Differential Effect of Trypsin on Infectivity of *Chlamydia trachomatis*: Loss of Infectivity Requires Cleavage of Major Outer Membrane Protein Variable Domains II and IV

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*Chlamydia trachomatis* is an obligate intracellular bacterium that infects mucosal epithelial cells of humans, causing blinding trachoma and sexually transmitted urogenital diseases (8). These diseases afflict millions throughout the world each year and are major public health problems for which control and prevention measures are clearly needed.

Chlamydiae are distinguished from other intracellular procaryotes by their complex life cycle. They exist as two distinct developmental forms which differ both morphologically and functionally. The structurally rigid elementary body (EB), which is 200 nm in diameter, is the infectious form of the parasite. It survives extracellularly and attaches to and enters host cells (13, 14). After entry, the EB resides within a host phagosome and quickly differentiates into a larger (1,000 nm in diameter) noninfectious reticulate body. The fragile reticulate body is metabolically active and divides by binary fission within the endosome. The replicated reticulate bodies then differentiate to become infectious EBs that are released from the infected cell and reinfect the infectious process. The surface components that function in the attachment of chlamydiae to host cells are clearly key virulence factors. Their identification and molecular characterization are crucial in our understanding of chlamydial pathogenesis and in developing a chlamydial vaccine.

The predominant protein species found in the chlamydial outer membrane is the ca. 40,000-molecular-weight major outer membrane protein (MOMP) (40K MOMP). It plays an important role in maintaining the structural integrity of the outer membrane of the infectious EB through intra- and intermolecular disulfide bonds (1a, 10-12). The MOMP is also an immunologically complex antigen. Serovar-specific determinants are associated with each MOMP in addition to more common antigenic determinants that confer subspecies-, serogroup-, and species-specific determinants (3, 18). All serovar-specific MOMP epitopes and some serogroup-specific MOMP epitopes are highly immunoreactive at the EB cell surface and are immunodominant epitopes recognized during chlamydial infection (18). Monoclonal antibodies (MAbs) specific for these MOMP epitopes passively neutralize chlamydial toxicity and infectivity in vivo but do not neutralize the in vitro infectivity of the organism for HeLa 229 cells (7). These differences between in vivo and in vitro neutralization results are not understood. In addition, the mechanism by which MOMP MAbs neutralize infectivity in vivo is unknown. MAbs specific to a surface-accessible epitope on chlamydial lipopolysaccharide do not neutralize infectivity in vivo, demonstrating that accessibility of surface antigens to antibody per se is insufficient for in vivo neutralization of chlamydial infectivity. We have interpreted these findings as evidence that implicates the MOMP in the pathogenesis of chlamydial infection.

Recently, the MOMP genes of *C. trachomatis* serovars L2 and B have been sequenced (1, 15). These genes encode amino acid sequences that are approximately 93% homologous. Nonhomologous amino acids reside within four variable domains (VDs). Three of these (VDs I, II, and IV) protrude toward the external environment at the chlamydial cell surface and are major antigenic sites whose structures have been defined (1, 15a). A limited number of potential trypsin cleavage sites exist within these domains. The serovar B MOMP and the serovar L2 MOMP each have four potential trypsin cleavage sites, one in each VD. The susceptible sites in VD I and VD IV are identical for both serovar B and L2 MOMPs, while the sites in VD II and VD III differ both in location of the lysine residues and in the surrounding amino acid sequences.

In this report, we have used trypsin proteolysis of viable chlamydiae to examine the possible role of MOMP VDs in
the interaction of chlamydiae with host cells. We present evidence that strongly supports a role for MOMP in the attachment of this parasite to its eucaryotic host cell.

**MATERIALS AND METHODS**

**Chlamydiae.** *C. trachomatis* serovar B (strain TW-5/OT) and serovar L2 (strain LGV/434/Bu) were propagated in HeLa 229 cells. EBs were purified from infected cells by Renografin density gradient centrifugation (5). Chlamydiae were metabolically radiolabeled with 14C-amino acids as previously described (6).

