Purification and Characterization of a 36 kDa Antigen of *Mycobacterium leprae*

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A 36 kDa antigen of *Mycobacterium leprae* was purified by phenol biphasic partition followed by preparative SDS-PAGE. The purified antigen appeared as a single band in SDS-PAGE and eluted as a single peak in ion-exchange chromatography. The antigen comprised epitopes which were cross-reactive with *M. tuberculosis*, as well as a species-specific epitope (recognized by MAb F47-9). Different treatments of the 36 kDa antigen suggested it to be largely protein in nature; the amino acid composition of 81% of the antigen was determined. A majority of sera from leprosy patients contained antibodies recognizing the 36 kDa antigen.

INTRODUCTION

Relatively little is known about the complex and diverse immune response, involving both cellular and humoral immune reactions, in leprosy patients. The use of purified antigens from *Mycobacterium leprae* will enable us to distinguish the functional epitopes involved in both protective and suppressive responses. An understanding of these is crucial for vaccine development and purified antigen has already been shown to be useful for serodiagnostic purposes (Cho *et al.*, 1983).

We have previously reported the identification of species-specific antigenic components of *M. leprae*, using sera of leprosy patients (Klatser *et al.*, 1984). Further characterization of one of these antigens, with a molecular mass of 36 kDa, became possible with the availability of the murine monoclonal antibody (MAb) F47-9, which recognizes an *M. leprae*-specific epitope on this antigen (Klatser *et al.*, 1985). We have also developed an ELISA-inhibition test using this MAb for the serodiagnosis of leprosy (Klatser *et al.*, 1985).

In this paper we extend our previous studies, describing a procedure for obtaining the purified 36 kDa antigen and its immunochemical characterization. Exploitation of this purified antigen in immunological tests should provide information as to its role during natural infection with *M. leprae*.

METHODS

**Purification of antigen.** *M. leprae* was purified from livers of experimentally infected nine-banded armadillos (*Dasypus novemcinctus* Linn.) (World Health Organization, 1980). Part of the purified *M. leprae* was obtained through Dr P. J. Brennan under NIH contract NO1 AI-52582. The suspension of *M. leprae* was sonicated (Klatser *et al.*, 1984) and a portion (8.97 mg dry wt; determined by freeze-drying) of the resultant extract was applied to a phenol biphasic partition system, as described by Westphal & Jann (1965). The phenol phase was extensively dialysed against distilled water then applied to preparative SDS-PAGE slab gels (6-46 mg freeze-dried material per 810 mm² per run). Slices (2 mm) were cut from the gel in the area of 36 kDa. The gel slices were mixed with

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*Abbreviations:* MAb, murine monoclonal antibody; PBST, phosphate-buffered saline containing 0.5% Tween 20; FCS, foetal calf serum; TMB, tetramethylbenzidine; DOSS, diocyl sodium sulphosuccinate; NGS, normal goat serum; Con A, Concanavalin A; LAM, lipoarabinomannan.

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15 ml 4 M-urea (AnalaR, BDH; absorbed with active charcoal) and the mixture was ground until the gel had visibly been dispersed. The polyacrylamide was separated out by centrifugation (12000 g, 10 min, 10 °C). The resulting pellet was re-extracted in the same way and the combined supernatants were extensively dialysed against distilled water and concentrated by lyophilization. The purity of the antigen preparation was investigated by SDS-PAGE, immunoblotting and ion-exchange chromatography.

**Slab SDS-PAGE.** This was done essentially by the method of Laemmli (1970). Samples (10–30 μg) were treated at 100 °C for 5 min with solubilizing buffer (62.5 mM-Tris buffer, 2% (w/v) SDS, 10% (v/v) glycerol and 0.002% (w/v) bromophenol blue). β-Mercaptoethanol was omitted because it was shown to cause background and point streaking in silver-stained gels (Gorg et al., 1987). Omitting β-mercaptoethanol had no effect on the relative mobility of the antigen. Electrophoresis was done at a constant current of 40 mA through a 3% (w/v) polyacrylamide stacking gel and an 11% (w/v) polyacrylamide slab separating gel. Gels were stained with Coomassie brilliant blue R-250 or with a silver stain for glycoconjugates (Tsai & Frasch, 1982). The molecular mass of the antigen was estimated by the relative mobility method of Weber et al. (1972); standards were obtained from Pharmacia.

