Identification of Mycobacterium avium Complex Isolated in Eastern and Central Japan by Using DNA Probes

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Department of Respiratory Medicine, Division of Cancer Control, Institute of Development, Aging and Cancer, Tohoku University, Sendai 980–77, *Department of Medicine, Sendai Teishin Hospital, Sendai 980, †Department of Respiratory Diseases, Iwate Prefectural Central Hospital, Morioka 020, ‡Department of Respiratory Diseases, Seirei Mikatahara Hospital, Hamamatsu 433, and §The First Department of Medicine, School of Medicine, Kurume University, Kurume 830

WATANABE, A., KIKUCHI, H., SHOJI, S., NUKIWA, T., MOTOMIYA, M., YOSHIDA, T., TAKIZAWA, S. and OIZUMI, K. Identification of Mycobacterium avium Complex Isolated in Eastern and Central Japan by Using DNA Probes. Tohoku J. Exp. Med., 1995, 175 (2), 139-142 — An attempt was made to identify Mycobacterium avium and Mycobacterium intracellulare in the M. avium intracellulare complex (MAC) isolated in the Tohoku (38 strains) and Tokai (30 strains) districts of Japan by using DNA probes which are specific for M. avium, M. intracellulare and M. tuberculosis complex, respectively. The incidence of M. avium infection (82%) by far exceeded that of M. intracellulare infection in the Tohoku district of eastern Japan. In the Tokai district of central Japan, the incidence of M. avium infection (57%) were slightly larger than that of M. intracellulare infection. Five of 68 strains showed a positive reaction with two different DNA probes. Thus a possibility of mixed infection could not be ruled out, because reference strains showed a positive reaction with only one species-specific DNA probe.

DNA probe; M. avium intracellulare complex; M. avium; M. intracellulare

There is a growing number of patients with atypical mycobacterial infections in Japan (Tsukamura et al. 1988). Therefore, it is important to identify species of atypical mycobacteria. Mycobacteria have been identified and classified mainly by a combination of biochemical tests. However it is difficult, on the basis of conventional biochemical reactions alone, to differentiate Mycobacterium avium (M.a.) from Mycobacterium intracel-
Therefore, these two species have been documented as the *M. avium intracellulare* complex (hereafter referred to as MAC) in most reports.

As a result of recent progress in DNA hybridization techniques, identification of mycobacteria has become much easier. Several reports (Saito et al. 1989; Goto et al. 1990; Mizutani 1990) have been published concerning the distribution of MAC in Japan using species-specific DNA probes. These reports showed the ratio of the incidence of *M. avium* infection to that of *M. intracellulare* infection (*M. avium/M. intracellulare* ratio) in eastern Japan was higher than that in western Japan, where the incidence of *M. intracellulare* infection exceeded that of *M. avium* infection. However, as to the *M. avium/M. intracellulare* ratio in the Tohoku district of eastern Japan, no report has been available yet. In the present study DNA hybridization techniques were applied and the data on *M. avium/M. intracellulare* ratio in two different districts (the Tohoku and the Tokai districts) are presented.

A total of 68 strains of niacin-negative clinical isolates of MAC during the period from 1988 to 1992 were included in the present study. Of these 68 strains, 38 strains were isolated in the Tohoku district (8 strains at a hospital affiliated to the Institute of Development, Aging and Cancer, Tohoku University, 23 strains at Sendai Kosei Hospital and 7 strains at Iwate Prefectural Central Hospital), and 30 strains were isolated at Seirei Mikatahara Hospital in the Tokai district in the central Japan. *M. avium* ATCC 25291, *M. intracellular* ATCC 15985 and *Mycobacterium tuberculosis* H$_3$7,RV were used as reference strains.

Mycobacterial species were identified using the Gen-Probe™ Rapid diagnostic system (Gen Probe Co., San-Diego, CA, USA) as described in the standard manual of the manufacturer. Colonies on 1% Ogawa medium (3 to 4 weeks old at 37°C) were suspended in distilled water and adjusted to a turbidity equivalent of MacFarland No. 1 barium sulfate standard. The suspension thus prepared was sonicated and ribosomal RNA was extracted. An $^{125}$I-labeled single strand DNA probe solution which is specific for *M. avium*, *M. intracellular* and *Mycobacterium tuberculosis* complex respectively was added to this extract. The resulting suspension was incubated at 72°C for 1 hr for annealing, adsorbed on hydroxyapatite and centrifuged. The sediment thus obtained was washed and the radioactivity on hydroxyapatite was counted with a γ-counter (A500C; United Technologies Packard, Meriden, CT, USA). Percentage hybridization of 10% or above was defined as “positive” in DNA hybridization tests for the identification of mycobacterial species (Drake et al. 1987).

