Use of an antigen-capture assay for characterisation of monoclonal antibodies to mycobacterial lipoarabinomannan

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Summary. Monoclonal antibodies directed to six separate antigen molecules of Mycobacterium leprae have been tested in an antigen-capture assay based on combined use of polyclonal ("capture") and monoclonal ("detector") antibody reagents. This approach provides a potentially versatile, sensitive and specific assay for detection and relative quantitation of M. leprae antigens. Characterisation of monoclonal antibodies to mycobacterial lipoarabinomannan (LAM-B) by the antigen-capture assay indicates that some of the antigenic determinants present on LAM-B from M. leprae may be either absent altogether or present at much lower concentrations on the corresponding LAM-B structure from M. tuberculosis.

Introduction

Assays for the detection of mycobacterial antigens in tissues and body fluids from infected individuals have a potential role in the diagnosis of disease and in monitoring the effectiveness of chemotherapy. In the case of leprosy, it is important that such assays distinguish between specific antigens of Mycobacterium leprae and the many cross-reactive antigens shared by related mycobacteria, such as M. tuberculosis, which may often be present at high frequencies in leprosy endemic areas. The availability of mouse monoclonal antibodies (MAbs) directed to individual antigenic determinants of M. leprae (Gillis and Buchanan, 1982; Ivanyi et al., 1983; Engers et al., 1985) provides an opportunity for the development of novel antigen detection assays which would have the attractions of being potentially species-specific and of being suitable for monitoring the presence of antigens of defined molecular nature.

Two reports of the use of MAbs for detection of mycobacterial antigens in clinical samples have been published. Chandramuki et al. (1985) used a MAb (ML34) directed to a common carbohydrate determinant of mycobacteria to detect antigen in cerebrospinal fluid from patients with tuberculous meningitis and Young et al. (1985b) used a MAb (PG2.B8F) in conjunction with immunoblotting to detect the species-specific phenolic glycolipid antigen in sera from patients with lepromatous leprosy. Antigen-capture (or "tandem") assays with MAbs have been used to detect antigens from other infectious agents (Voller, 1980) and development of such assays has been described with the mycobacterial ML34 MAb (Praputpittaya and Ivanyi, 1985), and also for the 28-Kda protein of M. leprae (Young et al., 1985a) and the 38-Kda protein of M. tuberculosis (Young et al., 1986). These assays involve binding of antigen to a solid-phase support through interaction with a "capture" antibody, followed by detection with a second antibody directed to a separate epitope on the same molecule, and can be employed either for an antigen with repeating epitopes (e.g., the ML34 assay) or for an antigen with two spatially separate epitopes, as in the case of the 28-Kda protein. The format of such assays is attractive for detection of antigens in biological samples because the "capture" step allows for an initial partial purification or concentration of the specific antigen. In this paper we report the development of an antigen-capture assay with polyclonal antibody directed to cross-reactive mycobacterial determinants for the "capture" step followed by detection with MAbs directed to M. leprae determinants. It can be predicted that this
strategy should be successful for antigens which contain a mixture of cross reactive and species-specific determinants, as has been shown for the 65-Kda and 28-Kda proteins of M. leprae (Gillis et al., 1985; Young et al., 1985a).

The antigen-capture assay has been used for analysis of MAb-defined determinants of the mycobacterial lipoarabinomannan-B antigen. Hunter et al. (1986) have recently shown that the previously described arabinomannan antigen of mycobacteria is a partially hydrolysed form of a more complex lipoarabinomannan structure (LAM-B) which contains inositol 1-phosphate, lactate, succinate and fatty acids in addition to arabinose and mannan. The results reported here suggest the presence of species-specific determinants associated with the native LAM-B molecule.

Materials and methods

Mycobacterial antigens

Soluble extract from M. leprae was obtained from the World Health Organization Leprosy programme through Dr R. J. W. Rees, Clinical Research Centre, London. M. tuberculosis H37Rv was grown for 8 weeks as a surface pellicle on Sauton’s medium and a soluble extract was prepared as described previously (Young et al., 1986). The protein concentration in mycobacterial extracts was determined by the method of Lowry et al. (1951), with bovine serum albumin (BSA) as standard. LAM-B was purified from M. leprae and M. tuberculosis as described previously (Hunter et al., 1986).

Monoclonal antibodies

The following MAbs were used in the study: IIIE9 (M. leprae 65-Kda protein; Gillis and Buchanan, 1982), L12 (M. leprae 65-Kda protein; Britton et al., 1985), SA1.B11H and SA1.D2D (M. leprae 28-Kda protein; Young et al., 1985a), L5 (M. leprae 18-Kda protein; Britton et al., 1985), and MLO4 and MLO6 (M. leprae 35-Kda and 12-Kda proteins; Ivanji et al., 1983). MAbs SA1.C7F and SA1.C11H were derived from a fusion with spleens from mice immunised with a soluble extract from M. leprae as described previously (Young et al., 1985a). Both antibodies belonged to the IgG1 subclass and recognised subtilisin-resistant determinants. All MAbs were obtained in the form of ascitic fluid and were used in assays at dilutions ranging from 1 in 1000 to 1 in 20 000.

