Specific Serological Diagnosis of Leprosy with a Recombinant *Mycobacterium leprae* Protein Purified from a Rapidly Growing Mycobacterial Host

JAMES A. TRICCAS,1* PAUL W. ROCHE,2† AND WARWICK J. BRITTON1,2

Centenary Institute of Cancer Medicine and Cell Biology, Locked Bag No. 6, Newtown, 2042,1 and Department of Medicine, University of Sydney, Sydney, Australia, 2006,2 New South Wales, Australia

Received 20 February 1998/Returned for modification 23 March 1998/Accepted 30 April 1998

In this report we demonstrate the utility of a monoclonal antibody inhibition enzyme-linked immunosorbent assay based on the *Mycobacterium leprae* 35-kDa protein, purified from the rapidly growing host *Mycobacterium smegmatis*, for the serodiagnosis of multibacillary leprosy. The assay proved highly specific (97.5%) and sensitive (90%) and compared favorably with two other established methods routinely utilized for leprosy serodiagnosis.

The development of improved specific diagnostic reagents to detect infection with *Mycobacterium leprae* and to monitor the effectiveness of community control programs is a major priority of leprosy control strategies (1, 5). Ideally, diagnostic reagents should detect all forms of leprosy, ranging from the tuberculoid or paucibacillary (PB) form, characterized by strong cell-mediated immunity (CMI) to *M. leprae* and a high level of antibody formation (2). This implies that a combination of tests will be required for the efficient detection of leprosy, i.e., a specific skin test for the detection of CMI to *M. leprae* and a serological assay for the detection of anti-*M. leprae* antibodies. Previous studies have established that assays detecting the monoclonal antibody (MAb) MLO4 epitope of the *M. leprae* 35-kDa protein using *M. leprae* sonicate (MLS) are specific and sensitive for the serodiagnosis of leprosy. Antibodies to the 35-kDa protein have been detected in up to 100% of untreated lepromatous patients but are generally absent from the sera of tuberculosis patients and control subjects (7, 10, 12, 14), while levels of anti-35-kDa protein antibodies correlate strongly with the antigenic load and decline with effective chemotherapy (3, 6, 9). One limiting factor of the assay is the requirement for MLS, which must be prepared from *M. leprae* purified from infected armadillos as *M. leprae* still cannot be cultivated in vitro. When the gene encoding the *M. leprae* 35-kDa protein was expressed at high levels in *Mycobacterium smegmatis* and recombinant protein was purified, the antigen exhibited properties suggesting its potential as a leprosy-specific diagnostic tool (15, 16). The protein was specifically recognized by the immune responses of the large majority of leprosy patients tested, while strong delayed-type hypersensitivity was elicited by the recombinant 35-kDa protein in *M. leprae*-sensitized guinea pigs but not in *Mycobacterium tuberculosis*- or *Mycobacterium bovis* BCG-sensitized animals. Furthermore, only the form of the antigen derived from recombinant *M. smegmatis*, and not that purified from *Escherichia coli*, retained conformational determinants and was recognized by leprosy sera. Therefore, we have assessed the suitability of the purified recombinant *M. leprae* 35-kDa protein for the routine serological diagnosis of leprosy.

Sera were obtained from 60 MB and 30 PB leprosy patients, previously untreated, who were diagnosed according to the Ridley-Jopling classification (8) at Anandaban Leprosy Hospital, Kathmandu, Nepal. Sera from 50 clinically well health care workers in Nepal served as the endemic control group. The tuberculosis (TB) group consisted of sera from 15 patients from Nepal with active, smear-positive, radiologically confirmed pulmonary TB (endemic TB) and sera from 15 patients from Royal Prince Alfred Hospital, Sydney, Australia, with culture-proven pulmonary TB, who had not been exposed to leprosy (nonendemic TB).

The *M. leprae* 35-kDa protein was purified from the sonicate of recombinant *M. smegmatis* transformed with pWL19 as previously described (15). Antibodies to the 35-kDa protein were detected by three methods. The first was a MAb inhibition enzyme-linked immunosorbent assay (ELISA), as initially described elsewhere (13), using a 10-μg/ml concentration of MLS and the MAb MLO4-peroxidase conjugate supplied by J. Iwai (MRC Unit for Tuberculosis and Related Infections, Hammersmith Hospital, London, United Kingdom). The dilution of sera causing 50% inhibition of binding of MLO4 to MLS compared to the maximum binding in the absence of serum (ID50) was calculated, and samples with ID50 greater than 10 were considered positive. Previous studies (10, 12, 14) had confirmed that this level discriminates between leprosy patients and endemic control subjects. The second assay was a similar MAb inhibition assay except that the coating antigen was the purified 35-kDa protein (r35 kDa-MIA). In initial optimization experiments, the protein was tested over a concentration range of 0.1 to 10 μg/ml. All sera were subsequently tested with a 0.5-μg/ml concentration of antigen. The final assay detected antibodies to the 35-kDa protein by direct ELISA, with the purified 35-kDa protein used at a concentration of 10 μg/ml and patients’ sera diluted 1 in 100. Samples with A405s greater than 0.42, which was the mean of 50 control serum samples plus 2 standard deviations, were considered positive. Immunoglobulin M antiphospholipid glycoprotein antibody (PGL-I) was measured by direct ELISA, with the dissacharide bovine serum albumin conjugate (provided by M. J. Colston, Laboratory for Leprosy and Mycobacterial Research, National Insti-
TABLE 1. Comparison of three serological assays to detect anti-M. leprae 35-kDa protein antibodies and an assay for immunoglobulin M anti-PGL-I antibodies in leprosy and tuberculosis patients and control subjects

