CORRESPONDENCE

This department is for the publication of informal communications that are of interest because they are informative and stimulating, and for the discussion of controversial matters. The mandate of this Journal is to disseminate information relating to leprosy in particular and also other mycobacterial diseases. Dissident comment or interpretation on published research is of course valid, but personality attacks on individuals would seem unnecessary. Political comments, valid or not, also are unwelcome. They might result in interference with the distribution of the Journal and thus interfere with its prime purpose.

Active Surveillance of Leprosy Contacts in Country with Low Prevalence Rate

ABSTRACT

For advanced control of leprosy in Pakistan where the World Health Organization leprosy elimination goal was achieved in 1996, we conducted surveillance of *Mycobacterium leprae*-seropositive patients and their contacts and drug resistant strains of *M. leprae*.

We measured anti-PGL-I antibody level in sera from leprosy patients and their contacts for early detection of *M. leprae* infection. Out of 34 leprosy patients undergoing treatment, 4 lepromatous leprosy patients were antibody positive, and 6.8 to 23.7 percent of occupational or household contacts were seropositive. Furthermore, three cases (1.2%) had a high antibody titer. For surveillance of drug resistant strains of *M. leprae*, dapsone and rifampin were targeted. Four out of 18 polymerase chain reaction (PCR) positive samples had mutation in *folP* gene, and among 10 PCR positive samples, one had a mutation in the *rpoB* gene.

These results indicate that serological analysis of patient contacts might be useful to find out high risk individuals, and there are *M. leprae* strains resistant to chemotherapeutic agents in Pakistan.

RÉSUMÉ

Dans le cadre du contrôle avancé de la lèpre au Pakistan où le programme de l’Organisation Mondiale de la Santé a atteint son but d’élimination en 1996, nous avons mené une étude d’épidémio-surveillance des patients séropositifs contre *Mycobacterium leprae*, de leurs contacts et des souches résistantes de *M. leprae* aux médicaments.

Nous avons mesuré les niveaux d’anticorps anti-PGL-I dans le sérum de patients lépreux et des personnes en contact avec ces derniers afin d’effectuer une détection précoce de l’infection par *M. leprae*. Parmi 34 patients actuellement sous traitement, 4 patients lépromateux étaient positifs à l’examen sérologique, et 6,8 à 23,7 pour cent des personnes en contact, soit professionnel, soit domestiques, furent séropositifs. De plus, 3 cas (1,2%) présentaient un titre élevé. La résistance à la dapsone et la rifampicine furent évaluées pour la surveillance des souches résistantes de *M. leprae*. Quatre des 18 échantillons positifs par PCR présentaient des mutations du gène *folP* et, parmi 10 échantillons positifs par PCR, une avait une mutation du gène *rpoB*.

Ces résultats indiquent que l’analyse sérologique des contacts proches de patients hanséniens pourrait bien être utile pour découvrir les individus à haut risque et qu’il existe des souches de *M. leprae* résistantes aux médicaments chimiothérapeutiques au Pakistan.

RESUMEN

Se hizo un estudio en Pakistán, donde la meta de la OMS de eliminación de la lepra se logró en 1996, para evaluar la evolución de los pacientes sero-positivos a *Mycobacterium leprae* y sus contactos, y para detectar cepas de *M. leprae* resistentes a las drogas antileprosas.
Se midió la presencia de anticuerpos anti-PGL-I en los sueros de los pacientes y sus contactos para detectar la infección temprana por *M. leprae*. De los 34 pacientes en tratamiento, 4 pacientes con lepra lepromatosa (11.7%) tuvieron anticuerpos anti-PGL-I, además de que 6.8% de los contactos ocupacionales y 23.7% de los contactos convivientes también fueron sero-positivos. Tres casos (1.2%) tuvieron anticuerpos anti-PGL-I a títulos elevados. También se estudió la resistencia de las cepas a dapsona y rifampina. Cuatro de 18 muestras positivas por la reacción en cadena de la DNA polimerasa (PCR) tuvieron una mutación en el gene *foIP*, y una de 10 muestras positivas por PCR tuvo una mutación en el gene *rpoB*.

Estos resultados indican que el análisis serológico de los pacientes puede ser útil para detectar a los individuos de alto riesgo, y que en Pakistán hay cepas resistentes a la quimioterapia.

**TABLE 1. Detection of anti-PGL-I antibody in sera from leprosy patients and their contacts.**

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of sera examined</th>
<th>No. of positive sera</th>
<th>Percent positivity</th>
<th>No. of positive sera at each serum dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1:32</td>
</tr>
<tr>
<td>Lepromatous leprosy patients</td>
<td>20</td>
<td>4</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td>Borderline leprosy patients</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Tuberculoid leprosy patients</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Household contacts (children)</td>
<td>61</td>
<td>7</td>
<td>11.5</td>
<td>0</td>
</tr>
<tr>
<td>Household contacts (adults)</td>
<td>132</td>
<td>9</td>
<td>6.8</td>
<td>4</td>
</tr>
<tr>
<td>Occupational contacts</td>
<td>59</td>
<td>14</td>
<td>23.7</td>
<td>2</td>
</tr>
<tr>
<td>Non contacts</td>
<td>14</td>
<td>3</td>
<td>21.4</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>300</td>
<td>37</td>
<td>12.3</td>
<td>6</td>
</tr>
</tbody>
</table>

*a Detection of anti-PGL-I antibodies in serially diluted sera by ELISA using NT-P-BSA antigen coated gelatin particles.

