Human Mannose-Binding Protein Inhibits Infection of HeLa Cells by Chlamydia trachomatis

ALBERTINA F. SWANSON,1 R. ALAN B. EZEKOWITZ,2 AMY LEE,1 AND CHO-CHOU KUO1 *

Department of Pathobiology, University of Washington, Seattle, Washington 98195, 1 and Department of Pediatrics, Harvard Medical School, Pediatric Service, Massachusetts General Hospital, Boston, Massachusetts 02114.

Received 5 November 1997/Returned for modification 6 January 1998/Accepted 27 January 1998

The role that collectin (mannose-binding protein) may play in the host's defense against chlamydial infection was investigated. Recombinant human mannose-binding protein was used in the inhibition of cell culture infection by Chlamydia trachomatis (C/TW-3/OT, E/UW-5/Cx, and L2/434/Bu), Chlamydia pneumoniae (AR-39), and Chlamydia psittaci (6BC). Mannose-binding protein (MBP) inhibited infection of all chlamydial strains by at least 50% at 0.098 μg/ml for TW-3 and UW-5, and at 6.25 μg/ml for 434, AR-39, and 6BC. The ability of MBP to inhibit infection with strain L2 was not affected by supplementation with complement or addition of an L2-specific neutralizing monoclonal antibody. Enzyme-linked immunosorbent assay and dot blot analyses showed MBP bound to the surface of the organism to exert inhibition, which appeared to block the attachment of radiolabeled organisms to HeLa cells. Immunoblotting and affinity chromatography indicated that MBP binds to the 40-kDa glycoprotein (the major outer membrane protein) on the outer surface of the chlamydial elementary body. Hapten inhibition assays with monosaccharides and defined oligosaccharides showed that the inhibitory effects of MBP were abrogated by mannose or high-mannose type oligomannose-oligosaccharide. The latter carbohydrate is the ligand of the 40-kDa glycoprotein of C. trachomatis L2, which is known to mediate attachment, suggesting that the MBP binds to high mannose moieties on the surface of chlamydial organisms. These results suggest that MBP plays a role in first-line host defense against chlamydial infection in humans.

Chlamydia is an obligate intracellular bacterium that infects mammalian and avian species. The species Chlamydia trachomatis is a major cause of ocular and genital infection in humans. Pathogenic microorganisms may use carbohydrates or carbohydrate-binding proteins to attach to or enter host cells (44). We have identified three glycoproteins in C. trachomatis with molecular weights of 40,000 (40k), 32k, and 18k (35–37) and determined that the 40-kDa glycoprotein is the major outer membrane protein (MOMP) (36). Attachment of C. trachomatis to HeLa cells appears to be mediated via the glycans that decorate the 40-kDa and the 32-kDa outer surface glycoproteins (38, 39). However, only the exogenously added glycan of the 40-kDa MOMP glycoprotein inhibited the infectivity of C. trachomatis to HeLa cells. The presence of mannose has been detected in all three glycoproteins, in addition to galactose, N-acetyl glucosamine, and fucose (36, 37). We have recently elucidated the carbohydrate structure of the C. trachomatis L2 strain by two-dimensional sugar mapping technique, showing that the major oligosaccharide component (>80%) of the 40-kDa MOMP glycoprotein was the high-mannose type oligomannose-oligosaccharide (20). Functional analysis, with structurally defined oligosaccharides, revealed that the oligomannose-oligosaccharides were the ligands mediating attachment and infectivity of C. trachomatis to HeLa cells.

Mannose-binding protein (MBP) is a collectin with collagen and lectin domains (5, 6). It is a mammalian C-type lectin that is synthesized by hepatocytes and secreted into circulating serum at low levels (8). Its production increases in response to stress, like trauma, surgery, and infection (33, 42). MBP appears to be a pattern recognition molecule that plays a role in first-line host defense. MBP may be considered an “ante-anti-body” (7). MBP recognizes a wide range of microorganisms (6). MBP is also known to activate both the classical (11, 13, 22) and alternate complement pathways (30). The three-dimensional structure of the MBP carbohydrate recognition domain bound to a ligand reveals that the oligosaccharides bind to a calcium site and that the calcium ion binds to the equatorial 3-0H and 4-0H of the terminal mannose (43). Classical complement pathway activation can occur in the absence of antibody. This lectin pathway utilizes two novel serum proteases, mannose-binding protein-associated protease I (26) and II (40a). Alternatively, MBP may act directly as an opsonin for phagocytosis of bacteria by macrophages (15).

Because surface-exposed glycoproteins of C. trachomatis are rich in mannose and involved in infectivity (20), we examined whether infection of HeLa cells by C. trachomatis and other chlamydial species is inhibited by human MBP (hMBP) and analyzed its inhibitory mechanisms.