**Antibodies.** The procedures for the production, characterization and peroxidase-labelling of MAb F47-9, which recognizes the 36 kDa antigen of *M. leprae* were described by Klatser et al. (1985). MAb F47-10, which recognizes the 64 kDa antigen of *M. leprae* and MAb ML04AI, which recognizes a 35 kDa antigen of *M. leprae*, were described by Engers et al. (1985). MAb ML04AI was kindly provided by Dr J. Ivanyi, MRC Tuberculosis Unit, Hammersmith Hospital, London, UK. A MAb against the lipoarabinomannan of *M. leprae* (Hunter et al., 1986) was a kind gift of Dr P. J. Brennan, Colorado State University, Denver, USA). The production of a polyclonal mouse serum against *M. leprae* sonicate was described by Klatser et al. (1985).

Individual leprosy sera were generously provided by Dr R. V. Cellona, Leonard Wood Memorial Center for Leprosy Research, Cebu, The Philippines. All of the patients were new, untreated cases, classified clinically and histopathologically according to the Ridley–Jopling scale (Ridley & Jopling, 1966).

**Western blot analysis.** Preparations were subjected to SDS-PAGE and the separated bands were electrophoretically transferred (25 V at room temperature for 18 h) to nitrocellulose (0.45 μm pore size, Schleicher and Schull) using 25 mM-Tris buffer containing 192 mM-glycine and 20% (v/v) methanol, pH 8.3, in a Bio-Rad ‘Transblot’ apparatus. After electrophoretic transfer, the nitrocellulose sheets were washed for 30 min with PBS (20 mM-Na2HPO4, 150 mM-NaCl, adjusted to pH 7.4 with HCl) containing 0.5% (v/v) Tween 20 (PBST). MAbs (ascites) or mouse serum diluted (1:1000 and 1:250, respectively) in 10% (v/v) foetal calf serum (FCS) in PBST were added to the membranes, which were then incubated with gentle agitation for 1 h at room temperature. The membranes were washed five times (10 min each wash) with PBST and incubated with secondary antibody (1:100 dilution of goat anti-mouse IgG-horseradish peroxidase conjugate; Pasteur) in PBST containing 10% (v/v) FCS for 1 h at room temperature with gentle agitation. The membranes were washed five times with PBST and immersed in the substrate solution [10 ml 0.1 M-acetate/citrate buffer (pH 6.0) containing 100 μl tetramethylbenzamidine (TMB, Sigma) in dimethylsulphoxide (10 mg ml⁻¹), 100 μl diocetyl sodium sulphosuccinate (DOSS, Sigma) in dimethylsulphoxide (33 mg ml⁻¹) and 100 μl 0.5% (v/v) H2O2]. After 5 min the blot was allowed to dry.