Antigen-capture assay

Antibody from a rabbit hyperimmunised with M. bovis BCG (Dako Ltd, High Wycombe, Bucks) was diluted 1 in 5000 in carbonate-bicarbonate buffer, pH 9-6, and coated overnight at 0-4°C to Immulon II microtitration plates (Dynatech Laboratories, Inc., Alexandra, VA). Plates were washed with phosphate-buffered saline, pH 7-2, containing Tween 20 0-05% v/v (PBS-T) and then incubated for 1 h at room temperature (RT) with BSA 1% w/v in PBS-T (BSA-PBS-T) to block non-specific binding. The BSA was discarded and antigens (mycobacterial extracts or LAM-B) were diluted in 1% BSA-PBS-T and added to the microtitration plate. After incubation for 1 h at RT, plates were washed three times with PBS-T and MAbs diluted in 1% BSA-PBS-T, were added for 1 h at RT. Plates were washed again and peroxidase-conjugated affinity-purified goat anti-mouse IgG antibody (Bio Rad, Richmond, CA) diluted 1 in 3000 in BSA-PBS-T was added. After 1 h at RT, plates were washed with PBS-T and colour was developed with tetramethyl-benzidine (dihydrochloride) 0-1 mg/ml in citrate buffer, pH 5, containing hydrogen peroxide 0-1% v/v. Reactions were stopped by adding sulphuric acid and absorbance was read at 450 nm. Separate control wells without polyclonal antibody, antigen, or MAbs, respectively, were included in each experiment.

Direct binding assay with human sera

A direct binding assay was performed by diluting LAM-B at different concentrations in carbonate-bicarbonate buffer, pH 9-6 and coating overnight at 0-4°C to Immulon II microtitration plates. After washing with PBS-T and blocking as described for the antigen-capture assay, human serum samples (diluted 1 in 300 in BSA-PBS-T) were added to the plates and incubated for 1 h at RT. Plates were washed with PBS-T and peroxidase-conjugated affinity-purified goat anti-human IgG antibody (Sigma Chemical Co., St Louis, MO) was added for 1 h at RT. Plates were washed again and colour was developed as described above. Results were corrected for any background binding of human serum antibody to uncoated wells of the microtitration plate. Human serum samples consisted of pooled sera from at least six separate donors with lepromatous leprosy, tuberculosis, or without mycobacterial disease.

Results

Antigen-capture ELISA

An antigen-capture assay was developed with hyperimmune rabbit anti-M. bovis BCG as “capture” antibody followed by detection with a variety of MAbs directed to M. leprae. The peroxidase-conjugated affinity-purified anti-mouse antibody used in the assay showed negligible binding to rabbit immunoglobulin and provided a convenient and versatile detection system which avoided the necessity of labelling individual mouse MAbs. Table I shows the results of the antigen-capture ELISA with MAbs IIIE9, L12, MLO4, SA1.B11H,
SA1.D2D, L5, MLO6, SA1.C11H, and SA1.C7F directed towards 65-Kda, 35-Kda, 28-Kda, 18-Kda, 12-Kda protein and LAM-B individual antigen molecules of *M. leprae*. The highest absorbance readings were obtained with antibodies directed to the 65-kda protein and to the LAM-B carbohydrate antigen. The assay was routinely performed in the presence of an excess of BSA (10 mg/ml) and was thus independent of the purity of the mycobacterial antigen extract used. Preliminary results (data not shown) indicated that normal human serum could be added to the assay with little adverse effect except for a general increase in the background binding of the peroxidase-conjugated antibody reagent.

### Table I. Screening of MAbs in antigen-capture assay

<table>
<thead>
<tr>
<th>MAb</th>
<th>Antigen recognised</th>
<th>Absorbance at 450 nm with <em>M. leprae</em> soluble extract (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IIIE9</td>
<td>65-Kda protein</td>
<td>1.17 0.94 0.61 0.26 0.07</td>
</tr>
<tr>
<td>L12</td>
<td>65-Kda protein</td>
<td>1.15 0.91 0.63 0.23 0.07</td>
</tr>
<tr>
<td>ML04</td>
<td>35-Kda protein</td>
<td>0.24 0.20 0.12 0.09 0.08</td>
</tr>
<tr>
<td>SA1.B11H</td>
<td>28-Kda protein</td>
<td>0.22 0.19 0.06 0.08 0.05</td>
</tr>
<tr>
<td>SA1.D2D</td>
<td>28-Kda protein</td>
<td>0.30 0.28 0.14 0.10 0.06</td>
</tr>
<tr>
<td>L5</td>
<td>18-Kda protein</td>
<td>0.18 0.15 0.13 0.0 0</td>
</tr>
<tr>
<td>MLO6</td>
<td>12-Kda protein</td>
<td>0.30 0.23 0.20 0.12 0.12</td>
</tr>
<tr>
<td>SA1.C11H</td>
<td>LAM-B</td>
<td>1.03 0.74 0.32 0.10 0.03</td>
</tr>
<tr>
<td>SA1.C7F</td>
<td>LAM-B</td>
<td>0.65 0.32 0.16 0.02 0.02</td>
</tr>
</tbody>
</table>

Different MAbs were used in the antigen-capture assay with *M. leprae* soluble extract as described in the text.