Table 1 shows the percent hybridization of reference strains as determined by using DNA probes which are specific for *M. avium*, *M. intracellular* and *Mycobacterium tuberculosis* complex.

Table 2 shows the results of DNA hybridization tests of 68 MAC strains from two districts of Japan. Thirty one of 38 strains (82%) from the Tohoku district, and 17 of 30 strains (57%) from the Tokai district reacted with the *M. avium* specific probe.

Of the total of 68 strains, 64 reacted with one probe and four reacted with two different probes. Two of the four strains reacted with *M. avium* and *M. intracellular* probes, and the remaining two reacted with *M. avium* and *Mycobacterium tuberculosis* complex probes.

Saito et al. (1989) and Mizutani (1990), based on their nationwide studies, found that

<table>
<thead>
<tr>
<th>Table 1. Percent hybridization of reference strains as determined by using species-specific DNA probes</th>
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<tbody>
<tr>
<td>Reference strain</td>
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<tr>
<td></td>
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<tr>
<td><em>M. avium</em> ATCC 25291</td>
</tr>
<tr>
<td><em>M. intracellular</em> ATCC 15985</td>
</tr>
<tr>
<td><em>Mycobacterium tuberculosis</em> H$_3$7,RV</td>
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Table 2. Results of DNA hybridization tests of 68 MAC strains isolated in two different districts of Japan as determined by using species-specific DNA probes

<table>
<thead>
<tr>
<th>District</th>
<th>Number of strains</th>
<th>Number of strains identified</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><em>M. avium</em></td>
<td><em>M. intracellulare</em></td>
</tr>
<tr>
<td>Tohoku</td>
<td>38</td>
<td>31</td>
<td>8</td>
</tr>
<tr>
<td>Tokai</td>
<td>30</td>
<td>17</td>
<td>14</td>
</tr>
<tr>
<td>Total</td>
<td>68</td>
<td>48</td>
<td>22</td>
</tr>
</tbody>
</table>

*a* one strain reacted with *M. avium* and *M. intracellulare* probes, and two strains reacted with *M. avium* and *M. tuberculosis* probes.

*b* one strain reacted with *M. avium* and *M. intracellulare* probes.

M.a. was more frequently isolated than M.i. in eastern Japan, while the incidence of M.i. infection exceeded that of M.a. infection in western Japan. However, in their study, no data have been presented on the M.a./M.i. ratio in the Tohoku district in the eastern Japan. In the present study, the DNA hybridization techniques were applied to identify M.a. and M.i. of MAC in two different districts of Japan.

Percentage hybridization of 10% or above in the Gen-Probe™ method for Mycobacteriaceae was defined as "positive" in the study of Drake et al. (1987). In the present study, the percentage hybridization in 210 of 213 tests with 68 clinical isolated strains plus three reference strains were above 15% or below 5%. It was between 5% and 15% only in three of 213 tests. Therefore, the above data justify the validity of 10% hybridiation as the cut-off point in our study.

In the present study, the incidence of M.a. infection in the Tohoku district by far exceeded that of M.i. infection as shown by M.a./M.i. index of 82%. The M.a./M.i. ratio in the Tokai district was lower than that in the Tohoku district, but still higher than that in the western Japan. Thus the findings by Saito et al. (1989) and Mizutani (1990) were confirmed and extended.

Of the 68 strains tested in the present study, four strains reacted with two different species-specific DNA probes. The M.a. probe and M.i. probe showed no cross reactions when tested with reference M.a. and M.i. strains. Neither of the above two probes cross-reacted with H37RV, a Mycobacterium tuberculosis hominis. Hence a possibility of mixed infection caused by two different strains can not be ruled out when test strains react with two different probes (Tomoka et al. 1991). Such a possibility must be taken into consideration when managing patients with atypical mycobacterial infection. Clinical studies showed that symptoms of M.a. infection tended to be severer (Urano et al. 1990), the results of retreatment were less satisfactory (Mizutani 1990) and that many M.a. strains were drug-resistant (Saito et al. 1989; Mizutani 1990; Urano et al. 1990). Therapeutic efficacy in patients with MAC in the eastern Japan has been less satisfactory than in the western Japan. This might be attributable to the fact that M.a. is isolated more frequently in the eastern than in western Japan as confirmed in the present study.

References