ELISA. *M. leprae* extract (20 μg ml⁻¹), phenol-phase (14.5 μg ml⁻¹), water-phase (0.57 μg ml⁻¹) or purified antigen (1.3 μg ml⁻¹) or purified antigen (1.3 μg ml⁻¹) was coated onto γ-irradiated Immulon 96-well microtitre plates (Greiner) by adding to each well 50 μl of antigen in carbonate buffer (0.015 M-Na2CO3, 0.035 M-NaHCO3, pH 9.6) and incubating for 18 h at 37 °C. Normal goat serum (NGS) (100 μl; 10%, v/v, in carbonate buffer) was added to each well and after 1 h incubation at 37 °C, the plates were washed four times with PBS containing 0.05% (v/v) Tween 20. Antibody samples (100 μl), either MAbs in 2-fold serial dilutions or human sera (1:1000) in PBS containing 10% (v/v) NGS and 0.1% (v/v) Tween 20, were incubated for 1 h at 37 °C. After washing, the plates were incubated for 1 h at 37 °C with peroxidase-labelled secondary antibody (1:1000 dilution of goat anti-mouse IgG or goat anti-human IgG + M; Pasteur) in PBS containing 10% (v/v) NGS and 0.1% (v/v) Tween 20, were incubated for 1 h at 37 °C. After washing, the plates were washed and incubated with 100 μl substrate (TMB solution), as described for Western blotting, except that DOSS was omitted. After 15 min at room temperature, the colour development was stopped with 50 μl 1 M-H2SO4 and the A450 measured in a Titertek Multiscan spectrophotometer (Flow Laboratories). The results were corrected for colour development in the absence of primary antibody or coating antigen.

To further investigate the purity of the final antigen preparation, ELISA was done as described, except that the purified antigen was coated in 2-fold serial dilutions starting from 70 μg ml⁻¹ and tested against a constant dilution of the MAbs (1:500).

Binding of peroxidase-conjugated Concanavalin A (Con A; Sigma) was tested in the ELISA as described, except that the incubation with MAb was omitted and the anti-globulin conjugate was replaced by the Con A conjugate.

**ELISA-inhibition.** Antibody titres inhibitory for the binding of peroxidase-labelled MAb F47-9 were determined as previously described (Klatser et al., 1985). Briefly, polystyrene microtitre plates coated with *M. leprae* extract were incubated simultaneously with peroxidase-labelled F47-9 and 2-fold serial dilutions of monoclonal or
Characterization of *M. leprae* 36 kDa antigen

polyclonal antibodies for 3 h at 37 °C. Colour development \(A_{450}\) using TMB as substrate was measured in the washed wells after addition of 50 μl 1 M H₂SO₄ and the 50% inhibitory dilutions were derived from graph plots. Human sera were tested at one dilution (1:5) and results are given as percentage inhibition at that dilution.

The 36 kDa antigen content was estimated in the ELISA-inhibition test as above. Antigen-containing samples were titrated in the assay and the amount of sample, determined by dry weight, required to produce 50% of the maximum binding level of peroxidase-labelled F47-9 to *M. leprae* in the absence of added antigen was determined. The results shown are based on a standard curve generated with purified 36 kDa antigen in the ELISA inhibition test. The amount of purified antigen required to give 50% inhibition was calculated to be 1.4 μg.

Ion-exchange chromatography. This was done on an FPLC apparatus (Pharmacia) using a Mono-Q-HR5/5 anion-exchange column. Purified antigen (10 μg) was applied to the column in 20 mM-Tris/HCl buffer, pH 7.6, and eluted with 30 μl of a linear gradient of 0-0.5 M NaCl in the same buffer. The absorbance of the eluate was monitored at 280 nm.

Biochemical characterization. Pronase and trypsin digestion, heat treatment and treatment with β-mercaptoethanol and periodate of the purified antigen were done as described by Vennegoor *et al.* (1985). Treatment with trifluoromethanesulphonic acid was done according to Edge *et al.* (1981). The binding of MAb F47-9 to the treated antigen preparations was then tested in a dot-blot assay, in which the preparations (1-3 μg ml⁻¹) were coated in serial dilutions onto nitrocellulose filters (0.45 μM pore size, Schleicher and Schuell). The filters were then incubated with MAb F47-9 (1:1000) as described above for Western blots. Results are expressed as the highest dilution of antigen giving visible staining of the dot-blot.

Amino acid analysis. A sample (50 μg) of the purified 36 kDa antigen was hydrolysed in 3 M HCl at 105 °C for 18 h; the amino acid composition was determined in a Beckman System 7300 high performance Amino Acid Analyzer.

