Protective Monoclonal Antibodies to *Chlamydia trachomatis*
Serovar- and Serogroup-Specific Major Outer Membrane Protein Determinants

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Monoclonal antibodies exhibiting *Chlamydia trachomatis* serovar specificity (serovar A, B-Ba, or C) and serogroup specificity (B, intermediate, or C serogroup) were produced and characterized. These antibodies reacted with the major outer membrane protein, recognized epitopes located at the chlamydial cell surface, and passively neutralized chlamydial toxicity for mice. The antibodies should be useful reagents for defining the molecular structure of these protective epitopes, a necessary step toward the development of a subunit or recombinant *C. trachomatis* vaccine.

*Chlamydia trachomatis* isolates are differentiated serologically into three serogroups with a total of 15 distinct serovars: the B serogroup (serovars B, Ba, D, E, L1, and L2), the intermediate serogroup (serovars F, G, K, and L3), and the C serogroup (serovars A, C, H, I, and J) (5, 12, 13). Serovars A, B, Ba, and C are the etiological agents of trachoma, the world's leading cause of preventable blindness (4). Serovars D through K are sexually transmitted pathogens that cause chlamydial urethritis, epididymitis, cervicitis, and pelvic inflammatory disease (PID).

Trachoma and PID are the most significant chlamydial diseases in terms of both severity and the number of persons afflicted. Trachoma is the leading cause of preventable blindness in the world; an estimated 500 million people in developing nations suffer from this disease (4). Chlamydial PID causes severe scarring of the fallopian tubes, which leads to tubal obstruction and may result in tubal blockage or ectopic pregnancy. It is estimated that chlamydial PID affects 400,000 women per annum in the United States (14).

Immunoprophylaxis has been suggested as a possible means for the control or prevention of blinding trachoma (6, 10) and may be a plausible approach for the control of sexually transmitted chlamydial infections. Protective immunity to chlamydial ocular infection is homotypic (6, 8), suggesting that serovar-specific antigenic determinants may be useful as candidates for the development of a chlamydial vaccine. Immunization with serovar-specific antigen(s) is a feasible approach for the development of a trachoma vaccine, since few serovars cause infection; however, it would not be a credible strategy in the case of sexually transmitted diseases because of the greater number of *C. trachomatis* serovars associated with these infections.

The major outer membrane protein (MOMP) is the primary chlamydial serotyping antigen; this molecule contains antigenic determinants which elicit serovar-, subspecies-, serogroup-, and species-specific antibodies (3, 9, 11). Monoclonal antibodies (MAbs) that recognize B serovar-specific and B complex-specific epitopes of the MOMP passively neutralize both chlamydial toxicity for mice and chlamydial infectivity for monkey conjunctivae (15). However, protective MAbs against the A or C serovar-specific MOMP epitopes or the conserved serogroup-specific MOMP determinants common to serovars of the intermediate or C serogroup have not been described. In this report we describe protective MAbs that recognize trachoma A and C serovar-specific MOMP determinants as well as MAbs against conserved MOMP determinants common to those serovars of the intermediate serogroup (F, G, K, and L3) and the C serogroup (A, C, H, I, and J).

Chlamydiae were grown in HeLa 229 cells, and elementary bodies (EBs) were purified by centrifugation on Renografin density gradients (2, 7). The procedures for immunization, generation of murine hybridomas, and screening of hybridoma supernatants for the presence of chlamydial antibodies have been described previously (15). Briefly, hybridoma supernatants were screened by the dot immunoblot assay with viable EBs as antigen. Positive supernatants were then screened by Western blot (immunoblot) to verify their binding to the MOMP. MAbs showing immunoreactivity in both assays were selected, since they most likely recognize contiguous MOMP determinants that were both immunogenic and antigenic on the native chlamydial cell surface. We sought contiguous protective determinants since our future objective is to express these determinants as fusion proteins at the cell surface of infectious enteric bacterial and viral vectors—vaccine-based vectors that require a minimal amount of inserted foreign sequence.

The MAbs were assayed for their ability to neutralize chlamydial toxicity for mice. The assay was done as previously described (15). Briefly, 50 µl of MAb (100 µg of protein) was mixed with 10 50% lethal doses of chlamydial EBs suspended in 1 ml of sucrose-phosphate-glutamic acid and incubated at 37°C for 1 h. Two hundred microliters (2 50% lethal doses) was then injected intravenously into the tail vein of 3- to 4-week-old (14- to 16-g) Swiss Webster mice. A total of 5 to 10 mice were tested for each MAb. Protection against toxic death was recorded between 2 and 18 h postinjection. Seven MAbs that demonstrated the desired specificities and binding properties by dot immunoblot and Western blot were identified (Fig. 1). MAb A-20 and C-20 reacted by dot immunoblot only with viable serovar A and C EBs, respectively. MAb B-B6, which has been described previously (15), was bispecific, reacting with serovars B and Ba. Each of these MAbs reacted with the MOMP of the

