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Mycobacteriology

Molecular identification of *Mycobacterium chimaera* as a cause of infection in a patient with chronic obstructive pulmonary disease

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Abstract

This report describes a case of *Mycobacterium chimaera* infection in a patient with a history of chronic obstructive pulmonary disease where the organism was identified by using molecular methods. *M. chimaera* was identified from fresh lung tissue and from an instrument-negative mycobacterial growth indicator tube broth culture. The utility of using sequence analysis of the internal transcribed spacer region for the rapid identification of a slow-growing nontuberculous *Mycobacterium* spp. where conventional culture methods were not successful was shown.

Keywords: Molecular identification; *Mycobacterium chimaera*; Chronic obstructive pulmonary disease

1. Introduction

Nontuberculous *Mycobacterium* (NTM) species continue to be identified as increasing causes of human disease (Glassroth, 2008). Tortoli (2003, 2006) estimated that the number of species in the known taxonomy of NTM grew by 42 between 1990 and 2003 and further increased by 30 in the subsequent 3 years (Iwen et al., 2006). One NTM species recently characterized was *Mycobacterium chimaera*, previously described as an undifferentiated member of the *Mycobacterium avium* complex (MAC). Subsequently, Lebrun et al. (2005) showed that the prior named species *Mycobacterium intracellulare* sequevar Mac-A should now be identified as *M. chimaera*. This newly described species has been recognized as a cause of respiratory disease in a series of 6 elderly patients during a 5-year period from 5 Italian hospitals (Tortoli et al., 2004).

Before its designation as a new species, *Mycobacterium chimaera* was identified as an *M. avium* complex by the AccuProbe test (Gen-Probe, San Diego, CA) or as a member of the MAIS group (*M. avium*/*M. intracellulare*/*Mycobacterium scrofulaceum*) by the INNO-LiPA-MYCOBACTERIA v1 (Lipav1) test (Innogenetics, Ghent, Belgium). Sequencing data using a variable region of the 16S rDNA target identified the species as *M. intracellulare* in both the GenBank (National Center for Biotechnology Information, Washington, DC) and the RIDOM databases (Harmsen et al., 2002). Nucleotide mismatches within the internal transcribed spacer (ITS) region between the 16S and 23S genes were used to identify *M. chimaera* as a new species (Tortoli et al., 2004; Turenne et al, 2007).

Overall, the identification of slow-growing NTM species is problematic for the clinical laboratory and frequently requires use of a molecular-based assay (Daley et al., 2008; Fend et al., 2007; Griffith et al., 2007; Lim et al., 2008; Tenover, 2007). In 11 cases of NTM-caused disease, Ooi and Fekete (2006) found that the median time for identification of the species that were not *M. avium* complex was 68 days (range, 47–292 days), and in 3 of these cases, treatment was not instituted because of this delay.

This present report adds to the cases of reported diseases caused by *M. chimaera* and shows the utility of using a molecular assay for the detection and identification of a pathogen from clinical material where conventional culture was negative.

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2. Case

The patient was a 69-year-old woman with a history of breast cancer (2.5 years previously) and chronic obstructive pulmonary disease (COPD). The patient was a prior smoker and was on oxygen for pulmonary support. As a follow-up to monitor for cancer, the patient underwent periodic repeat computed tomography (CT) scans. Two years before admission of the present illness, new small bilateral pulmonary nodules were noted on a routine CT scan. Because the patient was afebrile and the nodules were small and multiple and did not have characteristics for metastatic lung disease, the primary care physician decided at that time to continue monitoring the patient with serial CT scans to evaluate for progression of the nodules. At 8 weeks before admission, the nodule in the right upper lobe was noted to be increasing in size (from 8 to 11 mm). Because of this finding, a surgical consult was obtained with a subsequent recommendation to have the nodule excised to determine the etiologic source.

