Antigenic Analysis of the Major Outer Membrane Protein of *Chlamydia trachomatis* with Murine Monoclonal Antibodies

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We prepared monoclonal antibodies against prototype strains of the 15 serovars of *Chlamydia trachomatis* and identified a subset of reagents that reacted with the major outer membrane protein(s) (MOMPs) of one or more serovars. We then determined the specificities of these anti-MOMP monoclonal antibodies by radioimmunoassay and immunoblot assays against the 15 serovars of *C. trachomatis* and a *C. psittaci* strain. We identified 14 different anti-MOMP antibody specificities, including serovar-, several orders of subspecies-, and species-specific determinants. In addition, one antibody reacted with all *C. trachomatis* serovars and a *C. psittaci* strain, indicating the presence of a genus-specific epitope on MOMP. Many of the cross-reactions of the subspecies-specific antibodies were similar to those previously reported by use of the microimmunofluorescence technique. We also observed a number of cross-reactions that were unexpected but consistent with data derived from the microimmunofluorescence test. All antibodies, except the genus-specific antibodies, reacted with whole elementary bodies in a radioimmunoassay, suggesting surface exposure of the epitopes. These data confirm and extend previous observations that MOMPs among *C. trachomatis* serovars are antigenically complex and diverse. In addition, these data indicate that the cross-reaction patterns of some monoclonal antibodies directed against MOMP are similar to those detected by the microimmunofluorescence test and are consistent with the hypothesis that such determinants are contained within MOMPs.

The serological classification of clinical isolates of *Chlamydia trachomatis* has been accomplished largely by the microimmunofluorescence test (micro-IF) of Wang and Grayston (22). These studies have resulted in the classification of over 1,000 strains into 15 distinct serovars associated with human disease within the species *C. trachomatis* (7, 23). Micro-IF uses polyclonal mouse sera raised against both whole elementary bodies (EBs) of individual unknown strains of *C. trachomatis* and prototype strains representing the 15 known serovars of *C. trachomatis*. Such sera cross-react among Formalin-fixed whole EBs of each serovar in complex ways (for a review, see reference 22). Examples of observed specificities include genus-; species-; broad subspecies-, as in the B- and C-complex groups; narrow subspecies-, as in the close relationship of serovars E and D (22); and serovar-specific reactions. The molecular basis for such antigenic interrelationships has remained unknown, but recent studies have implicated chlamydial lipopolysaccharide (3) and surface-exposed outer membrane proteins (5, 11, 20).

Outer membrane proteins of *C. trachomatis*, particularly the major outer membrane protein (MOMP), have been implicated as structural determinants (4, 14), potential mediators of host-parasite interactions, and contributors to the antigenic structure of the organism (5, 13). Caldwell and Schachter (5) used isolated MOMP-sodium dodecyl sulfate (SDS) complexes to produce hyperimmune rabbit sera that reacted in micro-IF in a species- and subspecies-specific fashion. Of the six anti-MOMP sera tested, only the anti-serovar H MOMP serum sample showed appreciable serovar specificity. In studies with murine monoclonal antibodies, Stephens et al. (19) reported the presence of species- and subspecies-specific determinants in MOMPs, while Matikainen and co-workers (11, 20) first showed that MOMPs contain serovar-specific determinants. More recently, we have demonstrated (15) serovar-specific determinants in the MOMPs of 10 of the 15 *C. trachomatis* serovars (all except Ba, H, I, J, and L3). In addition, extensive immunoblot analyses (W. J. Newhall V, manuscript in preparation) of polyclonal mouse sera raised against all 15 *C. trachomatis* serovars confirm the presence of genus-, species-, serovar-, and diverse subspecies-specific determinants in MOMPs.

To explore further the antigenic diversity of MOMPs, we prepared murine monoclonal antibodies against prototype strains of *C. trachomatis*. We identified a subset of 14 monoclonal antibodies that illustrate the diversity and levels of antigenic relatedness among MOMPs.

**MATERIALS AND METHODS**

*Chlamydiae* representing each of the 15 known serovars of *C. trachomatis* and the meningopneumonitis and guinea pig inclusion conjunctivitis strains of *C. psittaci* were grown, and EBs were purified, as described previously (13). Murine monoclonal antibodies directed against *chlamydiae* were produced as described previously (15), and initial screening and specificity testing were performed in a radioimmunooassay (RIA) with Formalin-fixed whole EBs (15). Monoclonal antibodies reacting with *chlamydial* MOMPs were selected by immunoblotting (1, 13, 15) against the antigen of the immunizing serovar. In addition, the specificity of each antibody was tested by an immunoblot assay in which each of the 15 known *C. trachomatis* serovars and a representative *C. psittaci* strain were used as test antigens (13).

