Cervical Lymphadenitis due to an Unusual Mycobacterium

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A scotochromogenic acid-fast bacillus was isolated from a lymph node of a 2-year-old female. On the basis of conventional testing, the mycobacterium appeared to be *Mycobacterium scrofulaceum*. Its mycolic acid profile, however, was not identical to that of *Mycobacterium scrofulaceum* but was similar to that of *Mycobacterium interjectum*. Direct sequencing of the 16S rRNA gene revealed a unique nucleic acid sequence, suggesting that the isolate represents a previously undescribed pathogenic species.

Among mycobacterial infections that occur in childhood, cervical lymphadenitis ranks second in incidence after pulmonary tuberculosis. Apart from *Mycobacterium tuberculosis* (1), several species of nontuberculous mycobacteria can be involved. Although *Mycobacterium scrofulaceum* is the causative agent of cervical lymphadenitis (2), *Mycobacterium avium* complex (MAC) has increasingly been found to be responsible for this disease (3). In addition, *Mycobacterium malmoense*, at least in countries where this species is endemic, is a common agent of children's adenopathy (4). Several unknown or newly described mycobacterial species have been isolated from lymph nodes, particularly from very young patients (5–10). We present the characterization of a new mycobacterium isolated from a lymph node that was surgically removed from a 2-year-old patient.

Patient and Methods. A 2-year-old girl was hospitalized in July 1995 because of a right latero-cervical swelling, which had been treated unsuccessfully with clarithromycin. Ultrasonic investigation showed a nonhomogeneous, sonographically hypodense, oval mass (major axis = 3.5 cm). Abdominal sonographic scan and chest radiograph were normal. Serological tests for toxoplasmosis and mononucleosis were negative; biochemical and hematological data, including erythrocyte sedimentation rate, were normal. Mantoux test with 5 TU of PPD was positive, with 6 mm of induration. A diagnosis of tuberculous lymphadenitis was made, and the entire lymph nodal group was surgically removed. The patient was discharged three weeks after admission, completely healed; no relapse has occurred thus far.

Microscopic observation of the resected mass showed scanty acid-fast bacilli, which grew in culture three weeks later. Culture was performed according to standard procedures on Löwenstein-Jensen slants and in radiometric broth. Commercially available DNA probes, conventional cultural and biochemical tests, high-performance liquid chromatography (HPLC) of cell wall mycolic acids, and genomic nucleotide sequencing were used for identification.

DNA probes (11) for *Mycobacterium tuberculosis* complex, MAC, *Mycobacterium kansasii*, and *Mycobacterium gordonae* were used according to the manufacturers' recommendations. Conventional tests were performed according to standard procedures (12).

Mycolic acids extracted from colonies grown in culture were analyzed by the HPLC method described previously (13, 14), using a System Gold model instrument (Beckman, USA) equipped with a reverse-phase C_{18} Ultrasphere-XL cartridge column. For identification of peaks, the retention time ratios were calculated to a high molecular weight internal standard (Ribi, ImmunoChem, USA).

Nucleotide sequencing of a PCR-amplified 16S rRNA gene fragment was performed as described (15), and the regions corresponding to positions from 129 to 266 (hypervariable region A) and from 430 to 468 (hypervariable region B) of *Escherichia coli* were used for identification.

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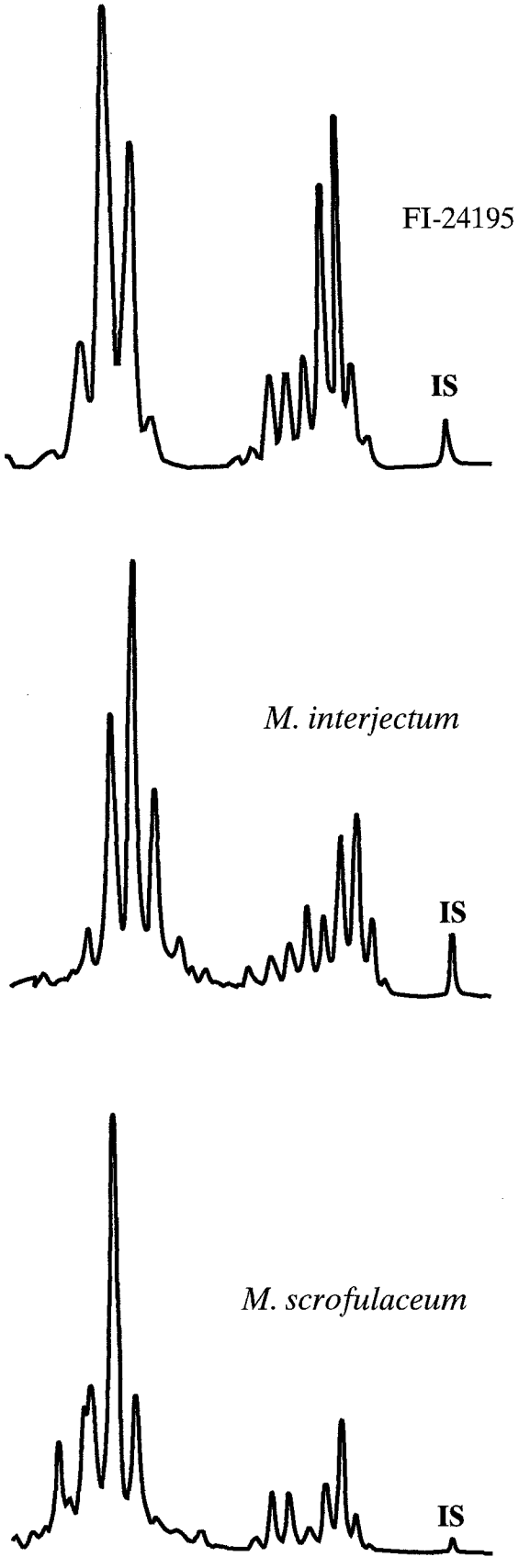