**Monoclonal antibodies to LAM-B**

Two of the MAbs listed in table I (SA1.C11H and SA1.C7F) were originally characterised as binding to alkali-sensitive, subtilisin-resistant determinants and were subsequently found to recognise the LAM-B antigen purified from *M. leprae*. The high absorbance readings observed with these antibodies in the antigen-capture assay are consistent with the presence of repeating epitopes on the carbohydrate antigen, and addition of purified *M. leprae* LAM-B in the antigen-capture assay showed that sub-nanogram amounts of LAM-B could readily be detected by these MAbs (fig. 1A). SA1.C11H gave consistently higher absorbance readings than SA1.C7F indicating either that the former antibody possessed a higher affinity for the antigen, or that its epitope was present at a higher frequency than that of SA1.C7F on the *M. leprae* LAM-B molecule. When LAM-B from *M. tuberculosis* was used in the assay (fig. 1B), a markedly higher concentration was required to produce absorbance values comparable to that of the *M. leprae* antigen with SA1.C11H (see table II) while no significant recognition of *M. tuberculosis* LAM-B by SA1.C7F was found even at high antigen concentrations. Analogous results were obtained when comparing extracts from *M. leprae* or *M. tuberculosis* in the antigen-capture assay with SA1.C11H showing preferential binding to the *M. tuberculosis* LAM-B.

![Fig. 1. Antigen-capture assay with purified LAM-B. LAM-B from *M. leprae* or *M. tuberculosis* was added at various concentrations to the antigen-capture assay described in the text and antigen detection was with MAb SA1.C11H (○) or MAb SA1.C7F (●).](image-url)
Table II. Quantitative comparison of LAM-B preparations from *M. leprae* and *M. tuberculosis*

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Antigen concentration (ng/ml) required for A₅₅₀ 0.2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>M. leprae</em></td>
</tr>
<tr>
<td>MAb SAl.C11H</td>
<td>1.2</td>
</tr>
<tr>
<td>MAb SAl.C7F</td>
<td>20</td>
</tr>
<tr>
<td>Pooled leprosy sera</td>
<td>0.3</td>
</tr>
<tr>
<td>Pooled tuberculosis sera</td>
<td>1.5</td>
</tr>
</tbody>
</table>

LAM-B purified from *M. leprae* or from *M. tuberculosis* was added at different concentrations to the antigen-capture and the direct binding assay as described in the text. The antigen concentration required to produce an absorbance reading of 0.2 was determined for each of the antibody preparations.

*leprae* extract and SA1.C7F showing no significant binding to *M. tuberculosis* (figs. 2A and 2B).

**Recognition of LAM-B by human sera**

Because of the apparent species-specificity of antigenic determinants on LAM-B, the purified preparations from *M. leprae* and *M. tuberculosis* were each analysed for recognition by antibodies in sera from patients with leprosy or tuberculosis. As shown in fig. 3, pooled sera from leprosy patients or tuberculosis patients bound strongly to LAM-B prepared from either mycobacterial species while a serum pool from uninfected individuals showed only a low degree of binding. Some quantitative differences between the two samples were observed with a lower concentration of the *M. leprae* LAM-B producing absorbance values of 0.2 compared to the *M. tuberculosis* LAM-B, particularly in the case of the lepromatous serum pool (table II).

**Discussion**

Monoclonal antibodies directed to several antigenic determinants of *M. leprae* were found to function well in an antigen-capture ELISA employing a polyclonal anti-mycobacterial antibody for the "capture" step. This indicates that these antibodies recognise either repeating epitopes (as in the LAM-B molecule) or *M. leprae*-specific epitopes on antigens which also possess separate cross-reactive epitopes. Thus the format of combined use of polyclonal and monoclonal antibodies in such assays appears potentially broadly applicable for monitoring several individual *M. leprae* antigens. While the use of a peroxidase-conjugated secondary antibody for screening a wide variety of MAbs was a convenient aspect of the study described here, further development of such tests with material from human clinical samples has indicated that use of directly conjugated MAbs may be necessary to avoid background binding of the anti-mouse secondary antibody to components in the biological extracts. The antigen capture ELISA provides a highly sensitive assay for antigen

![Fig. 2. Antigen-capture assay with mycobacterial extracts. Soluble extract from *M. leprae* or *M. tuberculosis* was added at various concentrations to the antigen-capture assay described in the text and antigen detection was with MAb SAl.C11H (○) or MAb SAl.C7F (●).](image-url)
Fig. 3. Recognition of LAM-B by antibodies in human sera. Various concentrations of LAM-B from *M. leprae* or *M. tuberculosis* were coated to microtitration plates for the direct binding assay described in the text. Assays were then developed with pooled human serum samples (1 in 300 dilution) from lepromatous leprosy patients (■■), tuberculosis patients (○○○) or uninfected individuals (▲).

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REFERENCES