**Monoclonal antibodies.** The production of hybridomas secreting MAbs and the characterization of these MAbs have been described in previous studies (3, 18), and a summary of the properties of these MAbs is shown in Table 1.

**Trypsin treatment of chlamydiae.** (i) *Infectivity assays.* Purified serovar B EBs (4.5 × 10^7 inclusion-forming units [IFU] per ml) or serovar L2 EBs (7.2 × 10^9 IFU/ml) were suspended in 1 ml of 0.25 M sucrose–10 mM sodium phosphate–5 mM L-glutamic acid (pH 7.2) containing 0.1, 1.0, or 10 μg of trypsin (type XIII, tosylsulfonyl phenylalanyl chloromethyl ketone treated; Sigma Chemical Company, St. Louis, Mo.). These suspensions were incubated for 15, 30, and 60 min at 37°C in a gyratory shaking water bath. Trypsin was inhibited by the addition of trypsin inhibitor (Type I-P, Sigma) (0.5 μg of inhibitor per 1 μg of trypsin). The suspensions were diluted in 0.25 M sucrose–10 mM sodium phosphate–5 mM L-glutamic acid (pH 7.2) to contain approximately 5 × 10^5 IFU/ml. A 0.2-ml volume was inoculated onto confluent HeLa 229 cell monolayers (3 × 10^5 cells) grown on glass cover slips. After 2 h of absorption at 37°C, the inoculum was removed and fresh medium was added to the cells. Cells were incubated for 48 h at 37°C and then were processed for the determination of IFU as previously described (10).

(ii) *Attachment assays.* A 1.0-ml volume of 14C-labeled serovar B EBs (6.3 × 10^7 IFU [3.1 × 10^6 cpm/ml]) was treated with 1 μg of trypsin at 37°C for 120 min. Control organisms were incubated in 0.25 M sucrose–10 mM sodium phosphate–5 mM L-glutamic acid (pH 7.2) buffer alone. The suspensions were diluted in 0.25 M sucrose–10 mM sodium phosphate–5 mM L-glutamic acid (pH 7.2) to give a final concentration of 6.3 × 10^9 IFU/ml (3.1 × 10^6 cpm/ml). Two hundred microliters of the suspension was inoculated onto each of four HeLa 229 cell monolayers, and the cells were incubated at 37°C for 2 h. The inoculum was removed, and the monolayers were washed three times with 500 μl of Hanks balanced salt solution. Two monolayer cultures were fed with minimal essential medium 10 and incubated at 37°C for 72 h and processed to determine IFU. The remaining two monolayers were lysed with 200 μl of 0.1 N NaOH at 70°C for 1 h. The solubilized cells were collected, and the wells were washed twice with 200 μl of 0.1 N NaOH. Lysates and their corresponding washes were pooled, mixed with 10 ml of liquid scintillation cocktail (Ready-Sov; Beckman Instruments, Inc., Fullerton, Calif.), and the radioactivity associated with each sample was determined with a liquid scintillation counter (LS 9000; Beckman).

The physical properties of trypsin-treated chlamydiae were examined by determining their osmotic stability and isoelectric point. Osmotic stability was determined by measuring the absorbance at 540 nm of trypsin-treated organisms in hypotonic (distilled water) or hypertonic (0.25 M sucrose) solutions. Isoelectric focusing of chlamydiae was done as described by Vance and Hatch (17).

