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Mycobacterium simiae and *Mycobacterium avium-M. intracellulare* Mixed Infection in Acquired Immune Deficiency Syndrome

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Acquired immune deficiency syndrome was diagnosed in a 43-year-old man, born and living in Congo. The patient presented a disseminated infection caused by mycobacteria which were recovered from blood, jejunal fluid, and duodenal and rectal biopsies. Identification, according to conventional tests and mycolate profile determination, showed that *Mycobacterium avium-M. intracellulare* and *M. simiae* were both involved.

Mycobacteria cause opportunistic infection in patients suffering from defects in cell-mediated immunity and are now well recognized in patients with acquired immune deficiency syndrome (AIDS) (8–10, 12, 17, 24, 25, 38). In 1985, Good described the species distribution for mycobacterial isolates from 212 patients identified at the Centers for Disease Control (10). Over 80% of these mycobacterial strains were *Mycobacterium avium-M. intracellulare*, whereas only 9% were *M. tuberculosis* strains. These percentages indicated that the species distribution of the mycobacterial isolates from AIDS and non-AIDS patients was significantly different: *M. tuberculosis* is worldwide the most frequent mycobacterial pathogen in non-AIDS patients (11). The subspecific distribution of the *M. avium-M. intracellulare* serotypes was also found to be different between AIDS and non-AIDS patients, who are more frequently infected by serotypes 4 and 8, respectively (10). The other main differences between AIDS and non-AIDS patients were the site and extent of the infection, which is usually disseminated in AIDS patients. Pulmonary infections, caused by either *M. avium-M. intracellulare* or *M. tuberculosis*, are more frequent in non-AIDS patients. Less frequently encountered mycobacterial species in AIDS patients include *M. asiaticum*, *M. flavescens*, *M. fortuitum*, *M. gordonae*, *M. kansasii*, *M. malmoense*, *M. scrofulaceum* and *M. xenopi* (9, 10, 17). Some of these species were found in association with *M. avium-M. intracellulare* (10).

In this paper, we report the case of an AIDS patient with disseminated infection due to both *M. avium-M. intracellulare* and *M. simiae*. We point out the usefulness of the determination of the mycolic acid profile for the identification of *M. simiae*.

Case report. The patient was a 43-year-old man, born and living in Congo (Brazzaville). He was a hospital attendant and denied any homosexuality or intravenous drug abuse. He had no medical history until March 1981, when he had dorsal herpes zoster. Diarrhea began in January 1982, first related to amebiasis but persisting despite specific therapy. In March 1983, he was febrile and cachectic, with a 17-kg weight loss, and was referred to France. According to Centers for Disease Control criteria (5), AIDS was diagnosed. Candidal esophagitis and acute colitis were evidenced by endoscopic examination. Blood and stool cultures yielded *Shigella flexnerii*. He had lymphopenia (900 lymphocytes per mm^3); typage of lymphocyte populations by immunofluorescence showed a low absolute number (100/mm^3) of T4 cells and a T4/T8 ratio of 0.125. Presence of antibodies to lymphadenopathy-associated virus/human T-cell lymphotropic virus type III was detected by enzyme-linked immunosorbent assay. Despite the healing of *Shigella* infection, diarrhea persisted. In June 1983, *Cryptosporidium* oocysts were found by stool examination. At the same time, he presented a lobar pneumonitis due to *Nocardia asteroides* from which he recovered with antimicrobial therapy. He was discharged from the hospital in September 1983 and was seen as an outpatient in December 1983. He showed a 15-kg weight gain and no diarrhea, despite the persistence of a few *Cryptosporidium* oocysts in stools. On May 1984, he was readmitted with massive malnutrition (25-kg weight loss). In sections of duodenal biopsies (Fig. 1), granulomas suggestive of a mycobacterial infection were not seen, and the lesions were similar to the histopathologic appearance of Whipple's disease, i.e., infiltration of the lamina propria with foamy macrophages including numerous acid-fast bacilli (Fig. 2). Consequently, the mycobacterial etiology was detected only by staining for acid-fast bacilli. Acid-fast bacilli were also found in smears of blood and jejunal fluid. Cultures of duodenal and rectal biopsies, jejunal fluid, and blood yielded mycobacteria. At his request, the patient returned to Africa and was lost to follow-up.

Identification procedures. Mycobacterial strains were identified by conventional methods (21, 31).

Determination of mycolate profile. The technique used was previously described (6, 19). Briefly, the bacteria were saponified for 2 h at 110°C in a solution of ethylene glycol monomethyl ether containing 5% (wt/vol) potassium hydroxide and 12% (vol/vol) distilled water. After acidification, the fatty acids were extracted into ether, and lipids were methylated with diazomethane freshly prepared from Diazogen (Janssen-Chimica, Beerse, Belgium). The methyl esters were spotted in duplicate on two K6 silica gel plates (Whatman, Inc., Clifton, N.J.). One plate was developed by four passages of petroleum ether-diethyl ether (90:10, vol/vol), and the other plate was developed by one passage of dichloromethane. The mycolate profile was determined by comparison with profiles of reference mycobacterial strains, specifically *M. avium* ATCC 25291 and *M. simiae* ATCC 25275.