RESULTS AND DISCUSSION

Purification of the 36 kDa antigen

The proportion of the 36 kDa antigen in the preparation from *M. leprae* increased 26-fold during the purification procedure; recovery was calculated to be 94% of the original antigen based on concentrations generated from the use of a standard curve obtained with the purified antigen in the ELISA-inhibition assay.

Several groups (Chakrabarty *et al.*, 1982; Britton *et al.*, 1985) have reported sugar-containing *M. leprae* antigens in the 30-40 kDa region, which were either contaminated with or equivalent to lipoarabinomannan (LAM) (Hunter *et al.*, 1986). In order to avoid any contamination with LAM, a phenol-biphasic partition was included in the purification procedure. The specific silver staining pattern of LAM was detectable in the starting material and the water phase, but not in the phenol phase or in the final preparation (result not shown).

The 36 kDa antigen of *M. leprae* appeared as a single band in Coomassie-blue-stained gels (Fig. 1). The purity of the antigen was further analysed by screening Western blots with a polyvalent antiserum having high activity against *M. leprae* antigens. This serum reacted with several antigens in the crude *M. leprae* sonicate (Fig. 2, lane 2), while only a single band of reactivity was seen with the purified antigen (Fig. 2, lane 4). This was the same band as that detectable with the MAb F47-9, known to recognize the 36 kDa antigen (Fig. 2, lanes 1 and 3). The purity of the antigen was further established by ion-exchange chromatography, in which the antigen eluted as a single peak (Fig. 3).

Analysis of the 36 kDa antigen with MAbs

Analysis of the purified antigen with MAbs recognizing certain major antigens of *M. leprae*, including a 64 kDa protein (F47-10), a 35 kDa protein (ML04AI) and lipoarabinomannan (anti-LAM), failed to detect contamination in the ELISA, although all MAbs could bind to the crude extract of *M. leprae* with comparable reactivities (Table 1). In addition, when the purified 36 kDa antigen was coated onto ELISA plates in serial dilutions starting with 70 μg ml⁻¹, these MAbs (1:500) failed to show any reactivity, even at the highest coating concentration (results not shown). Although MAb ML04AI does not react with the purified 36 kDa antigen, its reactivity appears to be lost as a result of phenol extraction, so it is still possible that the 35 and 36 kDa antigens are the same. A polyvalent mouse anti-*M. leprae* antiserum reacted with the purified antigen, but was unable to inhibit binding of monoclonal antibody F47-9 to the extract.
of *M. leprae* (Table 1), suggesting that the mouse serum did not contain antibodies to the *M. leprae*-specific epitope identified by that MAb. This might be explained by the fact that this mouse antiserum was raised against a crude, unfractionated extract of *M. leprae* and not against a soluble fraction of the extract, which has been shown to give rise to more specific antibodies (Ivanyi et al., 1983; Kolk et al., 1984). A polyvalent mouse anti-*M. tuberculosis* antiserum showed some reactivity to the purified 36 kDa antigen of *M. leprae*, which implies that cross-reactive determinants are also present on the antigen. This serum did not inhibit the binding of the *M. leprae*-specific MAb F47-9 to the antigen (Table 1). Thus, the 36 kDa antigen of *M. leprae* appears to contain several epitopes, both species-specific (that recognized by MAb F47-9) and cross-reactive (those recognized by the polyvalent anti-*M. leprae* and anti-*M. tuberculosis* sera). T-Helper cells from leprosy patients have also recently been shown to recognize several antigenic determinants on the 36 kDa antigen (Ottenhoff et al., 1986a).