The patient was admitted to the hospital in stable condition (day 0 postadmission [PA]), with plans to have a video-assisted thoracoscopy (VAT) with a wedge resection of the right lung nodule performed. The VAT occurred on day 1 PA, and a frozen section of the nodule at the time of surgery gave a preliminary report of "necrotizing granulomatous inflammation". Bacterial, fungal, and viral cultures were performed on the lung tissue including culture for acid-fast bacilli (AFB). An original AFB stain on a homogenate of the lung tissue was reported as "moderate AFB present". The AFB culture included inoculation of the specimen into nonradiometric mycobacterial growth indicator tube (MGIT) media with incubation in the BACTEC MGIT960 System (Becton Dickinson, Sparks, MD) and to Lowenstein–Jensen and Middlebrook 7H11 agar slants. The permanent sections for histopathology were consequently reported as "caseating granulomas with no evidence of malignancy [and] numerous AFB identified within the caseating granulomas". Because there was a potential diagnosis for tuberculosis (TB), the patient was placed in respiratory isolation. Because of the concern for TB, a sample of the fresh lung tissue was submitted for DNA extraction and molecular analysis using a technique as described by Mohamed et al. (2005). An amplified product from the ITS region of the rRNA gene was detected in the tissue and then sequenced. The sequence was submitted for species sequence analysis identification using the AMIS™ (Advanced Molecular Identification System) and the associated curated MycoAlign™ database as described previously (Olsen et al., 2007). The results of the sequence analysis comparison identified the molecular product as a 100% match with *M. chimaera*. An infectious diseases consult at the time of the histology report suggested withholding therapy until the patient became more stable and the culture results become available for optimal patient management. Fungal, viral, and routine bacterial cultures remained negative during the patient's hospitalization. After surgery and for the ensuing 10 days, the patient's pulmonary condition worsened with subsequent development of abdominal symptoms that required an exploratory laparotomy leading to a subtotal colon resection (day 10 PA). Because of worsening pulmonary symptoms, the patient was placed on a do-not-resuscitate status, and on day 16 PA, the patient died. The primary cause of death was reported as complications to COPD in a patient with a history of breast cancer. No postmortem examination was performed.

The solid media cultures remained negative after 8 weeks of incubation. The MGIT broth culture also remained instrument negative; however, a terminal AFB stain of the broth showed the presence of a few AFB. Subsequently, broth from the MGIT bottle was subcultured to solid AFB medium as done previously and also tested directly by the MycoAlign™ molecular assay. Although the solid medium remained negative after 10 weeks of incubation, the extracted DNA from the MGIT broth did amplify and the product was again identified as *M. chimaera*.

3. Discussion

M. chimaera, which has only recently been reported as a new pathogen, was described by Tortoli et al. (2004) as a cause of human respiratory disease in 6 patients. Although additional citations of case studies of *M. chimaera* infection were not found in the literature, many records of mycobacterial infections where a subset was likely caused by *M. chimaera* before its designation as a new species were recognized (Aksamit, 2002; Field et al., 2004; Piersimoni and Scarparo, 2008; Prince et al., 1989; Reich and Johnson, 1991; Rosenzweig, 1979). Lebrun et al. found that 10 of 35 MAIS isolates from patients with pulmonary disorders (bronchiectasias and lung cancer) were probably *M. chimaera*; however, no specific clinical records were presented (Lebrun et al., 2005).

Five of the 6 patients described by Tortoli et al. (2004) had an underlying disease involving the respiratory tract, with COPD as the most common in 3 of the cases. Three of the 6 patients had known recovery of disease, all treated with clarithromycin in combination with other antimycobacterial agents. No antimicrobial susceptibility results were available for any of the reported cases.

All 6 prior reported cases had positive cultures of *M. chimaera* from respiratory specimens. These cases all fulfilled the microbiologic diagnostic criteria of nontuberculous mycobacterial lung disease in a symptomatic person without another diagnosis from the American Thoracic Society and the Infectious Diseases Society of America (ATS/IDSA) (Griffith et al., 2007). These criteria included positive culture results from 1) at least 2 separate expectorated sputum samples, 2) at least 1 bronchial wash or lavage, or 3) a biopsy material with mycobacterial histopathologic features (granulomatous inflammation or presence of AFB). Although the present case did not fulfill