<table>
<thead>
<tr>
<th>Patient group</th>
<th>n</th>
<th>MLS-MIA</th>
<th>r35 kDa-MIA</th>
<th>35 kDa direct ELISA</th>
<th>PGL-I direct ELISA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endemic control</td>
<td>50</td>
<td>6 (133)</td>
<td>2 (76)</td>
<td>4 (0.200)</td>
<td>2 (0.211)</td>
</tr>
<tr>
<td>Endemic TB</td>
<td>15</td>
<td>6.7 (20)</td>
<td>6.7 (32)</td>
<td>13.4 (0.243)</td>
<td>0 (0.173)</td>
</tr>
<tr>
<td>Nonendemic TB</td>
<td>15</td>
<td>0 (0)</td>
<td>0 (0.193)</td>
<td>0 (0.130)</td>
<td>0 (0.130)</td>
</tr>
<tr>
<td>MB</td>
<td>60</td>
<td>90** (1,526)</td>
<td>90* (1,421)</td>
<td>83** (0.896)</td>
<td>85** (1.058)</td>
</tr>
<tr>
<td>PB</td>
<td>30</td>
<td>17 (188)</td>
<td>17* (88)</td>
<td>17 (0.270)</td>
<td>28** (0.333)</td>
</tr>
</tbody>
</table>

* Values in parentheses for MLS-MIA and r35 kDa-MIA are mean positive titers. Values in parentheses for the direct 35 kDa and PGL-I assays are mean absorbances (A405). Significant differences in the proportions of positive responders compared to endemic controls were determined by chi-square test for each assay. *, P < 0.05; ***, P < 0.001.

FIG. 1. Optimization of the r35 kDa-MIA for the detection of anti-M. leprae 35-kDa protein antibodies. Results are expressed as percentages of inhibition of binding of the MAb MLO4-peroxidase conjugate to the protein compared to the maximum binding in the absence of serum. The serum titer causing 50% inhibition of maximum binding of MAb MLO4 (ID50) is represented by the horizontal dashed line. LSP, pooled sera from 10 lepromatous leprosy patients.
The protein used in the form of a MAb inhibition assay was highly sensitive and specific for detecting MB leprosy and compared favorably with two previously established tests for leprosy serological diagnosis. This is due to the recombinant protein purified from *M. smegmatis* retaining the structural characteristics of the native antigen (15). Indeed, previous studies comparing structure (15), function (17), and immunogenicity (4, 11) of recombinant proteins purified from mycobacterial host systems have demonstrated considerable advantages over the same protein purified from *E. coli* expression systems. The obvious benefit of the use of this antigen is its relative abundance and ease of purification compared to MLS or purified PGL-I. Whereas extraction of large quantities is a time-consuming process, recombinant *M. smegmatis* is a fast-growing organism producing relatively large amounts of recombinant 35-kDa protein (1 to 2 mg/liter of culture). Furthermore, far less protein is required for the r35 kDa-MIA than for the MLS-MIA.

The *M. leprae* 35-kDa protein is a major and specific target of the cellular immune response to *M. leprae*, inducing T-cell proliferation and gamma interferon secretion by leprosy patients and contacts but not by *M. tuberculosis*-infected individuals (15). The protein also elicits a specific delayed-type hypersensitivity in mycobacterial-sensitized animals (15). This study shows that the same recombinant antigen can be utilized in a sensitive and specific assay of the humoral response to *M. leprae*. Thus, a combination of tests based on this single antigen may be of considerable benefit in both the diagnosis of clinical leprosy and the recognition of subclinical leprosy infection. Moreover, this study further illustrates the advantages of utilizing recombinant mycobacterial proteins derived from mycobacterial hosts, suggesting that refinement of such expression systems may prove beneficial for the specific diagnosis of other mycobacterial infections.

We are grateful for the assistance of the staff and patients of Anandaban Leprosy Hospital, Kathmandu, Nepal, which is fully supported by The Leprosy Mission International. This work was supported by the National Health and Medical Research Council of Australia. J.T. was a recipient of an Australian Postgraduate Award and P.R. was a recipient of a University of Sydney Medical Foundation Research Fellowship.

REFERENCES


