. **32:**2494–2500.
3. **De Bièvre, C.** 1991. Les *Alternaria* pathogènes pour l'homme: mycologie épidémiologique. J. Mycol. Med. **118:**50–58.
4. **Duffill, M. B., and K. E. Coley.** 1993. Cutaneous phaeohyphomycosis due to *Alternaria alternata* responding to itraconazole. Clin. Exp. Dermatol. **18:**156–158.
5. **Ellis, M. B.** 1971. Dematiaceous Hyphomycetes. Commonwealth Mycological Institute, Kew, United Kingdom.
6. **Guarro, J., G. S. de Hoog, M. J. Figueras, and J. Gené.** 1995. Rare opportunistic fungi, p. 241–670. *In* G. S. de Hoog and J. Guarro (ed.), Atlas of clinical fungi. Centraalbureau voor Schimmelcultures, Baarn, The Netherlands.
7. **Mitchell, A. J., A. R. Solomon, E. S. Beneke, and S. Anderson.** 1983. Subcutaneous alternariosis. J. Am. Acad. Dermatol. **8:**673–676.
8. **National Committee for Clinical Laboratory Standards.** 1992. Reference method for broth dilution antifungal susceptibility testing for yeasts. Proposed standard M27-P. National Committee for Clinical Laboratory Standards, Villanova, Pa.
9. **Repiso, T., N. Martin, P. Huguet, J. Luelmo, M. Roca, U. González-Castro, C. Margarit, and A. Castells.** 1993. Cutaneous alternariosis in a liver transplant recipient. Clin. Infect. Dis. **16:**729–730.
10. **Roosje, P. J., G. S. de Hoog, J. P. Koeman, and T. Willemse.** 1993. Phaeohyphomycosis in a cat caused by *Alternaria infectoria* E. G. Simmons. Mycoses **36:**451–454.
11. **Simmons, E. G.** 1981. *Alternaria* themes and variations (1–6). Mycotaxon **13:**16–34.
12. **Singh, S. M., J. Naidu, and M. Pouranik.** 1990. Ungual and cutaneous phaeohyphomycosis caused by *Alternaria alternata* and *Alternaria chlamydospora*. J. Med. Vet. Mycol. **28:**275–278.