Brief Report: Disseminated Mycobacteriosis Caused by Drug-Resistant Mycobacterium triplex in a Human Immunodeficiency Virus–Infected Patient during Highly Active Antiretroviral Therapy

Mycobacterium triplex is a novel species that, until now, has been isolated only from limited clinical samples, and its clinical relevance has been largely unknown. In this report, we describe the first case of disseminated disease caused by M. triplex in a human immunodeficiency virus–infected patient.

A 40-year-old man with a history of drug abuse who has been infected with HIV-1 since 1986 developed Toxoplasma encephalitis, diagnosed according to Centers for Disease Control and Prevention–presumptive criteria. He was treated successfully with sulfadiazine plus pyrimethamine. In spring 1997, at another center, after the resolution of toxoplasmosis, a tuberculous meningoencephalitis was diagnosed on the basis of CSF findings and the neuroradiological picture, although CSF cultures were negative for mycobacteria. He was successfully treated with rifabutin, isoniazid, and ethambutol until August 1998. Findings on a chest radiograph were negative at the beginning of treatment and during follow-up. The patient remained naive for antiretroviral therapy until March 1997, when zidovudine/lamivudine was started (baseline values: CD4 cell count 35 cells/µL; HIV RNA 125,000 copies/mL). Because of incomplete viral suppression, indinavir was added to his regimen in August 1997.

The patient presented at our facility in August 1998 for evaluation of a 15-day history of fever, night sweats, chills, weight loss, and articular pain localized to the right knee. Plasma HIV viremia was 15,000 copies/mL, and CD4 cell count was 98/µL. Physical examination demonstrated an erythematous fluctuating and painful monoarticular joint with a marked functional impairment. Conventional radiographs showed rarefaction and erosion of the tibial and femoral metaphyses of the right knee, and MRI revealed signs of synovitis and joint effusion. No lesions were found at the left knee. Cultures of the inflamatory joint fluid (obtained by arthrocentesis) were negative for bacteria and fungi (WBC count, <10,000 cells/µL). A wide-spectrum empiric treatment with clindamycin, teicoplanin, and ciprofloxacin was started, but after 2 weeks of therapy symptoms at the right knee worsened, and articular pain and local swelling at the left knee was observed. A new MRI showed altered signal areas with peripheral enhancement indicative of a colliquative abscess of the left knee, and a radionucleotide bone scan detected an increased uptake of 99mTc-MDP at the tibial and femoral metaphyses bilaterally. Necrotic tissue with purulent inflammatory reaction was removed during an open-field biopsy procedure, and at histologic examination a necrotizing granulomatous process with acid-fast bacilli was detected. Bone marrow biopsy also revealed granulomatous giant-cell necrotizing disease. Ultrasound and CT scan revealed an enlarged spleen with a single parenchymal abscess and an abdominal multiple lymphadenopathy. Two acid-fast isolates were recovered from synovial fluid, tibial bone of the left knee, and bone marrow specimens.

Initially, identification at the species level by means of biochemical testing [1] yielded a pattern resembling that of other slow growing mycobacteria, such as Mycobacterium avium complex (MAC). However, the isolate failed to hybridize with the commercially available genetic probe for MAC. PCR-reverse cross-blot hybridization [2] and PCR-restriction enzyme analysis of hsp65 gene [3] were unable to identify the microorganism (figure 1A and 1B), nor was high-performance liquid chromatography analysis of the mycolic acids [4] using Sherlock software (MIDI, Delaware). However, sequence analysis of the PCR products by amplification of the 16S rRNA gene revealed a complete homology to that gene in Mycobacterium triplex, a newly described species [5]. We then designed an oligonucleotide probe specific for M. triplex (pTrip: 5'-GACGCAATGCTCTGTTGGT-3'; nucleotide position 152-172 of the hypervariable 16S rRNA region) and used it in the PCR-reverse cross-blot hybridization assay (figure 1C).

We performed susceptibility tests on the two isolates from synovial fluid, tibial bone, and bone marrow specimens. Both isolates were susceptible to ethionamide (MIC, 0.0064 µg/mL) and clarithromycin (MIC, 1 µg/mL) but resistant to amikacin (MIC, 256 µg/mL), ciprofloxacin (MIC, 32 µg/mL), streptomycin (MIC, 256 µg/mL), isoniazid (MIC, 3 µg/mL), ethambutol (MIC, 6 µg/mL), levofloxacin (MIC, 32 µg/mL), and rifampin (MIC, 4 µg/mL). A combined regimen of clarithromycin and ethionamide was started. Antiretroviral therapy was modified to include quadruple combination of 2 new nucleoside analogues (didanosine and stavudine) and 2 protease inhibitors (ritonavir and saquinavir hard-gel capsules).

For 5 months after starting therapy, the patient’s conditions improved; he was persistently apyretic, and the local articular picture showed remission. No focal lesions were detected by ultrasound or CT scan of the spleen, and a control bone-marrow biopsy revealed remission of granulomatous features. However, after >8 months of in vitro susceptibility–driven treatment, he developed fever again, although his CD4 cell count was 34 cells/µL and plasma viremia level was 6500 HIV-RNA copies/mL. M. triplex with the same susceptibility pattern of the previous organism was again cultured from a colliquative abscess at the right knee. At this time the patient is continuing the same treatment and his condition is slowly worsening.

To our knowledge, this is the first report of disseminated disease due to M. triplex in an HIV-infected patient. Since 1996, when M. triplex was characterized as a novel species distin-
guishable from closely related species Mycobacterium simiae and Mycobacterium avium, this slow-growing organism has been isolated from only a limited number of samples (lymph node, sputum, and CSF) [5]; indeed, the clinical relevance and the clinicopathological spectrum of disease associated with the isolation of M. triplex has been unknown until now.

Most of the strains described previously showed a similarity to MAC in traditional susceptibility testing. All were resistant to the commonly used antituberculous drugs, as well as to kanamycin and capreomycin, whereas 90%–100% of strains were susceptible to ethambutol and ethionamide [5]. In the case reported here, the susceptibility pattern revealed an excellent MIC for ethionamide and a good MIC for clarithromycin, but complete resistance to ethambutol.

Its similarity to MAC infection may cause M. triplex infection to be misdiagnosed in immunocompromised individuals. The misidentification of this possibly emerging pathogen is particularly dangerous because M. triplex is resistant to drugs currently administered for disease due to MAC. However, an accurate diagnosis based on combined results from conventional biochemical testing, molecular methods, and 16S rRNA analysis should be reliable as demonstrated in our case. Moreover, in the era of potent antiretroviral therapy, in which the incidence of mycobacterial diseases due to MAC have been strongly decreasing [6], it might be hypothesized that a selective pressure towards newly emerging mycobacteria may be changing the pattern of mycobacterial infection.

The pathogenetic role of the immune reconstitution in the development of mycobacterial infections in patients starting antiretroviral therapy has been reported with increasing frequency [7]. However, our case experienced mycobacterial disease after a failure of highly-active antiretroviral therapy and had a persistently detectable plasma viremia level and low CD4 counts after 1 year of triple antiretroviral combination. This suggests that the pathogenetic hypothesis is unlikely and strongly suggests that M. triplex infection be considered an uncommon opportunistic infection.

In conclusion, this report suggests that M. triplex must be considered as a potential etiologic agent of disseminated mycobacterial disease. Moreover, the difficulty of identification and the peculiar susceptibility pattern makes an accurate surveillance of unidentified mycobacterial diseases among HIV-infected individuals crucially important, in order to estimate the real impact of newly emerging pathogens.

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