MATERIALS AND METHODS

Chlamydial organisms. C. trachomatis C/TW-3/OT, E/UW-5/Cx, and L2/434/Bu, Chlamydia pneumoniae AR-39, and Chlamydia psittaci 6BC were used. TW-3, UW-5, and 434 were grown in HeLa 229 cells (17). AR-39 and 6BC were grown in HL cells (18). All organisms were purified by density gradient centrifugation using Hypaque (Hypaque-76; Sanofi Winthrop Pharmaceuticals, New York, N.Y.) (16). The preparations contained at least 106 inclusion-forming units per ml of elementary bodies (EBs).

Human MBP. Recombinant hMBP (rhMBP) was produced from a Chinese hamster's ovary cells transfected with the cloned hMBP gene as previously described (15). Recombinant protein was purified from the culture supernatant by elution in a mannan-Sepharose affinity column with 50 mM mannose in Tris-buffered saline (TBS) plus 10 mM CaCl2.

Carbohydrates. Glycopeptides containing high-mannose type and hybrid type carbohydrates from ovalbumin were prepared by fractionation with a concanavalin A-Sepharose column (Pharmacia AB, Uppsala, Sweden) according to Krusius et al. (14). Structurally defined oligomannose-oligosaccharides containing 6, 8, or 9 mannose residues and a conserved trimannosyl core were obtained from Oxford GlycoSystems, Rosedale, N.Y. These oligosaccharides are analogs to those found in the C. trachomatis L2 strain (20). Oligomannose S,D1D3 has shown the highest activity in infectivity neutralization assays, while the tri-
In testing the experiments as to whether there was an additive effect of MBP and mannosyl core has shown the least activity (20). Monosaccharides, D-mannose and D-fructose, were obtained from Sigma Chemical Co., St. Louis, Mo.

**Antibodies.** Monoclonal antibodies used were anti-rhMBP (31), anti-C. trachomatis MOMP, which is specific against L.2 serovar (155-35), anti-C. trachomatis MOMP, which is species-specific (KK-12), and antichlamydial lipopolysaccharide, which is genus-specific (CF-2). 155-35 is a neutralizing antibody, but KK-12 and CF-2 are nonneutralizing, all of which have been described (25, 38).

**Inhibition of cell culture infection by chlamydial organisms with mannosyl-binding protein.** Assays of inhibition of cell culture infectivity by MBP were performed by using HeLa 229 (TW-3, UW-5, and 434) or HL (AR-39 and 6BC) cell monolayers. MBP was preincubated with 96-well microtiter plates in a fourfold series in a 96-well plate so that the final volume in each well for each dilution contained 90 μl. An equal volume of 2 × 10⁴ inclusion forming units/ml of organism suspension was added to each well. The range of MBP concentra-

**Tests for calcium dependency of MBP.** Two methods were used to test for calcium dependency of MBP: omission of 2 mM CaCl₂ from the buffer or chelation of CaCl₂ with 2 mM EDTA.

**Hapten inhibition.** MBP was preincubated with D-mannose or D-fructose (100 and 10 mM) for 1 h at room temperature before reacting with organisms in the infectivity assay or applying to nitrocellulose paper or enzyme-linked immuno-

**Radiolabeling of chlamydial organisms.** Chlamydial organisms were metabolically labeled by culturing with low leucine (1/10 of the normal concentration)-radioactivity was counted in a scintillation counter (LS-5800 series, Liquid Scin-

**Inhibition of attachment of titrated chlamydial organisms to HeLa cells by mannos-binding protein.** The assay for inhibition of attachment of titrated chlamydial organisms to HeLa cell monolayers in duplicate and incubated at room temperature for 30 min. MBP/mannose mixtures were incubated onto HeLa cell monolayers in duplicate and incubated at room temperature for 30 min. Inocula were removed and cell monolayers were washed three times with TBS. One milliliter of tissue solubilizer (Amersham, Arlington Heights, Ill.) was added per vial and incubated at room temperature overnight. Digested tissue suspension was dissolved in 10 ml of scintillation fluid (Amersham), and the radioactivity was counted in a scintillation counter (LS-5800 series, Liquid Scintilla-

**Inhibition of infectivity with MBP.** Inhibitory effects of MBP on cell culture infections by Chlamydia spp. with rhMBP

**Peroxidase Substrate System:** Kirkegaard & Perry Laboratories, Gaithersburg, Md.) was added to each well. The plate was read at 405 nm on a Thermomax microplate reader ( Molecular Devices Corp., Sunnyvale, Calif.). Slightly different parameters were used for the final washings: The protein bands were visualized with 4-chloro-1-naphthol as substrate.