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FIG. 1. Specificity and reactivity of MAbs by dot immunoblot and Western blot analyses. (A) Specificity of MAbs by dot immunoblot. Viable EBs of each C. trachomatis serovar are used as antigen(s) in the dot immunoblot assay. Reactivity patterns of MAbs in this assay demonstrate the antigenicity of the epitope on the native EB cell surface. (B) Relevant portion of a Coomassie brilliant blue-stained gel that was used for electrophoretic transfer to nitrocellulose for the processing of Western blots (C). Each of the MAbs is immunoreactive with the MOMP. The specificities of serovar-specific MAbs A-20 and C-20, bispecific MAb B-B6, and B-serogroup-specific MAB L2I-5 are identical by both dot immunoblot and Western blot. In contrast, MAbs GI-C3, HV-A5, and DIII-A3 are more broadly reactive by Western blot than by dot immunoblot, suggesting a different surface arrangement and exposure of these epitopes on different C. trachomatis serovars.

TABLE 1. Neutralization of chlamydial mouse toxicity with serovar- and serogroup-specific MAbs

<table>
<thead>
<tr>
<th>MAb</th>
<th>Isotype*</th>
<th>Specificity by dot immunoblot</th>
<th>Epitope location</th>
<th>Neutralization of mouse toxicity</th>
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<tr>
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<td>C. trachomatis</td>
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<td>serovar</td>
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<td>A-20</td>
<td>IgG3</td>
<td>A</td>
<td>MOMP</td>
<td>A</td>
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<tr>
<td>B-B6</td>
<td>IgG3</td>
<td>B, Ba</td>
<td>MOMP</td>
<td>B</td>
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<tr>
<td>C-20</td>
<td>IgG2b</td>
<td>C</td>
<td>MOMP</td>
<td>C</td>
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<tr>
<td>L2I-5</td>
<td>IgG3</td>
<td>B&lt;sup&gt;8&lt;/sup&gt;, Ba, D, E, L1, and L2</td>
<td>MOMP</td>
<td>B</td>
</tr>
<tr>
<td>GI-C3</td>
<td>IgG2a</td>
<td>F, G, K, and L3</td>
<td>MOMP</td>
<td>D</td>
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<tr>
<td>HV-A5</td>
<td>IgG3</td>
<td>A, C, H, I, and J</td>
<td>MOMP</td>
<td>G</td>
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<tr>
<td>DIII-A3</td>
<td>IgG2b</td>
<td>B, Ba, D, E, L1, L2, F, G, and L3</td>
<td>MOMP</td>
<td>C</td>
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<tr>
<td>L2I-6</td>
<td>IgG3</td>
<td>All serovars</td>
<td>LPS&lt;sup&gt;a&lt;/sup&gt;</td>
<td>D</td>
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* IgG, Immunoglobulin G.
<sup>a</sup> MAbs L2I-5 reacts very weakly with serovar B by dot immunoblot.
<sup>b</sup> LPS, Lipopolysaccharide.
with the MOMP of the intermediate serogroup serovars plus the MOMP of C-complex serovars C, H, I, J, and L1 (weakly). MAb HV-A5 reacted more strongly with the MOMP of serovars G and F than with the MOMP of C-complex serovars. MAb DIII-A3 reacted with the MOMP of all serovars (except K). These findings show that the serogroup-specific MOMP epitopes studied here, with the exception of the L2I-5 determinant, were antigenically related to MOMP determinants of serovars in other serogroups. These differences in reactivities by dot immunoblot and Western blot most likely reflect differences in the surface topography of the MOMP in those regions of the molecule where these epitopes reside among chlamydial serovars.

The ability of these MAbs to neutralize chlamydial toxicity in mice is shown in Table 1. Both serovar-specific and serogroup-specific MAbs passively protected mice from toxic death. The ability of antibody to neutralize toxicity correlated with the surface accessibility of its MOMP epitope. For example, MAb L2I-5 reacted poorly with serovar B by dot immunoblot assay and did not protect mice from toxic death. However, this antibody reacted strongly with the cell surface of serovar D EBs and neutralized the toxicity of this serovar for mice. MAb L2I-6, which reacts with a surface-accessible, genus-specific epitope located on chlamydial lipopolysaccharide (1), does not protect mice, indicating that binding of antibody to the cell surface in itself is insufficient to promote neutralization. These findings support a functional role for MOMP in the pathogenesis of chlamydial toxicity in vivo.

We have identified MAbs specific to trachoma serovars (A, B, Ba, and C) and MAbs that recognize serogroup-specific epitopes. Epitopes recognized by these MAbs were located on the MOMP and were highly accessible to antibody at the chlamydial cell surface. MAbs specific to these MOMP epitopes were protective in the mouse toxicity test. The ability of these MAbs to passively protect against chlamydial infection was not tested. However, in a previous study (15) we observed that MAb B-B6 was protective in the mouse toxicity test and neutralized the infectivity of serovar B for the monkey conjunctivae, suggesting a functional relation between antibody-mediated protection of mouse toxicity and chlamydial infectivity.

We believe that the MAbs described here will be useful reagents for epitope mapping and for defining the molecular structure of these MOMP determinants, a critical step in evaluating their potential as antigens in the development of a synthetic or recombinant chlamydial vaccine.

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**LITERATURE CITED**