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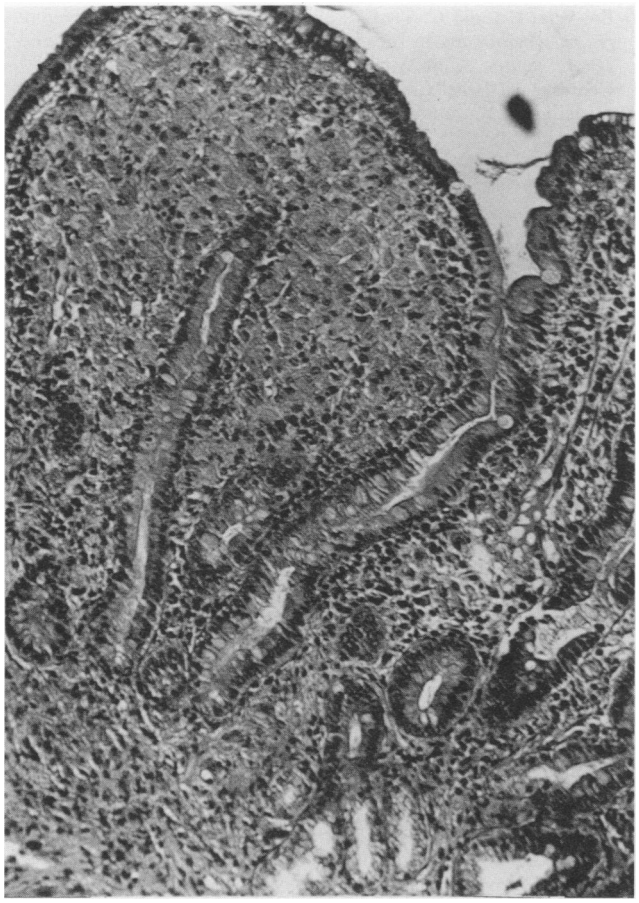

FIG. 1. Duodenal biopsy: pseudo-Whipple aspect with infiltration of the lamina propria. Stain, hematenine-phloxine-safran.

Mycobacterial identification. Mycobacteria were isolated from samples from rectal biopsy, jejunal fluid, blood, and feces at different periods from May to October 1984. Cultures from feces and from a sample of jejunal fluid yielded strains identified as *M. avium-M. intracellulare*, according to current bacteriological tests and mycolate composition.

Mycobacterial cultures obtained from another sample of jejunal fluid, rectal biopsy, and blood yielded data in agreement with the identification of *M. avium-M. intracellulare*. The mycolate profile showed α, α′, keto, and dicarboxylic mycolates (Fig. 3, lane 2). However, single-colony isolation yielded two strains. One was identified as *M. avium-M. intracellulare* with the mycolate profile of α, keto, and dicarboxylic mycolates, and the other was identified as *M. simiae* with the characteristic mycolate profile of α, α′, and keto mycolates (Fig. 3, lanes 1 and 3).

All the *M. simiae* strains slowly accumulated a yellow pigment; however, the strain from a rectal biopsy showed a strongly positive niacin test and a negative urease test, whereas strains from blood and jejunal fluid were urease positive and niacin negative. These positive tests were obtained only with old cultures (more than 8 weeks).

Discussion. In this report we describe an AIDS patient with a mixed mycobacterial infection caused by *M. avium-M. intracellulare* and *M. simiae*, and the difficulties in differentiating these two mycobacterial species are underscored.

Knowledge about *M. simiae* has evolved considerably since the formal description of the species in 1965 (14). The first strains were isolated from monkeys and characterized by slow growth, positive photochromogenicity, and positive niacin test (14). In 1971, Valdivia et al. described "*M. habana*," a causative agent of pulmonary infection in humans (28, 29), but bacteriological studies, antigenic profiles, pathogenicity for animals, and comparative reciprocal sensitin testing as well as DNA-DNA hybridization established its synonymy with *M. simiae* (1, 3, 4, 20, 27, 36). Epidemiologic studies in different countries such as France, Israel, Thailand, and the United States pointed out the role of *M. simiae* as a pulmonary pathogen (2, 3, 15, 16, 26). A single report recognized *M. simiae* as the cause of a disseminated infection (23). Like other nontuberculous mycobacteria, *M. simiae* has been recovered from tap water (37).