**RESULTS**

**TABLE I. Inhibition of cell culture infection by Chlamydia spp. with rhMBP**

<table>
<thead>
<tr>
<th>Strains</th>
<th>MIC₅₀ of rhMBP (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. trachomatis</td>
<td>0.098</td>
</tr>
<tr>
<td>C/TW-3/OT</td>
<td>0.098</td>
</tr>
<tr>
<td>C/1/UW-5/Cx</td>
<td>6.25</td>
</tr>
<tr>
<td>L2/343/Ba</td>
<td>6.25</td>
</tr>
<tr>
<td>C. pneumoniae</td>
<td>6.25</td>
</tr>
<tr>
<td>AR-39</td>
<td>6.25</td>
</tr>
<tr>
<td>C. psittaci</td>
<td>6.25</td>
</tr>
<tr>
<td>6BC</td>
<td>6.25</td>
</tr>
</tbody>
</table>

“Repeated tests gave identical endpoints.”

**Peroxidase Substrate System:** Kirkegaard & Perry Laboratories, Gaithersburg, Md.) was added to each well. The plate was read at 405 nm on a Thermomax microplate reader ( Molecular Devices Corp., Sunnyvale, Calif.). Slightly different conditions were used for the final washings: the 0.05% Tween 20 was used for blocking, and MBP was absorbed at room temperature overnight.

**Dot blot.** Five microliters of EB suspensions were applied onto nitrocel-

**Immunoblotting.** Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) followed by immunoblotting for detection of specific chlamyd-

**Determination of chlamydial proteins that bind to mannose-binding protein.** (i) **Immunoblotting.** Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) followed by immunoblotting for detection of specific chlamyd-

**Inhibition of infectivity with MBP.** Inhibitory effects of MBP on cell culture infections by Chlamydia spp. were examined. As shown in Table 1, all chlamydial strains tested were susceptible to inhibition by MBP. Differences in susceptibility among chlamydial strains were observed. The trachoma biovar of C. trachomatis was more sensitive than the lymphogranu-

**RESULTS**

**Inhibition of infectivity with MBP.** Inhibitory effects of MBP on cell culture infections by Chlamydia spp. were examined. As shown in Table 1, all chlamydial strains tested were susceptible to inhibition by MBP. Differences in susceptibility among chlamydial strains were observed. The trachoma biovar of C. trachomatis was more sensitive than the lymphogranu-

**Downloaded from http://iai.asm.org/ on October 14, 2017 by guest**
inhibition, which was similar to untreated organisms. As expected, calcium depletion also abolished the inhibitory effect of MBP on infectivity. Inhibition of infectivity by MBP was only 29% without calcium versus 67% with calcium.

Supplementation with complement produced only a single fourfold dilution difference in the endpoint. The inhibitory concentration of MBP was 1.56 μg/ml in the presence of complement, compared to 6.25 μg/ml in the absence of complement. However, this difference was not statistically significant (P > 0.05).

Whether MBP would augment complement-dependent antibody neutralization of infectivity was also examined. The same protocol with and without complement was followed. MBP at an inhibitory concentration of 6.25 μg/ml did not enhance antibody neutralization of infectivity. The average percent inhibition of two experiments in the absence of complement was 32% for antibody alone, 62% for MBP alone, and 65% for antibody plus MBP. When complement was added the percent inhibition was 64, 73, and 81%, respectively.

**Inhibition of attachment of chlamydial organisms to HeLa cells with MBP.** Whether MBP inhibits the attachment of organisms to HeLa cells was examined. The inhibition assays were performed using tritium-labeled organisms. As shown in Table 2, attachment of chlamydial EBs to HeLa cells was inhibited by greater than 50% by MBP at 100 and 25 μg/ml. These results suggest that MBP inhibits infectivity by inhibiting attachment of organisms to the host cell surface.

**Binding of MBP to whole chlamydial organisms.** Whether MBP binds to the surface of chlamydial organisms to exert inhibition was determined by ELISA (Table 3) and dot blot (Fig. 1) by using UW-5, L2, and AR-39 strains. Both tests showed that MBP bound to the surface of organisms.

**Identification of chlamydial proteins that bind to MBP.** Three different methods were used to determine that the chlamydial protein that binds to MBP was the MOMP. First, a hapten inhibition assay was conducted to see whether MBP binds to mannose on the glycoprotein of the bacterial cell surface. The results showed that the binding of MBP to EB was inhibited effectively by mannose (Fig. 2). Oligomannose-oligosaccharides found in the 40-kDa MOMP glycoprotein also inhibited the binding of MBP to EB (Fig. 3). Nearly complete inhibition was observed at concentrations of 50 ng or greater. As previously seen in inhibition of infectivity (20), mannose-8,D1D3 was the most effective oligosaccharide tested. Binding of anti-MOMP monoclonal antibody KK-12 to EB was not inhibited by mannose (Fig. 2).