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RESULTS

In Table 1 are summarized the specificities of 14 anti-MOMP monoclonal antibodies, as assessed by RIA with intact, formalin-fixed EBs as antigens. In Fig. 1 are illustrated the binding patterns determined by immunoblot assays for seven of the anti-MOMP monoclonal antibodies. The most broadly reactive was M.1/2E5/E2, which bound the MOMPs of all 15 C. trachomatis strains as well as that of the meningoencephalitis strain of C. psittaci (Fig. 1). In another experiment (data not shown), this genus-reacting anti-MOMP antibody also bound the MOMP of a second C. psittaci strain, the guinea pig inclusion conjunctivitis agent. Although this antibody reacted well in immunoblot assays, it did not react with whole EBs of any chlamydial strain in the RIA (Table 1). These data suggest that the epitope identified by this monoclonal antibody is not surface exposed. In fact, this monoclonal antibody was one of five antibodies exhibiting this pattern of reactivity that was produced from fusions which utilized purified MOMPs as immunogen rather than whole EBs.

Another broadly reactive antibody, L1/2/C5/B8, recognized the MOMPs of the C. trachomatis serovars but not of the C. psittaci strain (Table 1 and Fig. 1). This species-specific anti-MOMP antibody appeared to react with a surface-exposed epitope based on its positive reactivity in the RIA (Table 1) and is one of several independently derived clones that exhibited this cross-reactivity pattern. Although not shown in Fig. 1, this antibody reacted in an immunoblot with the MOMP of the mouse pneumonitis agent (16) as well as with whole EBs in the RIA. Thus, this antibody reacted with all three biotypes of C. trachomatis. However, the possibility of a reaction with C. psittaci strains other than the meningoencephalitis strain and the guinea pig inclusion conjunctivitis agent has not been assessed.

Many of the anti-MOMP monoclonal antibodies gave subspecies-specific reaction patterns that were similar to those defined by micro-IF. According to Wang and Grayston (22), the B complex consists of serovars B, Ba, D, E, L1, and L2, whereas the C complex consists of serovars C, J, A, H, and I. Serovars F and G are related to the B complex, while serovars K and L3 are related to the C complex but serve as an antigenic bridge between the B and C complexes. One monoclonal antibody reacted with B-complex strains (L5/27/B1/2), while another reacted with C-complex strains plus K and L3 (H-197/A3) (Table 1). The immunoblot reactions of the latter antibody are shown in Fig. 1. In addition, another antibody was identified that reacted with B-complex strains plus F and G (G/102/C2/1), and still another was identified that reacted with B-complex strains plus F, G, K, and L3 (L1/6/6/F1/G11) (Table 1). In the immunoblot, G/102/C2/1 gave the same reactivity as in the RIA (data not shown), but L1/6/6/F1/G11 reacted strongly only with B-complex strains and minimally or not at all with K, L3, F, and G (Fig. 1). These results illustrate (i) that the MOMPs of C. trachomatis serovars contain epitopes that may be responsible for the broad divisions of antigenic relatedness described by micro-IF, (ii) the antigenic bridging of the B and C complexes by the K and L3 serovars, and (iii) that antichlamydial monoclonal antibodies may not react identically in different assay systems.

A number of narrow subspecies specificities were identified that correlated very closely with antigenic relationships that have been defined by the micro-IF test. These include F/G (Table 1 and Fig. 1); B/Ba, which we reported previously (15); and C/J (Table 1). The C/J-specific anti-MOMP antibody described here (J/151/D3) differs from the C/J-specific antibody we have used as a serotyping reagent (15) in that the latter does not react with MOMP in the immunoblot. When tested by RIA, the J/151/D3 monoclonal antibody reacted more strongly with J than with C, while it seemed to react equally strongly with C and J MOMPs in the immunoblot. A similar C/J-specific antibody has been described by Stephens et al. (19) that reacts primarily with J in micro-IF but that does not react in an immunoblot assay. In addition to these expected pairs, we have also observed an anti-MOMP antibody that reacts with serovars C and L3 (15), a relationship not previously appreciated, whereas monoclonal antibody specificities that correlate with other known