**Nature of the 36 kDa antigen**

The nature of the 36 kDa antigen reacting with MAb F47-9 was studied in a dot-blot assay, using serial dilutions of the treated, purified antigen (Table 2). The antigenic determinant recognized by the MAb was preserved after treatment at elevated temperature as well as after reduction with β-mercaptoethanol. The latter observation indicates that MAb F47-9 does not recognize a disulphide-dependent configuration in the antigen. The epitope was sensitive to peptide digestion by pronase and to a lesser extent by trypsin. Oxidation of carbohydrates with periodate and deglycosylation with trifluoromethanesulphonic acid did not affect the antibody-binding, thus excluding the association of the antigen binding site with carbohydrates. Furthermore, Con A did not show any binding to the purified antigen (Table 1), indicating that
Characterization of *M. leprae* 36 kDa antigen

Fig. 2. Immunoblot analysis of the 36 kDa antigen of *M. leprae*. Extract of *M. leprae* (lanes 1 and 2) and purified 36 kDa antigen (lanes 3 and 4) were separated by SDS-PAGE and electrophoretically transferred to nitrocellulose paper. The paper strips were then incubated with MAb F47-9 (lanes 1 and 3) or a polyclonal mouse serum against extract of *M. leprae* (lanes 2 and 4); blots were probed with peroxidase-conjugated goat anti-mouse immunoglobulins G.

Fig. 3. Ion-exchange chromatogram of the purified 36 kDa antigen of *M. leprae* on a Mono-Q–HR5/5 column equilibrated with 20 mM-Tris/HCl, pH 7.6. The column was eluted with a linear gradient of NaCl; the absorbance of the eluate was monitored at 280 nm.

Table 1. *Analysis of the 36 kDa antigen of M. leprae with monoclonal and polyclonal antibodies*

<table>
<thead>
<tr>
<th>Antiserum</th>
<th>Extract of <em>M. leprae</em></th>
<th>Phenol phase</th>
<th>Water phase</th>
<th>36 kDa antigen</th>
<th>MAb F47-9 50% inhibitory dilution†</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAb F47-9</td>
<td>&gt; 500</td>
<td>&gt; 500</td>
<td>&lt; 10</td>
<td>&gt; 500</td>
<td>1000</td>
</tr>
<tr>
<td>MAb F47-10</td>
<td>&gt; 500</td>
<td>100</td>
<td>&lt; 10</td>
<td>&lt; 10</td>
<td>NA</td>
</tr>
<tr>
<td>MAb ML04AI</td>
<td>&gt; 500</td>
<td>&lt; 10</td>
<td>&lt; 10</td>
<td>&lt; 10</td>
<td>NA</td>
</tr>
<tr>
<td>MAb anti-LAM</td>
<td>&gt; 500</td>
<td>&lt; 10</td>
<td>500</td>
<td>&lt; 10</td>
<td>NA</td>
</tr>
<tr>
<td>Mouse anti-<em>M. leprae</em></td>
<td>&gt; 500</td>
<td>400</td>
<td>100</td>
<td>200</td>
<td>&lt; 10</td>
</tr>
<tr>
<td>Mouse anti-<em>M. tuberculosis</em></td>
<td>500</td>
<td>200</td>
<td>100</td>
<td>100</td>
<td>&lt; 10</td>
</tr>
<tr>
<td>Mouse nonimmune</td>
<td>&lt; 10</td>
<td>&lt; 10</td>
<td>&lt; 10</td>
<td>&lt; 10</td>
<td>NA</td>
</tr>
<tr>
<td>Con A</td>
<td>&gt; 500</td>
<td>&lt; 10</td>
<td>&gt; 500</td>
<td>&lt; 10</td>
<td>NA</td>
</tr>
</tbody>
</table>

NA, Not applicable.

* Antisera were tested in ELISA at dilutions ranging from 1:10 to 1:500.
† Antisera were tested in the ELISA-inhibition test at dilutions ranging from 1:10 to 1:1000.
Table 2. Antigen activity after different treatments of the purified 36 kDa antigen of M. leprae

The binding of MAb F47-9 to the treated antigen preparations was tested in a dot-blot assay. The preparations (1-3 µg ml⁻¹) were coated in serial dilutions onto nitrocellulose filters and incubated with MAb F47-9 (1:1000).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Antigen activity relative titre (%)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 °C, 5 min</td>
<td>100</td>
</tr>
<tr>
<td>β-Mercaptoethanol</td>
<td>100</td>
</tr>
<tr>
<td>Trypsin</td>
<td>25</td>
</tr>
<tr>
<td>Pronase</td>
<td>&lt;5</td>
</tr>
<tr>
<td>Periodate</td>
<td>100</td>
</tr>
<tr>
<td>Trifluoromethanesulphonic acid</td>
<td>100</td>
</tr>
</tbody>
</table>

* Compared with the untreated 36 kDa antigen.