(iii) **Protease accessibility of chlamydial surface proteins.** A 100-μl volume of EBs intrinsically labeled with 14C-amino acids (serovar B, 6.3 × 10^8 IFU/ml, 6.1 × 10^6 cpm/ml; serovar L2, 8.0 × 10^8 IFU/ml, 9.5 × 10^6 cpm/ml) were incubated with 1 μg of trypsin for various times at 37°C. Trypsin was inhibited by the addition of 2 mM phenylmethylsulfonyl fluoride. The suspensions were pelleted and suspended in H2O and then mixed with 100 μl of Laemmli sample buffer and placed in a boiling water bath for 10 min. A 20-μl volume of the solubilized suspensions was electrophoresed on a 12.5% polyacrylamide gel. Fluorography of polyacrylamide gels was produced by the procedure of Bonner and Laskey (2).

**Dot blot and Western blot (immunoblot).** Chlamydiae were treated with trypsin as described above, and the immunoreactivity of MOMP was determined by both dot blot and Western blot assays with the group of MAbs described in Table 1. The methods used for these assays have been described previously (3, 18). Briefly, organisms were treated with trypsin for various times; the enzyme was then inhibited by the addition of 2 mM phenylmethylsulfonyl fluoride. For the dot blot test, chlamydiae were diluted in phosphate-buffered saline to a concentration of 5 μg of protein per ml. A 50-μl volume of this suspension was then used in the dot blot assay by the methods previously described (18). For Western blots, trypsin-treated organisms were pelleted and

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**TABLE 1. Properties of MOMP monoclonal antibodies and locations of epitopes in MOMP primary sequences**

<table>
<thead>
<tr>
<th>MAb</th>
<th>MAb specificity (serovar[s])</th>
<th>Immuno-accessibility of epitope*</th>
<th>Mouse/monkey in vivo neutralization*</th>
<th>MOMP variable domain (serovar)</th>
<th>Location of epitope</th>
<th>Amino acid sequences (sequence no.) of variable domains and epitopes*</th>
</tr>
</thead>
<tbody>
<tr>
<td>B-B6</td>
<td>B, Ba (bispecific)</td>
<td>+</td>
<td>+/+/+</td>
<td>VD II (B)</td>
<td>NNENQTFK VSNGA FVPNMSLDQS (139-160)</td>
<td></td>
</tr>
<tr>
<td>B-B5</td>
<td>B, Ba, D (subspecies-specific)</td>
<td>+</td>
<td>+/+</td>
<td>VD IV (B)</td>
<td>SAETIFDV TLNP TIA GDV KTSAEGQLG (288-317)</td>
<td></td>
</tr>
<tr>
<td>L21-45</td>
<td>L2 (serovar-specific)</td>
<td>+</td>
<td>+/ND*</td>
<td>VD II (L2)</td>
<td>DNNENHATVSDS KLV PNMSLDQS (139-160)</td>
<td></td>
</tr>
<tr>
<td>L21-10</td>
<td>All <em>C. trachomatis</em> serovars (A-L3) (species-specific)</td>
<td>-</td>
<td>-/-</td>
<td>VD IV (L2)</td>
<td>SATTYFDV TLNP TIA GDV KTSAEGQLG* (288-317)</td>
<td></td>
</tr>
</tbody>
</table>

* Accessibility of MOMP epitope to antibody, at the EB cell surface (+) was determined by dot blot assays with viable chlamydiae as the antigen.

* Neutralization of mouse toxicity and neutralization of serovar B for monkey conjunctivae (+).

* Epitope amino acid sequence is italicized, and lysine (K) residues are identified by asterisks.

* ND, Not done.

* The L21-10 epitope is identical in both B and L2 serovars.

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suspended in Laemmli sample buffer, boiled, and electrophoresed on a 12.5% polyacrylamide slab gel. Western blots of gels were done as previously described (3).

Two-dimensional peptide mapping. 131Iodine peptide maps of MOMP and its tryptic peptide fragments were produced as described by Swanson (4, 16), with the following modifications. Precast thin-layer chromatography plastic sheets (cellulose) (E. Merck AG, Darmstadt, Federal Republic of Germany) were used. Electrophoresis was done at a constant voltage of 1,200 V for 22 min at 13°C.

