

Infections Due to the Newly Described Species *Mycobacterium parascrofulaceum*

Enrico Tortoli,^{1*} Leonardo Chianura,² Lionella Fabbro,³ Alessandro Mariottini,^{1,4}
Nuria Martín-Casabona,⁵ Gianna Mazzarelli,^{1,6} Cristina Russo,⁷
and Mario Spinelli⁸

Regional Reference Center for Mycobacteria, Microbiology and Virology Laboratory,¹ Cytogenetics and Genetics Unit,⁴ and Microbiological and Virological Serum-Immunology Laboratory,⁶ Careggi Hospital, Florence, Infectious Diseases Unit, Niguarda Hospital, Milan,² Social Hygiene Dispensary, Turin,³ Microbiology Laboratory, Bambino Gesù Hospital, Rome⁷, and Clinical Laboratory, Sant'Anna Hospital, Como,⁸ Italy, and Microbiology Laboratory, Vall d'Hebron Hospital, Autònoma University, Barcelona, Spain⁵

Received 29 March 2005/Returned for modification 29 April 2005/Accepted 18 May 2005

We report on four cases of infection by the recently described species *Mycobacterium parascrofulaceum*. In two cases the mycobacterium was isolated from AIDS patients, while in the others it was responsible for pulmonary disease in elderly men. Our findings suggest that *M. parascrofulaceum* is an opportunistic pathogen, like many other nontuberculous mycobacterial species.

CASE REPORTS

Case 1. A 35-year-old male with AIDS presented, in 1997, along with a very low CD4 lymphocyte count (7/ml) cerebral neurotoxoplasmosis, cytomegalovirus disease, and systemic cryptococcosis. His major symptoms included chorea, fever, and diarrhea. From his sputa, which were smear negative, a nontuberculous mycobacterium, not identified at that time (FI-97251), was isolated twice. The patient was treated with ethambutol and rifabutin until death, which came 1 month later.

Case 2. An unidentified mycobacterium (FI-00121) was grown in 2000 from a blood culture of a 40-year-old AIDS patient. The man was severely immunocompromised (39 CD4 lymphocytes/ml) and presented esophageal candidosis, cytomegalovirus infection, and fever. He was not treated with antimycobacterial drugs and died 6 months later.

Case 3. A 67-year-old Spanish male with epidermoid carcinoma of the left lung and mediastinum presented with chronic obstructive pulmonary disease and emphysema since 1997. Between August 2003 and April 2004, a nontuberculous mycobacterium (FI-03077), at first identified as *Mycobacterium scrofulaceum*, was isolated seven times from the sputum, which was microscopically negative for acid-fast bacilli. No information is available about treatment and follow-up.

Case 4. A 63-year-old patient, previously subjected to lobectomy because of bronchiectasis, presented in 2004 a cavitation of the left lung. A clinical sample obtained by means of bronchial aspiration was strongly positive for acid-fast bacilli and yielded in culture a strain (FI-04199) which was initially identified as *M. scrofulaceum*. Treatment with isoniazid, ethambutol, and rifampin was undertaken but did not produce any radiological improvement, the inability of the patient to produce sputum did not allow a microbiological follow-up. The

man died 4 months later because of worsening respiratory insufficiency.

An a posteriori reexamination of a cluster of mycobacteria showed that the four strains reported above belong to the newly described species *M. parascrofulaceum*.

The description of *M. parascrofulaceum* was based on a thorough analysis of multiple genetic regions of unidentified mycobacteria (7) previously named MCRO 33 (3). It was realized that several strains of unnamed mycobacteria reported in the literature or listed in public-domain nucleic acid databases in fact belong to the newly established species (7), to which also must be assigned several reference strains previously assigned to the species *M. scrofulaceum* and *M. simiae*. (7).

We were stimulated to investigate the presence of *M. parascrofulaceum* by the paper describing the new species (7), in which the authors refer to two strains of ours reported in an article concerning unidentified mycobacteria (5). In addition to the above strains, others were detected by sequencing the first 500 bp of the 16S rRNA gene (8) and the gene encoding the 65-kDa heat shock protein (*hsp65*) (7) in a group of 45 clinical mycobacteria collected in our laboratory since 1987. This group had been selected by screening the collection database on the basis of a number of phenotypic traits: slow growth at 25 and 37°C but not at 45°C, scotochromogenicity, negative Tween 80 hydrolysis, positive urease, and a high-performance liquid chromatography profile (HPLC) characterized by three peak clusters as reported by Turenne and coworkers for *M. parascrofulaceum* (7). Forty-five strains fit this pattern; 6 of them were unidentified, while 39 had been assigned to the species *M. scrofulaceum*, mostly on the basis of the identification obtained with the commercial inverse hybridization test INNO-LiPA Mycobacteria (LiPA; Innogenetics, Ghent, Belgium).