Figure 1: Comparison of mycolic acid patterns obtained by HPLC analysis of FI-24195, *Mycobacterium interjectum*, and *Mycobacterium scrofulaceum*. IS, internal standard.

Susceptibility testing was performed in radiometric broth using a previously described macrodilution method (16) that was well adapted to our isolate because the growth rate of our isolate in broth was equal to that of MAC.

Results and Discussion. Within three weeks, cultures yielded a scotochromogenic mycobacterium (FI-24195) that failed hybridization with commercially available DNA probes (*Mycobacterium tuberculosis*, *Mycobacterium kansasii*, MAC, *Mycobacterium gordonae*). Colony appearance and results of conventional tests appeared compatible with *Mycobacterium scrofulaceum*, with the exception of quantitative catalase (< 45 mm of foam), tellurite reduction (negative), and inhibition by hydroxylamine (500 μg/ml).

The profile obtained by HPLC analysis resembled that of *Mycobacterium scrofulaceum* and the newly described species *Mycobacterium interjectum* (10), although some differences were observed (Figure 1).

Partial sequencing of 16S rRNA revealed a unique sequence (Figure 2-I) clearly different from that of previously described species belonging to the genus *Mycobacterium* (15). Figure 2-II compares two short stretches from the hypervariable region A of several mycobacterial species. Moreover, our isolate exhibits a long helix 18 in the hypervariable region B. This finding excludes any relation to *Mycobacterium interjectum*, which exhibits a short helix 18, or to *Mycobacterium scrofulaceum*, which is characterized by a very distinct secondary structure of its long helix 18, with a deletion of three nucleotides when compared to other slow growers.

Minimal inhibitory concentrations in vitro suggest resistance to ethambutol (16 μg/ml) and moderate susceptibility to amikacin (2 μg/ml), ciprofloxacin (2 μg/ml), and streptomycin (2 μg/ml). Clarithromycin (0.12 μg/ml), clofazimine (0.06 μg/ml), rifabutin (0.03 μg/ml), rifampin (0.12 μg/ml), and sparfloxacin (0.25 μg/ml) appear effective.

The present case of lymphadenitis in a 2-year-old girl corresponds with the typical description of this disease: unilateral infection, female predominance, median age of 2.9 years, and positive PPD (3). Two nontuberculous mycobacteria are usually involved in cervical lymphadenitis in children: MAC and *Mycobacterium scrofulaceum* (3). Other species are rare. As discussed by Wolinsky (3), the long-lasting predominance of *Mycobacterium scrofulaceum* as the causative agent of childhood

I

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CGGCGTGCTT AACACATGCA AGTCGAACGG AAAGGTCTCT TCGGAGATAC TCGAGTGGCG AACGGGTGAG TAACACGTGG GCAATCTGCC
CTGCACTTCG GGATAAGCCT GGGAAACTGG GTCTAATACC GGATATGACC TCGAGGCGCA TGCCTTGTGG TGGAAAGCTT TTGCGGTGTG
GGATGGGCCC GCGGCCTATC AGCTTGTTGG TGGGGTGATG GCCTACCAAG GCGACGACGG GTAGCCGGCC TGAGAGGGTG TCCGGCCACA
CTGGGACTGA GATACGGCCC AGACTCCTAC GGGAGGCAGC AGTGGGGAAT ATTGCACAAT GGGCGCAAGC CTGATGCAGC GACGCCGCGT
GGGGGATGAC GGCCTTCGGG TTGTAAACCT CTTTCACCAT CGACGAAGGT CCGGGGTTTC TCGGATTGAC GGTAGGTGGA GAAGAAGCAC
CGGCCAACTA CGTGCCAGCA GCCGCGGTAA TACGTAGGGT GCGAGCGTTG TCCGGAATTA CTGGGCGTAA AGAACTCG
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II