RESULTS

The effect of trypsin on chlamydiales infectivity. *C. trachomatis* serovars B and L2 were assayed for infectivity after treatment with 0.1, 1.0, and 10 μg of trypsin for various periods of time (Fig. 1). Serovar B showed an 80% reduction in infectivity for HeLa 229 cells after treatment with 10 μg of trypsin for 15 min. Incubation with 1 μg of trypsin resulted in a more gradual loss of infectivity, with an 80% reduction occurring after 2 h of treatment. Incubation with 0.1 μg of trypsin had no effect on the infectivity of serovar B. In contrast, a similar effect of trypsin on the infectivity of serovar L2 was not observed.

The effect of trypsin on chlamydiales attachment. Next, we examined the ability of trypsin-treated serovar B EBs to attach to HeLa cells. Tryptsin-treated organisms showed a marked decrease in their ability to attach to cells compared with untreated control organisms (Table 2). Treatment of EBs with 1 μg of trypsin for 2 h resulted in a 60% decrease in cell-associated radioactivity. This decrease in attachment was paralleled by a similar decrease in IFU. Trypsin-treated serovar L2 EBs showed no decrease in either attachment or infectivity for HeLa 229 cells (data not shown).

The osmotic stability and isoelectric point of serovar B EBs were not significantly different after trypsin treatment (data not shown). These findings indicated that the reduction of chlamydiales infectivity was not a result of physical damage to the outer membrane.

Trypsin susceptibility of chlamydiales surface proteins. We examined the polypeptide profiles of trypsin-treated serovar B and L2 EBs to determine whether we could associate the susceptibility of surface proteins to trypsin with those differences observed in infectivity assays. We treated serovar B and L2 EBs, which had been intrinsically labeled with 14C-amino acids, with 1 μg of trypsin for 15, 30, 60 and 120 min at 37°C and subjected them to sodium dodecyl sulfate-polyacrylamide gel electrophoresis and fluorography (Fig. 2). The MOMP of both chlamydiales serovars was the primary protein cleaved by trypsin. Serovar B MOMP was rapidly cleaved, producing tryptic peptide fragments 29K (F1), 20K (F2), 16K (F3), and 6K (F4) within 15 min of proteolysis. The 29K fragment was an intermediate proteolytic product of MOMP, since this peptide was not detectable after 120 min of trypsin treatment. The 20K, 16K, and 6K polypeptides were stable proteolytic MOMP fragments that were present

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**FIG. 1.** The effect of trypsin treatment on the infectivity of *C. trachomatis* serovars B and L2. Serovars B and L2 EBs were treated with different concentrations of trypsin for the periods indicated and then assayed for infectivity. Serovar B EBs were treated with 0.1 (■), 1 (▲), and 10 (●) μg of trypsin. Serovar L2 EBs were treated with 10 μg of trypsin (○). The percent reduction in infectivity = [(control IFU - trypsin IFU)/control IFU] × 100.

**FIG. 2.** Trypsin accessibility of *C. trachomatis* surface proteins. Serovars B and L2 EBs were intrinsically labeled with 14C-amino acids and treated with 1 μg of trypsin. At the times indicated (0, 15, 30, 60, and 120 min), the chlamydiales were lysed and subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis and fluorography. (A) Fluorogram of serovar B EBs. (B) Fluorogram of serovar L2 EBs. Trypsin cleaved primarily the MOMP of both serovars. The serovar B MOMP was cleaved to give fragments (F) 29K, 20K, 16K, and 6K. The serovar L2 MOMP was cleaved to give fragments 36K and 6K.