The taxonomy of *M. simiae* has been difficult to establish, as the strains of this species, including the type strain, yield poor reproducibility in highly standardized tests, such as pigment production or niacin accumulation, which have been shown to be reproducible for other mycobacteria (32–35). The discrepant phenotypes evoke the MAIS intermediate strains described by Hawkins (13) for strains which share in various combinations the features of *M. avium*, *M. intracellulare*, or *M. scrofulaceum*. Actually, the classical scheme of identification may lead to a misidentification of the *M. simiae* strains as members of one of the MAIS species

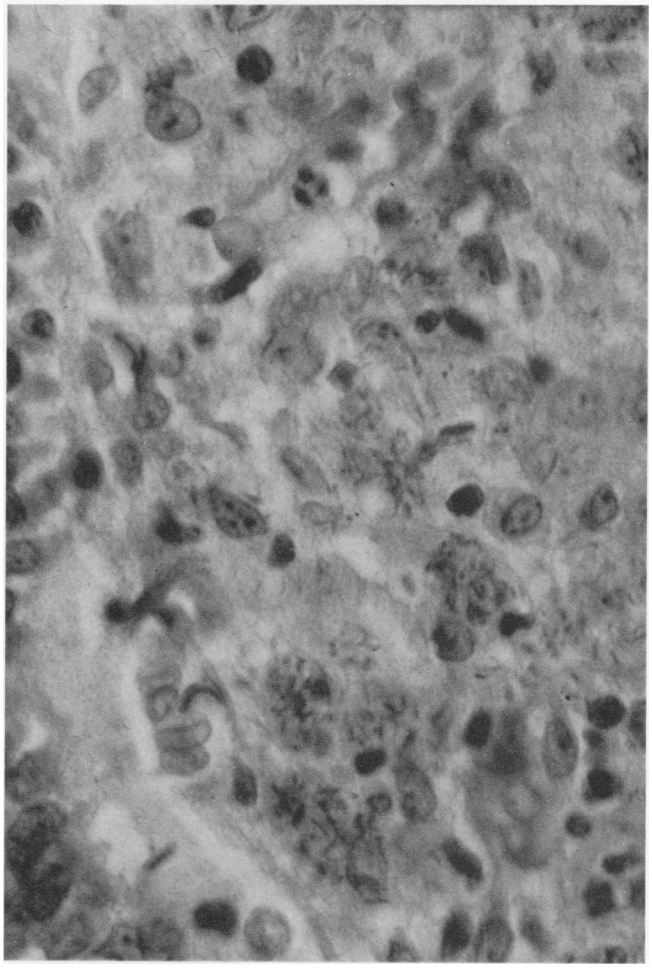

FIG. 2. Duodenal biopsy: foamy macrophages including acid-fast bacilli. Stain, Ziehl-Neelsen technique.

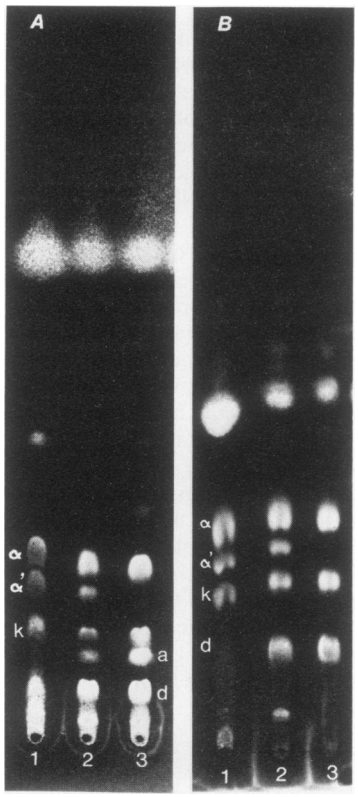

FIG. 3. Thin-layer chromatography of methylated lipids extracted from mycobacterial isolates. The solvents used were (A) petroleum ether-diethyl ether (90:10, vol/vol) and (B) dichloromethane. Lane 1, *M. simiae* strain; lane 2, mixed culture; lane 3, *M. avium-M. intracellulare* strain. α, Nonoxygenated mycolate; α′, short nonoxygenated mycolate; k, keto mycolate; a, alcohol-esterifying dicarboxylic mycolate; d, dicarboxylic mycolate.

(30, 34). The chemotaxonomic criterion based on mycolic acid determination allows an easy and definitive differentiation of *M. simiae* from the MAIS complex strains. All strains of *M. simiae* present a characteristic pattern including α, α′ (short nonoxygenated), and keto mycolates (6, 19, 22), whereas the pattern shown by the MAIS complex (α, keto, and dicarboxylic mycolates) is shared by many mycobacterial species (6, 7, 18, 19, 22). Six *M. malmoense* strains, including the type strain, studied by numerical taxonomy analysis (35) showed mycolate patterns identical to the *M. simiae* profile, i.e., α, α′, and keto mycolates (19). However, the ability to hydrolyze Tween 80 coupled with differences in tolerance to antituberculous drugs, other than isoniazid (30), served to differentiate *M. malmoense* from *M. simiae* and from the MAIS complex.

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