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129                                 172
TGA TCT GCC CTG CAC TTC / TAC CGG ATA GG-ACCA CGG GAT GCA TGTCT-TGT GGT     M. tuberculosis
.A. ... ... ... ... ...   ... ... ... ..-...T ..A .GC ... ..C..-... ...   M. interjectum
CA. ... ... ... ... ...   ... ... ... T.-...T ..A .GC ... ..C..-... ...   FI-24195
CA. ... ... ... ... ...   ... ... ... ..-.... .TT .GC ... ..C..-... ...   M. scrofulaceum
.A. ... ... ... ... ...   ... ... ... ..-.... .TT .GC ... ..G..-... ...   M. simiae
... ... ... ... ... ..T   ... ..A ... TTCC.TA TT. .TC ... ..G.CTG.. A.G   M. flavescens
... ... ... ... ... ..T   ... ..A ... ..-...G .AT .C. ... ..GTG-... ...   M. nonchromogenicum
... ... ... ... ... .CT   ... ... ... ..-.... T.. ... ... ...TC-... ...   M. terrae
... C.. ... ... ... ...   ... ... ... ..-.... TTC TGC ... ...GG-G.. ...   M. xenopi
.A. ... ... ... ... A..   ... ..A ... ..-.... .A. ..C A.. ....C-... ...   M. gordonae
C.. ... ... ... ... ...   ... ... ... ..-.... ... ... T.. ....C-... ...   M. marinum/M. ulcerans
CA. ... ... ... ... ...   ... ... ... ..-.... .TT .GC ... ..C..-... ...   M. scrofulaceum
.A. ... ... ... ... ...   ... ... ... ..-...C ..A .GC ... ..C..-..G ...   M. szulgai
.A. ... ... ... ... ...   ... ..A ... ..-...C ..A .GC ... ..C..-..G ...   M. malmoense
CA. ... ... ... ... AC.   ... ... ... ..-.... .TT .GC ... ..C..-... ...   M. gastri/M. kansasii
CA. ... A.. ... ... ...   ... ... ... ..-...T .AA ..C ... .....-.C. ...   M. avium
CA. ... ... ... ... ...   ... ... ... ..-...T TTA .GC ... .....-.TA ...   M. intracellulare
.A. ... ... ... ... ACT   ... ... ... ..-...T .TC .GC ... ..C..AG.A ...   M. intermedium
.A. ... ... ... ... ...   ... ... ... T.-.... ... A.C ... ...T.-... ...   M. genavense
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Figure 2: I. 16S rRNA sequence of FI-24195; the first and the last nucleotides correspond to *Escherichia coli* positions 40 and 581, respectively. II. Partial alignment within hypervariable region A of selected mycobacterial 16S rRNA sequences (underlined in I). *Mycobacterium tuberculosis* was used as the reference sequence. Only nucleotides different from those in the *Mycobacterium tuberculosis* sequence are shown; dashes indicate deletions. The respective *Escherichia coli* 16S rRNA positions are indicated.

lymphadenitis was abruptly replaced by MAC in the 1970s. Although MAC involvement is hardly disputable, mainly because of the worldwide diffusion of commercial DNA probes, it is likely that several difficult-to-identify mycobacteria (primarily *Mycobacterium interjectum*) are loosely labeled together as *Mycobacterium scrofulaceum*. The mycobacterium presented here supports this hypothesis, as it would usually, albeit erroneously, be identified as *Mycobacterium scrofulaceum* because of its phenotypic similarity to that species.

There is no reason to doubt the pathogenic significance of our isolate, which was grown from primarily sterile tissue obtained by surgical excision concomitant with clinical and pathological evidence of chronic lymphadenitis. Although of limited clinical relevance given the universally accepted surgical treatment of cervical lymphadenitis, the susceptibility pattern of our isolate is characterized by MICs only slightly lower than those of MAC. No comparison with *Mycobacterium scrofulaceum* is possible because of the lack of quantitative data concerning that species.

As a first attempt to define its features, FI-24195 appears as an acid-alcohol-fast rod-shaped coccobacillus that does not form spores or capsules. Diluted inocula grow smooth scotochromogenic yellow colonies on solid media in two to three weeks at temperatures ranging from 25 to 37°C. Positive biochemical reactions include thermostable catalase and urease, while niacin, nitrate reduction, Tween 80 hydrolysis, and tellurite reduction are negative. The HPLC profile is different from those of other species, though it is not easily differentiated from that of either *Mycobacterium interjectum* or *Mycobacterium scrofulaceum*. However, nucleic acid sequences of hypervariable regions of the 16S rRNA (Figure 2) show divergence from *Mycobacterium scrofulaceum* of more than 12 nucleotides (in a stretch of almost 200 nucleotides). This finding definitively rules out

the possibility that FI-24195 is simply a variant of such species.

The combined data of phenotypical and nucleic acid analyses leave no other option but to suggest that FI-24195 represents a new species.

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