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**TABLE 2.** Effect of treatment of *C. trachomatis* serovar B EBs with trypsin on chlamydiales attachment to HeLa 229 cells and infectivity

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Attachment (cpm)</th>
<th>Infectivity</th>
<th>% Reduction in attachment</th>
<th>% Reduction of infectivity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EBs inoculated</td>
<td>EBs attached (%)</td>
<td>No. of inclusions*</td>
<td></td>
</tr>
<tr>
<td>Trypsin</td>
<td>51,790 ± 146</td>
<td>2,628 ± 89 (5.1)</td>
<td>58</td>
<td>10,097 ± 106</td>
</tr>
<tr>
<td>Control</td>
<td>45,287 ± 202</td>
<td>5,454 ± 93 (12.0)</td>
<td></td>
<td>25,296 ± 213</td>
</tr>
</tbody>
</table>

* Number of inclusions per 10^4 HeLa 229 cells.
throughout the period of trypsin treatment. However, a different trypsic peptide profile was observed for serovar L2 MOMP. Serovar L2 MOMP was less rapidly degraded by trypsin, and only two proteolytic fragments, 36K and 6K, were detected. The rate of trypsin cleavage differed significantly for each serovar. The cleavage of the majority of serovar B MOMP into the 29K and 16K fragments occurred within 15 to 30 min, whereas cleavage of the majority of serovar L2 MOMP into its two peptide fragments occurred between 60 and 120 min. This suggested that the initial cleavage site on serovar B MOMP was highly accessible to trypsin and that a similarly exposed site may not exist on serovar L2 MOMP. The total molecular mass of the trypsinic peptide fragments produced from both MOMP were nearly equal to that of their respective monomeric MOMP, indicating that all fragments remained associated with the outer membrane after cleavage.

Two-dimensional peptide mapping of MOMP trypsin fragments. To ascertain the relationships of the four trypsin fragments of serovar B MOMP (29K, 20K, 16K and 6K) to each other and to the intact MOMP, we mapped the peptides of each fragment and of the monomeric MOMP. The peptides generated from chymotrypsin-digested monomeric MOMP (Fig. 3A) were assigned numbers to facilitate their identification in maps of each trypsin fragment. Two-dimensional peptide maps generated from the 20K, 16K, and 6K trypsinic fragments showed that together they represent all of the peptides found in the monomeric MOMP. The peptides found in the 20K and 6K fragments were also present in the map of the 29K trypsinic fragment, demonstrating that these two fragments were derived from the larger 29K polypeptide. These data and those shown in Fig. 2 suggested that at the cell surface, serovar B MOMP was cleaved at two different locations. The initial rapid cleavage produced polypeptides 29K and 16K. The 29K fragment was then further cleaved to produce fragments of 20K and 6K. In contrast, the serovar L2 MOMP appeared to have a single trypsin-accessible site which was cleaved to produce 36K and 6K fragments.

Antigenicity of MOMP trypsin fragments. To identify the trypsin cleavage sites on the serovar B and L2 MOMP, we used previously characterized MAbs to determine the antigenicity of each proteolytic fragment (Table 1). MAbs B-B6 and L2I-45 are specific to serovars B and L2, respectively, and bind to epitopes located in VD II (Baehr et al., in press; Stephens et al., in press). MAb B-B5 is subspecies specific (to serovars B, Ba, and D) and reacts with an epitope located in VD IV of serovar B MOMP. All of these determinants are accessible to antibodies on the native chlamydial cell surface. MAb L2I-10 binds to a highly conserved species-specific epitope located in VD IV. This epitope is not accessible to antibodies on the surfaces of serovars B and L2.

The effect of trypsin on the antigenicity of B-B6, B-B5, and L2I-10 epitopes of the serovar B MOMP was determined by dot blot and Western blot analysis (Fig. 4). The dot blots showed the effect of trypsin on the antigenicity of MOMP at the EB cell surface. The Western blots demonstrated the antigenic properties of polypeptides derived from trypsin cleavage of MOMP at the cell surface. The dot blots showed that both epitopes, B-B6 and B-B5, were destroyed by trypsin at the chlamydial cell surface. Epitope B-B6 was destroyed more rapidly than epitope B-B5. Interestingly, the loss of B-B6 and B-B5 antigenicity was accompanied by the surface exposure of the conserved species-specific L2I-10 determinant. The antigenicity of the L2I-10 determinant was trypsin resistant.