Table 3. Amino acid analysis of the purified 36 kDa antigen*

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Concn found (mol%)</th>
<th>Amino acid</th>
<th>Concn found (mol%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Threonine</td>
<td>3.57</td>
<td>Methionine</td>
<td>1.59</td>
</tr>
<tr>
<td>Serine</td>
<td>18.85</td>
<td>Isoleucine</td>
<td>1.39</td>
</tr>
<tr>
<td>Aspartate/asparagine</td>
<td>6.55</td>
<td>Leucine</td>
<td>1.79</td>
</tr>
<tr>
<td>Glutamate/gluatamine</td>
<td>6.75</td>
<td>Tyrosine</td>
<td>0.99</td>
</tr>
<tr>
<td>Proline</td>
<td>8.13</td>
<td>Lysine</td>
<td>7.34</td>
</tr>
<tr>
<td>Glycine</td>
<td>20.24</td>
<td>Histidine</td>
<td>6.75</td>
</tr>
<tr>
<td>Alanine</td>
<td>8.53</td>
<td>Arginine</td>
<td>1.19</td>
</tr>
<tr>
<td>Valine</td>
<td>6.35</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Acid hydrolysis destroys tryptophan which may or may not have been present; asparagine and glutamine are converted to the corresponding dicarboxylic acids; phenylalanine and cysteine were not detected.

glycosyl and/or mannosyl residues were not present. These results, including the staining of the purified antigen by Coomassie blue, all suggest that the 36 kDa antigen is mostly protein in nature. This is in accordance with the finding that the 36 kDa antigen could be expressed by a recombinant phage clone (Young et al., 1985; Klatser et al., 1987), although until now only as the 2 kDa C-terminal part of the antigen.

Table 3 shows the results of amino acid analysis of the purified 36 kDa antigen: 81.1% of the antigen was composed of amino acids. The presence of tryptophan remains uncertain as we analysed only acid-hydrolysed material. Consistent with the absence of effect of reducing agents on denaturation, we found no cysteine residues. The remaining 18.9% of ninhydrin-positive material could not be related to the elution pattern of normally occurring amino acids. Numerous other amino compounds have been described as giving ninhydrin-positive reactions (reviewed in Applications Data DS-656, Beckman Instruments). Further investigation is needed to elucidate the complete composition and structure of the 36 kDa antigen.

Serological activity of the 36 kDa antigen

A total of 57 sera from leprosy patients and 39 sera from control subjects were assayed for binding to the 36 kDa antigen (Table 4). Of the leprosy patients 57% showed positivity for binding to the 36 kDa antigen, while 70% scored positive for binding to the M. leprae-specific epitope on the 36 kDa antigen. Of the normal control subjects, 5% showed binding to the 36 kDa antigen and 2.5% to the M. leprae-specific epitope. However, since these subjects were from an endemic area, we cannot exclude the possibility that they had been infected with M. leprae. Higher seropositivity rates in control subjects from endemic areas compared to those from non-endemic areas have been reported before in studies using the 36 kDa antigen (Klatser et al., 1985) or the phenolic glycolipid antigen of M. leprae (Douglas et al., 1987).
Characterization of \textit{M. leprae} 36 kDa antigen

Table 4. Detection of human antibodies to the purified 36 kDa antigen of \textit{M. leprae} in ELISA and ELISA-inhibited