Four strains turned out to belong to the new species *M. parascrofulaceum*; two of these had not been identified before,

* Corresponding author. Mailing address: Centro Regionale di Riferimento per la Diagnostica dei Micobatteri, Piastra dei Servizi, Ospedale di Careggi, viale Morgagni 85, 50134 Firenze, Italy. Phone: 39-055-4279199. Fax: 39-055-4279830. E-mail: e.tortoli@libero.it.

```

77          440
AAGGTCTCTTCGGAGATACT    ACCAGGGACGAAG-----CGCAAGT-GACGGTACCTGCAGAA M. parascrofulaceum
....C.C.....G.G.....    ....TC.....GCTCACTTT.TGG..T.....GG..G..... M. scrofulaceum

```

FIG. 1. Alignment of traits of the 16S rRNA gene suitable for differentiation of *M. parascrofulaceum* from *M. scrofulaceum*. Base pair position is indicated according to the *Escherichia coli* sequence.

while two had been previously considered to belong to the species *M. scrofulaceum*. With LiPA, all such strains hybridized, in addition to the genus specific probe, also with the ones recognizing the group *M. avium-M. intracellulare-M. scrofulaceum* and the species *M. scrofulaceum*. With another commercial inverse hybridization assay, GenoType Mycobacterium CM (Hain, Nehren, Germany), the line probes specific for gram-positive bacteria characterized by high guanidine-cytosine content were positive, as was the one recognizing the whole genus *Mycobacterium* and lines 9 and 10, which pattern is considered distinctive of *M. scrofulaceum*.

Differentiation of *M. parascrofulaceum* from *M. scrofulaceum* is a major problem. The biochemical and cultural tests are not discriminative, and the same is true for HPLC of mycolic acids. Both commercial DNA probes intended for the identification of *M. scrofulaceum* assign *M. parascrofulaceum* to the latter species; this is surprising, as they are aimed at different genetic targets, the 16S-23S internal transcribed spacer in LiPA (6) and the 23S rRNA gene in GenoType (2). Genetic sequencing seems therefore the only approach providing reliable identification, whatever the region used (the 16S rRNA gene, the 23S rRNA gene, the internal transcribed spacer, or *hsp65*) (7). In the first 500 bp of the 16S rRNA gene, the most popular sequencing target for identification purposes, the distinction between *M. parascrofulaceum* and *M. scrofulaceum* is clear-cut (Fig. 1), with 4 mismatches in a short tract between nucleotides 80 and 90 and as many as 18 in hypervariable region B (positions 444 to 480). In our opinion, genetic sequencing of the strains identified biochemically, by HPLC, or by commercial DNA probes as *M. scrofulaceum* is advisable. In fact, the probability that such strains actually belong to the species *M. parascrofulaceum* is about 10%.

From the clinical point of view, *M. parascrofulaceum* seems to behave like the majority of nontuberculous mycobacteria (1, 4); its preferred target appears to be the lung, in particular in

elderly patients with predisposing conditions. In one such patient, as in the case reported in the description of the new species, *M. parascrofulaceum* was responsible for cavitations. In severely immunocompromised patients, in particular those with AIDS, it can be responsible for pulmonary or disseminated infection. None of the strains investigated so far was responsible for neck lymphadenitis, which is considered the most frequent disease due to the closely related species *M. scrofulaceum* (9).

From the present cases, little can be said about the response to antimycobacterial treatment. Therefore, the value of the in vitro susceptibility pattern, showing sensitivity to rifampin, clarithromycin, and amikacin (7) in all the strains investigated so far, remains uncertain.

REFERENCES

1. American Thoracic Society. 1997. Diagnosis and treatment of disease caused by nontuberculous mycobacteria. *Am. J. Respir. Crit. Care Med.* **156**:S1-S25.
2. Sarkola, A., J. Makinen, M. Marjamaki, H. J. Marttila, M. K. Viljanen, and H. Soini. 2004. Prospective evaluation of the GenoType Assay for routine identification of mycobacteria. *Eur. J. Clin. Microbiol. Infect. Dis.* **23**:642-645.
3. Springer, B., L. Stockman, K. Teschner, G. D. Roberts, and E. C. Böttger. 1996. Two-laboratory collaborative study on identification of mycobacteria: molecular versus phenotypic methods. *J. Clin. Microbiol.* **34**:296-303.
4. Tortoli, E. 2004. Clinical features of infections caused by new nontuberculous mycobacteria. *Clin. Microbiol. Newsl.* **26**:89-95, 97-100.
5. Tortoli, E., A. Bartoloni, E. C. Böttger, S. Emler, C. Garzelli, E. Magliano, A. Mantella, N. Rastogi, L. Rindi, C. Scarparo, and P. Urbano. 2001. Burden of unidentifiable mycobacteria in a reference laboratory. *J. Clin. Microbiol.* **39**:4058-4065.
6. Tortoli, E., A. Mariottini, and G. Mazzarelli. 2003. Evaluation of INNO-LiPA MYCOBACTERIA v2: improved reverse hybridization multiple DNA probe assay for mycobacterial identification. *J. Clin. Microbiol.* **41**:4418-4420.
7. Turenne, C. Y., V. J. Cook, T. V. Burdz, R. J. Pauls, L. Thibert, J. N. Wolfe, and A. Kabani. 2004. *Mycobacterium parascrofulaceum* sp. nov., novel slowly growing, scotochromogenic clinical isolates related to *Mycobacterium simiae*. *Int. J. Syst. Evol. Microbiol.* **54**:1543-1551.
8. Turenne, C. Y., L. Tschetter, J. Wolfe, and A. Kabani. 2001. Necessity of quality-controlled 16S rRNA gene sequence databases: identifying nontuberculous *Mycobacterium* species. *J. Clin. Microbiol.* **39**:3637-3648.
9. Wolinsky, E. 1979. Nontuberculous mycobacteria and associated diseases. *Am. Rev. Respir. Dis.* **119**:107-159.