The immunoreactivity of serovar B MOMP trypsin peptides with the same panel of MAbs is shown by the Western blots in Fig. 4B. Peptides 29K, 20K, 16K, and 6K were produced from MOMP after trypsin treatment of serovar B EBs (Fig. 2). The 16K and 6K trypsin MOMP fragments did not react with any of the MAbs. The reactivity of MAbs by Western blotting with the monomeric MOMP and with the 29K and 20K fragments corroborated the results obtained with dot blots. The majority of the reactivity of MAb B-B6 was lost after cleavage of the monomeric MOMP. The 29K fragment reacted slightly with MAb B-B6, indicating that...
enough of the critical amino acids remained in this peptide to result in low-avidity binding by MAb B-B6. MAb B-B5 reacted with both the monomeric MOMP and the 29K intermediate fragment; however, cleavage of the 29K fragment into the 20K and 6K peptides resulted in a loss of reactivity with MAb B-B5. MAb L21-10 reacted with the monomeric MOMP and the 29K and 20K fragments, and its reactivity with the 20K fragment was trypsin resistant. These findings showed that serovar B MOMP was cleaved in both VD II and VD IV by trypsin. The trypsin site located in VD II was within the B-B6 epitope and was rapidly cleaved by trypsin. The trypsin site located in VD IV was within the B-B5 epitope but was not within the L21-10 epitope, and it was not cleaved as rapidly as the site within VD II.

A different result was observed with trypsin-treated serovar L2 EBs (Fig. 5). The dot blot reactivity of MAb L21-45, which recognizes the surface-accessible, serovar-specific epitope located in VD II of serovar L2 MOMP, was not affected by trypsin. Like the serovar B EBs, trypsin-treated serovar L2 EBs demonstrated an enhanced binding of MAb

FIG. 4. The effect of trypsin on C. trachomatis serovar B MOMP antigenic determinants. (A) Dot blots of control (no trypsin) and trypsin-treated serovar B EBs probed with MABs specific for accessible (B-B6 and B-B5) and nonaccessible (L21-10) MOMP surface epitopes. (B) Western blots of trypsin-treated serovar B EBs probed with the same group of MABs used in panel A. MAB B-B6 recognizes an epitope located in VD II, and MABs B-B5 and L21-10 recognize epitopes in VD IV. The periods of trypsin digestion (0, 15, 30, 60, and 120 min) are shown above panels A and B. The results shown by the dot blot reflect the antigenic properties of MOMP at the chlamydial cell surface. The Western blot results show the antigenic properties of MOMP peptides generated by surface proteolysis with trypsin. Note that the B-B6 epitope is rapidly destroyed at the cell surface by trypsin and that this epitope is only present on the monomeric MOMP. The B-B5 epitope shows an intermediate sensitivity to trypsin at the cell surface and is associated with the monomeric MOMP and the 29K intermediate fragment. Immuno-reactivity of the conserved species-specific epitope L21-10 occurs only after exposure to trypsin and is trypsin resistant. The L21-10 epitope is present on the monomeric MOMP and the 29K and 20K peptide fragments. The 16K and 6K fragments (Fig. 2) were not immunoreactive.

L21-10 after trypsin treatment. Western blots of serovar L2 MOMP tryptic fragments showed that the 36K fragment reacted with both MABs L21-45 and L21-10. The 6K fragment (Fig. 2) was not immunoreactive. These findings indicated that trypsin does not cleave the serovar L2 MOMP in VD II but cleaves only in VD IV, resulting in fragments of 36K and 6K. Therefore, the only difference in cleavage of serovar B and L2 MOMP by trypsin was within VD II. A small amount of a fragment of 25K was observed after 60 min of digestion. This fragment reflects additional cleavage of MOMP in or near VD IV.