**TABLE 1. Cross-reactivities of anti-MOMP monoclonal antibodies in the RIA**

<table>
<thead>
<tr>
<th>Designation</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>M.1/2E5/E2</td>
<td>Not reactive</td>
</tr>
<tr>
<td>L1/2/C5/B8</td>
<td>C. trachomatis species</td>
</tr>
<tr>
<td>L1/6/6/F1/G11</td>
<td>B, Ba, D, E, L1, L2, F, G, K, L3</td>
</tr>
<tr>
<td>G/102/C2/1</td>
<td>B, Ba, D, E, L1, L2, F, G</td>
</tr>
<tr>
<td>L5/27/B1/2</td>
<td>B, Ba, D, E, L1, L2</td>
</tr>
<tr>
<td>F/2/3/G8</td>
<td>B, Ba, D, F, L1</td>
</tr>
<tr>
<td>Ba/243/5G4/G7</td>
<td>B, Ba, L2</td>
</tr>
<tr>
<td>L1/6/6/F1/G9</td>
<td>D, F, L1</td>
</tr>
<tr>
<td>G/220/F2</td>
<td>G, L3</td>
</tr>
<tr>
<td>Ba/222/1/C8</td>
<td>B, Ba</td>
</tr>
<tr>
<td>J/151/D3</td>
<td>C, J</td>
</tr>
<tr>
<td>G/238/4G10/G6</td>
<td>F, G</td>
</tr>
<tr>
<td>L1/66/2/F9</td>
<td>C, L3</td>
</tr>
</tbody>
</table>
antigenic relationships such as D/E, L1/L2, and K/L3 have not yet been identified.

Subspecies specificities were also observed that have not been previously reported. These include B/Ba/L2 (Table 1 and Fig. 1), D/F/L1 and B/Ba/D/F/L1 (Table 1), and G/L3 (Table 1 and Fig. 1). The antibody that bound the MOMP's of G/L3 illustrated bridging between the B and C complexes, while each of the other antibodies gave reactions that were consistent with the close antigenic ties shared among B-complex strains.

**DISCUSSION**

Our data confirm and extend previously reported data that have described the cross-reactivities of antichlamydial monoclonal antibodies (6, 9, 10, 12, 16-18, 21, 23). Although we did not perform an exhaustive antigenic survey of MOMPs of *C. trachomatis* strains, we found that each MOMP possesses a broad hierarchy of epitopes, ranging from those that are shared between species (genus specific) to those that are serovar specific. Subspecies epitopes on MOMP's shared among serovars in complex ways, interrelated serovars and B- and C-complex groups in ways consistent with the relationships previously noted in studies with micro-IF. We also described a few monoclonal antibodies that define relationships among serovars that have not been previously appreciated but that are consistent with reported micro-IF reactivity patterns. These data strongly support the hypothesis that the antigenic diversity among serovars of *C. trachomatis* defined by micro-IF is a reflection of the antigenic diversity of the various MOMPs.

For this hypothesis to be true, epitopes would need to be on a region of the MOMP that is surface exposed so that they would be available for reaction in micro-IF, which uses Formalin-fixed, whole EBs as the test antigen. Using the presence of reactivity in whole EB-based immunosays (RIA) as a crude indicator of surface exposure, all of the epitopes defined by the monoclonal antibodies in this study were surface exposed to some degree, except for the genus-specific epitope. In addition, differential surface exposure of an epitope may explain differences in reactivity of a particular antibody with different serovars. For example, one antibody, J/151/D3, reacted equally strongly against the MOMPs of serovars C and J in the immunoblot, which represented reactivity with the isolated MOMP-SDS complex. In contrast, when tested by RIA, an assay employing whole EBs, it reacted much more strongly with J than with C. These observations could be explained by poor surface availability for antibody binding in serovar C relative to that in serovar J.

We also observed differences in the degree of cross-reactivity of some monoclonal antibodies that depended on the assay used to test them (8). For example, we found several antibodies, both serovar specific and bispecific, that reacted in RIA but not in immunoblotting (15). These observations might be explained by a number of factors. The epitopes may be heat, SDS, or reduction sensitive; may not be resolved by SDS-polyacrylamide gel electrophoresis; or do not transfer from the gel or adsorb to the nitrocellulose. A different kind of discrepancy noted in this study was the monoclonal antibody that identified a genus-specific epitope on MOMP. The antibody reacted in the immunoblot, but not in the RIA, indicating that the epitope was either not surface exposed or, if so, was sterically hidden and therefore not available for binding in whole-EB assays. A third kind of discrepant binding was an antibody that reacted broadly in the RIA with B, D, E, L1, L2, F, G, K, and L3, but that reacted in the immunoblot only with B, Ba, D, E, L1, and L2. To say that the epitope was heat, SDS, or reduction sensitive is not sufficient, because in some MOMPs it reacted well in the immunoblot, despite such treatments. Additional factors, such as the nature of the binding to nitrocellulose or the three-dimensional conformation of the MOMPs on the nitrocellulose, may be important. Stephens et al. (17, 19) also observed such differences in reactivity of several monoclonal antibodies with different assays.

Thus, our data support the hypothesis that the antigenic diversity among serovars of *C. trachomatis* reflects the antigenic diversity of the various MOMPs. Undoubtedly, many epitopes that define additional relationships among serovars, or that define new serovars or antigenic variations within serovars, will be found as more antibodies are produced and evaluated.

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**LITERATURE CITED**