Case Report

Discordant molecular characterization results in a *Mycobacterium avium* complex strain isolated from an AIDS patient

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This report describes an unusual strain of *Mycobacterium avium* complex isolated from the sputum of an immunocompromised AIDS patient, which did not react with the MAC probe of the BDProbe Tec system, but was identified as *Mycobacterium intracellulare* by 16S rRNA gene sequencing. Its PCR restriction-enzyme analysis pattern was compatible with an allelic variant of *M. avium*. It was scotochromogenic, slow-growing and phenotypically identified as *Mycobacterium scrofulaceum*. Its clinical significance is not certain.

Introduction

The *Mycobacterium avium* complex (MAC) consists of two closely related species, *M. avium* and *Mycobacterium intracellulare*, and possibly other less-well-defined organisms. MAC is a group of environmental mycobacteria that are found widely in soil, water and aerosols, and cause disease in animals and humans.

There appear to be significant differences between *M. avium* and *M. intracellulare* clinically as well as genetically. However, the biochemical tests for the identification of the species of MAC do not accurately speciate the two. Recent studies have emphasized DNA-based methods. Tests to further discriminate between the two MAC species include hybridization with DNA probes (Beggs *et al.*, 2000; Tomioka *et al.*, 1993; Saito *et al.*, 1990), PCR or real-time PCR with specific primers (Smole *et al.*, 2002; Nishimori *et al.*, 1995), and amplification of conserved mycobacterial sequences followed by either hybridization with species-specific probes to variable regions within the amplified target (Ferrario *et al.*, 2001) or gene sequencing (Novi *et al.*, 2000; Frothingham & Wilson, 1994). In addition, identification of species using PCR restriction-enzyme analysis (PRA) based on analysis of polymorphisms of restriction digests of the gene has been

demonstrated. More than 34 mycobacterial species and subspecies, including *M. avium* and *M. intracellulare*, can be differentiated by the algorithms established (Telenti *et al.*, 1993; Taylor *et al.*, 1997; Devallois *et al.*, 1997). However, allelic variants in clinical isolates that are not included in the algorithm may result in erroneous identification. These variants can be misidentified if no other identification method is employed.

The BDProbeTec ET MAC assay was recently developed to detect MAC in cultures without further speciation. The test system utilizes homogeneous strand displacement amplification (SDA) technology and fluorescent energy transfer (ET) to detect the MAC from cultures. It is an easy-to-perform and cost-effective method to be used in a clinical setting.

In this report, an unusual strain of MAC was characterized by biochemical tests, PRA and BDProbeTec MAC assay, and confirmed by 16S rRNA gene sequencing at the 5' region.

Case report

A 41-year-old heterosexual man was diagnosed as having AIDS in February 2002, when he presented with symptoms of dry cough and intermittent fever for 3 weeks. His CD4 count was 5 mm⁻³, and human immunodeficiency virus (HIV) viral load was 34 781 copies ml⁻¹. Pneumonia of the right lower lung was diagnosed by chest roentgenography. The patient's serum was positive for cryptococcal antigen with a titre of 1 : 4. There were no significant pathogens identified in

Abbreviations: CAT, computed tomography imaging; ET, energy transfer; MAC, *Mycobacterium avium* complex; NTM, nontuberculous mycobacteria(l); PRA, PCR restriction-enzyme analysis.

sputum smears by Gram-staining and acid-fast staining. A computed tomography imaging (CAT) scan of the chest demonstrated lobar consolidation with a central air bronchogram in the right lower lobe and a large subcarinal lymph node (2 cm). The tentative diagnosis was mycobacterial infection. An attempt to perform a CAT-guided biopsy of the pulmonary lesion resulted in failure. The patient received highly active antiretroviral therapy with combivir and nevirapine. He also received fluconazole, rifampicin, isoniazid, ethambutol, pyrazinamide and clarithromycin as pre-emptive treatment for the diagnosis of cryptococcal and mycobacterial infections. The chest roentgenography follow-up 1 month later showed the pneumonia to be less dense and smaller in size. The initial mycobacterial culture of sputa resulted in growth of MAC in two of the six sputum specimens. The patient was treated at an outpatient clinic and improved clinically.

Methods

Biochemical methods. Culture features, i.e. growth rate, colony morphology, the nature of the pigment produced in the dark or after photoinduction, and the ability to grow at temperatures ranging from 25 to 42 °C, were investigated on 7H11 agar plates. Other biochemical features, including sodium chloride (5%), niacin accumulation, nitrate reductase, 3-d arylsulfatase, semiquantitative catalase, heat-stable catalase (pH 7, 68 °C), Tween 80 hydrolysis and urease were assessed.

PRA. The bacterial DNA was prepared by QIAamp DNA Mini Kit (Qiagen). An aliquot (5 µl) of purified DNA was used for PCR. Amplification and restriction enzyme analysis were performed according to the procedure described by Telenti *et al.* (1993).

BDProbeTec MAC assay. The standard ProbeTec assay was performed according to the instructions of the manufacturer using a BDProbeTec ET instrument (Becton Dickinson Microbiology Systems). Both positive and negative controls were included in each assay run. An internal amplification control was used to confirm the validity of the amplification reaction and to identify potential inhibition from the processed specimen.

16S rRNA gene sequencing. The sequence of the hypervariable fragment A was determined as reported elsewhere (Wayne *et al.*, 1996; Springer *et al.*, 1996) by the dideoxynucleotide chain-termination method using the Taq DyeDeoxy Terminator Cycle sequencing kit (Applied Biosystems Division, Perkin-Elmer), a GeneAmp PCR System 9600 (Perkin-Elmer) and ABI PRISM 310 Genetic Analyser (Perkin-Elmer/Applied Biosystems). The results obtained were interpreted by comparison to known sequences in the RIDOM and NCBI databases.

Results and Discussion

Growth of the nontuberculous mycobacterial (NTM) isolate was observed on 7H11 agar plates after 2 weeks of incubation at temperatures ranging from 25 to 37 °C. Colonies were smooth and scotochromogenic. The strain produced thermoresistant catalase, nitrate reductase, tellurite reductase and urease activities, but was negative for accumulation of niacin, Tween hydrolysis and 3-d arylsulfatase test. Based on these features, the NTM isolate was identified as *Mycobacterium scrofulaceum*.

However, molecular characterization showed discordant results. The strain did not react with the MAC probe of the BDProbeTec system. The pattern of PRA was the same as the allelic variant of *M. avium* as reported elsewhere (Leao *et al.*, 1999). The strain was finally identified as *M. intracellulare* by 16S rDNA sequencing.

PRA is a simple, rapid and useful tool in the clinical microbiology laboratory. It can be used to identify the majority of clinically significant NTM. However, due to the heterogeneity of MAC organisms, which may include taxonomically ill-defined species other than *M. avium* and *M. intracellulare* (Baess, 1983; McFadden *et al.*, 1992), PRA often results in restriction patterns that cannot be interpreted using previously established algorithms. The presence of allelic variation can lead to misidentification.

16S rRNA gene sequencing has been particularly helpful in clarifying the rather confusing taxonomy within the MAC (Frothingham & Wilson, 1994; Soini *et al.*, 1996) and has allowed better characterization of MAC organisms. Although 16S rRNA sequencing remains relatively cumbersome, it is the only way to confirm the taxonomy within the MAC. Identification by PRA or a probe-hybridization method such as BDProbeTec MAC assay alone could give inaccurate results, which would delay the initiation of appropriate antibiotic therapy, and possibly adversely influence the clinical outcome.

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