DISCUSSION

In this study, we examined the effect of trypsin proteolysis of viable chlamydiae on their binding to and infection of HeLa 229 cells. Trypsin substantially reduced binding affinity and infectivity of serovar B EBs for HeLa cells but had no effect on serovar L2 EBs. By using trypsin and a panel of previously characterized MABs, we identified surface-exposed domains of the MOMP which may be involved in interactions of chlamydiae with host cells. MOMP VDs II and IV are critical for chlamydial infectivity, and these structures, either separately or cooperatively, play a role in the attachment of chlamydiae to host cells.
A model of serovar B and L2 MOMP trypsin cleavage sites at the cell surface is presented in Fig. 6. The primary amino acid sequences of serovar B and L2 MOMPs in VDs II and IV are depicted in Fig. 6A; both the potential trypsin cleavage sites and the MAb binding sites are illustrated. The schematic shown in Fig. 6B summarizes the cleavage sites of the MOMPs, the kinetics of proteolysis, and the resulting tryptic fragments. The serovar B MOMP was cleaved twice by trypsin; the first cleavage was complete within 15 to 30 min and occurred in VD II between lysine 145 and valine 146. This destroyed the B-B6 epitope and generated the 29K and 16K peptide fragments. The 29K fragment was then cleaved in VD IV between lysine 309 and threonine 310. This second cleavage occurred more slowly, destroyed the B-B5 epitope, and generated peptide fragments 20K and 6K. In contrast, the serovar L2 MOMP was cleaved once at lysine 309 in VD IV, generating fragments 36K and 6K. The trypsin site at lysine 150 in VD II of serovar L2 MOMP was not cleaved by trypsin. Either it was inaccessible to the enzyme or the local microenvironment reduced trypsin activity. Cleavage within VD IV of both MOMPs resulted in accessibility of the conserved species-specific L2I-10 epitope to antibody. The L2I-10 epitope was resistant to trypsin as it was used in these experiments.

The kinetics of loss of infectivity of serovar B EBs (Fig. 1) correlate with the kinetics of trypsin cleavage (Fig. 2) in VD IV. The total cleavage of VD II (30 min) does not correspond to maximal loss in infectivity. Maximal reduction in infectivity occurred when both VD II and VD IV were cleaved by trypsin. There are several possible explanations for this effect: (i) cleavage of both domains sufficiently alters the tertiary or quaternary structure of MOMP and prevents the appropriate binding domains from interacting with the host cells; (ii) VD II and VD IV form a combined binding site, and cleavage in both domains is required to alter binding; and (iii) only one domain (i.e., VD II) may be involved in actual binding to host cells; however, the other domain may be essential to maintain conformational integrity through disulfide cross-linking or side chain interactions with this domain.

Conformational changes in MOMP do occur, as demonstrated by the binding of MAb L2I-10 to the cell surface after trypsin treatment (Fig. 3A). This MAb does not bind to native chlamydiae; however, following trypsin treatment, this epitope becomes immunoaccessible in both serovars. The L2I-10 epitope is located within amino acids 296 and 304 and is 4 amino acid residues toward the amino terminal from the trypsin cleavage site in VD IV.

The MAbs that bind to the trypsin-sensitive epitopes in MOMP VDs II and IV did not neutralize infectivity of chlamydiae for HeLa 229 cells. In contrast, these MAbs do neutralize infectivity in vivo (7). This apparent paradox has been difficult to understand. Because these MAbs neutralize infectivity in vivo, the failure to neutralize infectivity in vitro may be a property of HeLa cells and not the antibodies. It is possible that HeLa cells possess receptors for an immunoglobulin(s), and thus, through endocytosis, antibody-bound chlamydiae may infect the cell via these receptors. Such a mechanism would circumvent normal chlamydial-host cell mechanisms of attachment and uptake but may provide an equally functional environment for chlamydial growth. Thus, two mechanisms may exist for attachment of chlamydiae to HeLa cells. One is through binding of chlamydial MOMP to a HeLa cell receptor, and the other occurs in the presence of chlamydia-specific antibodies and may be mediated by immunoglobulin receptors on HeLa cells.

