Possession of the macrophage-induced gene by isolates of the *Mycobacterium avium* complex is not associated with significant clinical disease

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The *Mycobacterium avium* complex (MAC) is the most frequently isolated species among non-tuberculous mycobacteria (NTM) clinical isolates. Physicians pay attention to the differential diagnosis of the disease caused by MAC from tuberculosis because of their similar clinical presentations. Expression of the macrophage-induced gene (*mig*) is one of the virulence phenotypes in MAC, but it has not been determined whether the presence of the *mig* gene itself has any relationship with clinical disease or whether it is merely a marker for MAC. To uncover the significance of the *mig* gene among MAC clinical isolates, positive cultures from respiratory specimens from patients in a tertiary referral centre were identified by sequencing the 16S rRNA gene. The *mig* gene was also evaluated using PCR and sequence analysis. The medical records from the patients were reviewed retrospectively. The diagnostic criteria from the American Thoracic Association were adopted for the diagnosis of NTM lung disease. A total of 45 MAC clinical isolates were identified over a period of 1 year. Following 16S rRNA sequencing, all of the 23 *M. avium* isolates were categorized as sequevar I. Among the 22 *Mycobacterium intracellulare* isolates, 18 strains were identified as *M. intracellulare* sequevar I and the remaining four consisted of one each of sequevars II, III, IV and V. The proportion of cases that fitted the diagnostic criteria of NTM lung disease was 26.7% (12/45). The *mig* PCR results were 100% positive for the MAC isolates studied, irrespective of their species, sequevar or disease-causing properties. However, following bootstrap analysis of the *mig* sequences, we observed definite grouping between *M. avium* and *M. intracellulare*. Thus the *mig* gene is a species-specific marker with distinct sequence diversity between the two species *M. avium* and *M. intracellulare*, but there is poor correlation between disease-causing properties and specific *mig* sequences.

**INTRODUCTION**

Non-tuberculous mycobacteria (NTM) were generally regarded as contaminants in Korea where the incidence of tuberculosis is still high (WHO, 2004). However, NTM have come to attention as pathogens since cases of NTM lung disease were reported in the 1990s (Bai et al., 1993; Koh et al., 2004, 2006; Lew et al., 1992; Pae et al., 1999). In Korea, the *Mycobacterium avium* complex (MAC) is the most common species (48%), followed by *Mycobacterium abscessus* (33%), isolated from patients with NTM lung disease, which is estimated to account for 25% of culture-positive cases (Koh et al., 2006). The isolation frequencies from clinical specimens are 54% for MAC and 18% for *M. abscessus* (Shin et al., 2006).

MAC comprises two genetically distinct species, *M. avium* and *Mycobacterium intracellulare*, which are indistinguishable by conventional culture and biochemical methods (Inderlied et al., 1993).

In developed countries, where the incidence of tuberculosis is low, *M. avium* is more prevalent than *M. intracellulare* in the blood cultures of AIDS patients (Kulski et al., 1995). In contrast, *M. intracellulare* is more prevalent in human immunodeficiency virus (HIV)-seronegative patients (Kyriakopoulos et al., 1997). Specific MAC subtypes or

**Abbreviations:** AFB, acid-fast bacilli; MAC, *Mycobacterium avium* complex; NTM, non-tuberculous mycobacteria.
the presence of particular plasmids primarily influence the virulence of mycobacteria that cause human infections. However, it may also be important to understand the survival and growth of mycobacteria within their host cells, which are usually monocytes and macrophages. There have been several studies on macrophage-induced genes (mig) that are expressed only when \textit{M. avium} proliferates within macrophages (Plum & Clark-Curtiss, 1994; Plum et al., 1997). For clinical strains, the mig gene has been used as a genetic marker for \textit{M. avium} (Beggs et al., 2000; Menendez et al., 2001). However, only 64\% of MAC isolates are reported to have the mig gene in West Africa (Koivula et al., 2004). Thus the clinical significance of the mig genetic marker remains to be clarified.

The aim of this study was to characterize the mig gene in MAC isolates obtained from respiratory specimens and to evaluate the clinical significance of MAC isolates over a 1-year period from a tertiary hospital in Seoul, Korea. For the diagnosis of NTM lung disease, all three categories of clinical, radiological and microbiological criteria had to be met as recommended by the American Thoracic Society (1997), although the microbiological criteria used were more lenient (American Thoracic Society Documents, 2007). Because MAC lung disease, in particular the upper-lobe cavitory form, resembles pulmonary tuberculosis, which is prevalent in Korea, it is difficult to differentiate these two diseases by radiological and clinical features alone unless there is microbiological evidence of NTM.

In Korea, however, it has become common practice to make a presumptive diagnosis of pulmonary tuberculosis and then to prescribe treatment to a patient when the results of sputum smear staining for acid-fast bacilli (AFB) are positive. It is only recently that physicians have become aware of NTM culture isolates as the possible cause of the disease. In particular, positive culture results for NTM, along with negative staining for AFB, was until recently presumed to be a result of contaminants and to be clinically insignificant. Therefore, it is likely that the correct diagnosis and treatment of MAC lung diseases are not made early in the course of the disease. This study looked for a correlation between the mig gene and MAC clinical isolates, which would be helpful for physicians when deciding the significance of culture results.