Serum dilution of more than 1:32 showing agglutination was taken as positive.*
all lepromatous leprosy patients. However, borderline or tuberculoid leprosy patients had no antibodies against PGL-I. We then examined 193 household and 59 occupational contacts. Among household contacts, 11.5% of children had the antibody as did 6.8% of adult contacts (Table 1). Furthermore, 23.7% of occupational contacts had the antibody. Three out of 14 non-contacts were antibody positive. Further studies should be conducted with a larger number of non-contacts, but presently, we could not obtain informed consent from them. The titers among child contacts and occupational contacts are surprisingly high, which may indicate that some individuals were exposed to \textit{M. leprae}. This is in accordance with a report that the seroprevalence rate was 26 to 28% in the high endemic area, and 7% in the low endemic area in Sulawesi, Indonesia (7). When we measured the antibody in a semi-quantitative fashion, individuals having high antibody titer were found in household and occupational contacts. The titers of antibody varied from low (1:32) to high (1:>512) values. Three cases out of 252 (1.2%) samples showed quite high (1:>512) antibody titer. These individuals should have a clinical examination to monitor the leprosy manifestation. It has been reported that anti-PGL-I antibody level can reflect the disease activity (8). Therefore, it might be reasonable to speculate that the antibody production was suppressed by successful MDT treatment.

**Detection of drug resistant \textit{Mycobacterium leprae}**. Multi-bacillary (MB) type leprosy patients, either under or after MDT treatment, were targeted to obtain bacilli in the biopsy specimen. \textit{M. leprae} genomic DNA was extracted from the specimens as described previously (8).

To detect drug resistant \textit{M. leprae}, based on the previous studies (4, 6, 8), we targeted mutations of the fol\textit{P} gene encoding dihydropteroate synthase (DHPS) for dapsone (5), and the \textit{rpoB} gene for rifampin resistance (4, 8). The polymerase chain reaction (PCR) conditions and primers for \textit{folP} and \textit{rpoB} are as described previously (5, 6). The amplified products from each primer pair were sequenced by using the ABI Prism 310 Genetic Analyzer (Perkin-Elmer Applied Biosystems, Norwalk, CT, U.S.A.).

Thirty-nine skin samples were taken from leprosy patients in endemic areas of Pakistan such as Karachi, Peshawar, and Balakot, to detect gene mutations relating to drug resistance (Table 2). The number of samples successfully amplified using primers for \textit{folP} gene from 39 biopsy specimens was 18. Among amplified samples, four samples showed \textit{folP} mutations (22.2%). The \textit{folP} gene mutations were found at position 158th (the numbering system following that of reference 5) in three samples, and position 164th in one sample. These mutations induce amino acid changes from threonine to isoleucine at position 53rd of DHPS and from proline to arginine at 55th, respectively (not shown). These mutations have most commonly been observed in dapsone resistant strains (5). Although a larger number of samples should be analyzed, these observations may indicate that there are dapsone-resistant \textit{M. leprae} in Pakistan. In contrast to \textit{folP} gene, primer pair for \textit{rpoB} less frequently amplified the DNA. The possible reason for the failure might be the presence of less than detectable level of \textit{M. leprae} bacilli. In our hands, the detection limit is approximately ten bacilli per biopsy sample. Also the different amplification efficiency between \textit{folP}

<table>
<thead>
<tr>
<th>Place</th>
<th>No. of samples</th>
<th>No. amplified \textit{folP}</th>
<th>Mutation</th>
<th>No. amplified \textit{rpoB}</th>
<th>Mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Karachi</td>
<td>24</td>
<td>8</td>
<td>1</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Peshawar</td>
<td>5</td>
<td>5</td>
<td>1</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Balakot</td>
<td>10</td>
<td>5</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>39</td>
<td>18</td>
<td>4</td>
<td>10</td>
<td>1</td>
</tr>
</tbody>
</table>

*Drug resistance related-genes, \textit{folP} and \textit{rpoB} were amplified by PCR, sequenced, and compared with control \textit{M. leprae} strain, Thai 53.

† Number of samples successfully amplified by PCR.
and rpoB might depend on a difference of the specificity of primers for each gene. Among ten rpoB gene samples amplified from the 39 biopsies, one sample showed the gene mutation at position 550th of the M. leprae β subunit gene of RNA polymerase. This position was not a so-called “hot spot” of rpoB-associated resistant mutations (6); however, it induced a change of amino acid residue from aspartic acid to glycine (not shown). There was no relationship among the resistant samples, and no double mutation encoding both folP and rpoB genes was observed.

It is not easy to determine whether the resistant strain developed before or after introduction of MDT. However, there might be some patients who are inadequately treated with MDT due to economical or other social reasons. These patients have a higher risk to produce multidrug-resistant strain than patients adequately treated. Active surveillance is required for control of the spread of drug resistant M. leprae.

Taken together, we showed that some leprosy patient contacts have been infected with M. leprae. Also, dapsone resistance has been detected in Pakistan.

Acknowledgment. We thank Dr. Akira Kobayashi and Dr. Tetsu Nakamura (Peshawar-Kai Hospital, Peshawar, Pakistan) for supplying clinical samples. This work was supported in part by a Health Science Research Grants-Research on Emerging and Re-emerging Infectious Diseases, Ministry of Health, Labour and Welfare, Japan.

—Masanori Kai, Ph.D.,
Yumi Maeda, Ph.D.,
Shinji Maeda, Ph.D.,
Yasuo Fukutomi, Ph.D.,
Kazuo Kobayashi, M.D., Ph.D.,
Yoshiko Kashiwabara, Ph.D.,
Masahiko Makino, M.D., Ph.D.

Department of Microbiology, and Department of Host Defense
Leprosy Research Center, National Institute of Infectious Diseases

—Mohammad Ali Abbasi, M.D.

REFERENCES