A variety of proteases are present at mucosal surfaces and would theoretically be capable of cleaving sensitive sites within the immunodominant VDs of MOMP. Depending upon the sequence and structure of the serovar-specific epitopes, some serovars may be more susceptible than others to proteolytic cleavage. If cleavage occurs, the immunogenicity of the MOMP could be altered, resulting in the loss of protective immune responses directed at type-specific epitopes. This may allow chlamydiae to escape clearance by the immune response. In addition, individuals infected with serovars whose MOMP serovar-specific epitopes are protease sensitive may not produce antibodies directed at these epitopes. Therefore, serologic profiles of these individuals...
may show antibody responses that are not serovar specific but are directed at more conserved protease-resistant epitopes of MOMP.

Loss of infectivity of chlamydiae following proteolytic cleavage within the variable domains depends upon the sites and numbers of cleavages. Because of the different amino acid sequences within the variable domains, each serovar may differ in loss of infectivity following proteolytic cleavage at specific sites within the variable domains.

The MOMP genes from all serovars for which these proteins have been sequenced have potential trypsin cleavage sites within either VD I or VD II. Protective type-specific epitopes have not been defined for all serovars. Identification and characterization of protective type-specific epitopes and of MOMP-binding domains of other serovars should lead to greater understanding of the immune response to MOMP and of the role of MOMP in attachment to host cells.

Previously, Hackstadt and Caldwell (9) reported that MOMP did not have a role in the binding of chlamydiae to host cells. This conclusion was reached following similar experiments in which serovar L2 MOMP was extensively digested with trypsin without effect on the infectivity of the organisms for HeLa 229 cells. Both studies showed that trypsin treatment had no effect on the infectivity of serovar L2 and that immunoreactivity with MAb L21-45 was maintained at the cell surface after trypsin digestion. The major difference observed between the two studies is the sensitivity of the L21-10 epitope to trypsin. This difference can be explained at least in part by comparing the proteolysis conditions used in each of the experiments. In our initial study, a trypsin/chlamydia ratio of 0.4:1 (wt/wt) was used. These conditions resulted in a more extensive digestion of serovar L2 MOMP, which generated four small immune-reactive fragments that were detected with a monospecific polyclonal antiserum raised against purified serovar L2 MOMP. In this study, a trypsin/chlamydia ratio of 0.04:1 (wt/wt), two MOMP fragments were generated which together accounted for the entire MOMP molecule. We believe that these differences can be explained by the additional cleavage of MOMP at lysine 287 by using higher concentrations of trypsin. This residue occurs in the sequence at the start of VD IV and is 8 amino acid residues before the start of the L21-10 epitope. If MOMP were cleaved with trypsin at this site and at the already identified site in VD IV (lysine 309), the entire L21-10 epitope would be cleaved from the protein. It is likely that this fragment would be totally lost from the cell surface, as it lacks cysteine residues by which disulfide crosslinking would have enabled the small fragment to remain attached to MOMP at the cell surface. Therefore, we now conclude that MOMP may play a role in binding to host cells. The most conclusive evidence for this comes from data accumulated by studying serovar B and comparing these findings with those obtained from studies of serovar L2.

The data presented in this paper suggest that chlamydial MOMP functions in the attachment of chlamydiae to host cells; however, conclusive evidence for the role of MOMP can only be obtained by using purified MOMP which has maintained its native conformation. Because it is unlikely that such a preparation of MOMP will ever be isolated, we are using recombinant and synthetic peptides of the various variable domains of serovar B and L2 MOMP to further investigate the role of MOMP in binding and attachment to host cells.

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