### RESULTS AND DISCUSSION

**Identification of clinical MAC isolates**

16S rRNA gene analysis of the 45 clinical MAC isolates showed that 23 were \textit{M. avium} and 22 \textit{M. intracellulare}. All of the \textit{M. avium} isolates were categorized as \textit{M. avium} sequevar I, which includes \textit{M. avium} subsp. \textit{avium}, \textit{M. avium} subsp. \textit{paratuberculosis} and \textit{M. avium} subsp. \textit{silvaticum} (Sanderson et al., 1992). Among the \textit{M. 

### Table 1. Summary of the 45 clinical MAC strains according to diagnosis using the criteria of the American Thoracic Society

<table>
<thead>
<tr>
<th>Strain</th>
<th>No. of strains (%)</th>
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<tr>
<td></td>
<td>Disease</td>
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<tr>
<td>\textit{M. avium} (n=23)</td>
<td>4 (17%)</td>
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<tr>
<td>\textit{M. intracellulare} (n=22)</td>
<td>8 (36%)*</td>
</tr>
<tr>
<td>Total</td>
<td>12 (27%)</td>
</tr>
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</table>

*Seven of \textit{M. intracellulare} sequevar I and one of \textit{M. intracellulare} sequevar III. †Eleven of \textit{M. intracellulare} sequevar I and one from each category of \textit{M. intracellulare} sequevars II, IV and V.
intracellulare isolates, 18 were found to be \textit{M. intracellulare} sequevar I and the remaining four consisted of one from each category of sequevars II, III, IV and V. Of the 45 patients from whom MAC was isolated, none was immunocompromised: none had antibodies against HIV. Twelve (26.7\%) of the 45 cases could be clinically diagnosed as lung disease caused by MAC, comprising four cultured isolates of \textit{M. avium} and eight of \textit{M. intracellulare} (Table 1).

16S rRNA sequencing has been established as an important means of microbial identification, but its usefulness is limited in the case of mycobacteria due to the low variability among species (Domenéch \textit{et al.}, 1994; Picardeau \textit{et al.}, 1997). However, MAC strains can be identified to species level and designated to a specific sequevar using the RIDOM database (Harmsen \textit{et al.}, 2002).

### Analysis of the \textit{mig} gene

All of the 45 MAC isolates were positive for PCR of the \textit{mig} gene, with an amplification product of 373 bp (Fig. 1). The \textit{mig} gene sequences of our clinical \textit{M. intracellulare} isolates showed 13–17\% variation when compared with that of \textit{M. avium} GenBank accession no. U43598. When a phylogenetic tree based on the partial sequences of the \textit{mig} gene was constructed using the neighbour-joining method...
(bootstrap value 1000), M. avium and M. intracellulare were clearly separated into two groups, but the isolates that had caused lung disease did not group into specific clades (Fig. 2). Thus there was no specific mig gene sequence associated with a particular sequevar or disease-causing property.

In MAC species, the mig gene is expressed when the mycobacteria proliferate within macrophages (Plum & Clark-Curtiss, 1994) and encodes a medium-chain acyl-CoA synthetase (Plum et al., 1997). This enzyme contributes to the supply of energy and carbon to the mycobacteria within the phagolysosome and thus seems to be implicated in the intracellular survival of the microbes (Lorenz & Fink, 2001; McKinney et al., 2000). This has been shown by the fact that strains of Mycobacterium smegmatis were more resistant to the intracellular killing action of macrophages in an infection model in which the expression of mig had been artificially induced in the mycobacteria (Plum et al., 1997). It has been reported that the MAC isolates from nine AIDS patients showed a specific RFLP pattern of their mig genes, the presence of which was common in all MAC strains (Meyer et al., 1998). However, some studies have reported that the presence of the mig gene is peculiar to MAC (Menendez et al., 2001) or is specific for M. avium (Beggs et al., 2000). Our results of 100% positivity for mig PCR among the MAC isolates irrespective of 16S rRNA sequevar or the presence of lung disease supports the suggestion that mig is common in MAC rather than a marker for M. avium.

The presence of the mig gene in only 64% of MAC isolates in the study by Koivula et al. (2004) was probably due to the difference in the studied MAC isolates, which were defined by hybridizing with a MAC probe (AccuProbe), whilst our isolates were determined by sequencing of the 16S rRNA gene.

In conclusion, PCR and sequence analysis of the mig gene were performed for the clinical MAC isolates in this study. The presence of the mig gene was 100% positive for clinical MAC isolates by the PCR. However, even with sequence analysis, we found poor correlation between the genetic diversity of the mig gene and disease-causing properties, but could distinguish M. avium from M. intracellulare.

**REFERENCES**