Leprosy patients were classified according to the Ridley–Jopling scale: LL, lepromatous leprosy; BL, borderline lepromatous leprosy; BT, borderline tuberculoid leprosy; TT, tuberculoid leprosy; N, normal controls. For ELISA microtitre plates coated with purified 36 kDa antigen (1.3 \( \mu \text{g ml}^{-1} \)) were reacted with human sera (1:1000) and peroxidase-antihuman immunoglobulin G + M conjugate and substrate. Absorbance was measured at 450 nm. For ELISA-inhibition microtitre plates coated with extract of \textit{M. leprae} were reacted simultaneously with peroxidase-conjugated MAb F47-9 (recognizing a \textit{M. leprae}-specific epitope on the 36 kDa antigen) and human sera (1:5). Absorbance was measured at 450 nm. Inhibition was calculated as the percentage of the maximum obtained with peroxidase-conjugated MAb in the absence of human serum.

| Diagnosis | No. of sera in each diagnosis category | ELISA \( A_{450} \) Mean (SD) | % Positivity* | ELISA-inhibition Mean (SD) | % Positivity
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td>LL</td>
<td>20</td>
<td>1.07 (0.53)†</td>
<td>95</td>
<td>49.5 (21.9)†</td>
<td>100</td>
</tr>
<tr>
<td>BL</td>
<td>11</td>
<td>0.55 (0.54)†</td>
<td>64</td>
<td>14.2 (12.7)†</td>
<td>73</td>
</tr>
<tr>
<td>BT</td>
<td>15</td>
<td>0.21 (0.08)†</td>
<td>33</td>
<td>6.7 (8.9)†</td>
<td>45</td>
</tr>
<tr>
<td>TT</td>
<td>11</td>
<td>0.11 (0.07)</td>
<td>9</td>
<td>0.1 (0.7)</td>
<td>2.5</td>
</tr>
<tr>
<td>N</td>
<td>39</td>
<td>0.11 (0.06)</td>
<td>5</td>
<td>0.1 (0.7)</td>
<td>2.5</td>
</tr>
</tbody>
</table>

* Criterion for positivity: > mean + 2 SD of controls.
† Significantly different than normal values (Student's t-test, \( P < 0.05 \)).

The results of this pilot study showed a positive correlation (Pearson correlation test, \( P < 0.01 \)) between MAb F47-9 competition and 36 kDa antigen-binding. Although leprosy sera could be detected with a higher sensitivity by the ELISA-inhibition test, further study is necessary to find out whether the \textit{M. leprae}-specific epitope is the immunodominant epitope on the 36 kDa antigen.

**Conclusions**

Isolated antigens of \textit{M. leprae} are necessary for elucidation of their roles in the immune reactivity, or lack thereof, in leprosy patients; they are also exquisite tools for the development of diagnostic tests. In this paper we have described the isolation of the 36 kDa antigen of \textit{M. leprae} and have determined that at least the major part of it is protein, containing both a species-specific and cross-reactive antigenic determinants. The antigen has already been shown to be involved in both the humoral- and cellular-immune responses of leprosy patients (Klatser \textit{et al.}, 1985; Ottenhoff \textit{et al.}, 1986\textit{a, b}). Continuation of the study at the molecular level, both on the natural (isolated) antigen and on recombinant products, followed by functional immunological testing, should further our understanding of the 36 kDa antigen during infection with \textit{M. leprae}. Such studies are now under way.

We are obliged to Dr P. J. Brennan for his gift of \textit{M. leprae}, lipoarabinomannan and the MAb against lipoarabinomannan. We thank Dr J. Ivanyi for his gift of MAb ML04, Dr R. V. Cellona for his gift of human sera, Dr A. H. J. Kolk for MAb F47-10 and Dr R. B. H. Schutgens for performing the amino acid analysis. We are grateful to Dr E. P. Wright for critical reading of the manuscript. This work was supported by the Netherlands Leprosy Relief Association (NSL), the Immunology of Leprosy (IMMLEP) component of the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases, the Commission of the European Communities Directorate General for Science and Development TSD.043 and the Q. M. Gastmann-Wichers Foundation.

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