Second, immunoblotting was used to identify which chlamydial proteins bind to MBP. Immunoblotting of whole EB lysates revealed that only the 40-kDa MOMP bound consis-
The MBP is a pattern recognition molecule that appears to play a role in first-line host defense against bacteria, yeast, viruses, and protozoans rich in mannose (9, 12, 15, 29, 30, 33). MBP has also been detected in human amniotic fluid (24) and secretions of the upper respiratory tract, i.e., secretions of the nasopharynx and effusions of the middle ear in children (10). Interestingly, antimicrobial activity of amniotic fluid against C. trachomatis has been reported (41). The MIC at which 50% of the isolates are inhibited (MIC \(_{50}\)) for the trachoma biovar of C. trachomatis is within the range of the normal concentrations of MBP in amniotic fluids (0.304 \(\mu\)g/ml [0.084 to 0.64 \(\mu\)g/ml] at 32 weeks of gestation and 1 \(\mu\)g/ml [0.048 to 2.282 \(\mu\)g/ml] at 35 weeks of gestation) (24). Therefore, it seems imperative to study how MBP in these body fluids affects the outcome of infection of fetuses and newborns by mothers having cervical C. trachomatis infection, since maternal infection often results in fetal death or premature birth (25) and infantile pneumonia (1).

MBP may also work in the line of defense against hematogenous dissemination of chlamydia in humans. Systemic disease is common in C. psittaci infection in animals, especially in avians, but not in non-LGV serovars of C. trachomatis. Recent studies suggest that C. pneumoniae spreads systemically via infected macrophages in humans and animals. In humans, C. pneumoniae has been detected in cervical lymph nodes, spleen, and liver. A few case studies showed severe systemic manifestation (19). The detection of C. pneumoniae in 50% of atheromatous plaques of major arteries has presented the strongest evidence for systemic disease caused by C. pneumoniae (19). In mice, C. pneumoniae has been shown to disseminate readily from lungs following intranasal inoculation to spleen and peritoneal macrophages in Swiss Webster mice (45) and to atheromatous lesions in the aorta in ApoE mice (27). When systemic infection with C. pneumoniae occurred, bacteremia was seen, but the organisms were only found intracellularly in mononuclear phagocytes (28). These are exciting findings because bacteremia has been rarely demonstrated in...
human chlamydial infection. When it is demonstrated, as has been observed in psittacosis, a disease contracted from *C. psittaci*-infected birds, the organisms are usually recovered from the buffy coat (32). The observations that systemic dissemination of chlamydia is by infected macrophages and not by free particles suggest a possible role of serum MBP in inactivating infectivity of chlamydia if the organism gets into circulation in a free form. This hypothesis is supported by the observations of this study and the fact that the normal serum levels of MBP (0.995 μg/ml [0.01 to 4.47 μg/ml] (21, 34) to 1.67 μg/ml [0.28 to 8.67 μg/ml] (10) in Caucasians and 1.645 μg/ml [1.395 to 2.065 μg/ml] (21) to 1.72 ± 1.15 μg/ml (40) in Asians), are inhibitory in vitro. The lack of observation of systemic spread of non-LGV *C. trachomatis* coupled to the systemic spread of *C. psittaci* and *C. pneumoniae* may be correlated with sensitivity to the MBP.

MBP effectively inhibited *C. trachomatis* infection of HeLa cells in the presence and absence of complement. However, MBP did not enhance the neutralization of chlamydial infectivity with antibodies. It is possible that any small inhibitory effect by MBP on chlamydial infection was masked by the large effect of complement-dependent antibody neutralization. These findings show chlamydia is another infectious agent against which MBP could play a role in host defense infection (7). Because MBP may activate complement and facilitate the phagocytosis and killing of microorganisms, further in vitro studies should be conducted with polymorphonuclear and mononuclear phagocytic cells.

In conclusion, we have shown that MBP may play a role in the first-line host defense against chlamydia. The experimental data support our previous structural and functional analyses showing the presence of a specific high-mannose type oligosaccharide linked to the 40-kDa major outer membrane protein of *C. trachomatis* which mediates attachment and infectivity of the organism to HeLa cells (20). We have also demonstrated that the differential sensitivity to inhibition by MBP among chlamydial strains may be correlated with the ability of chlamydial strains to disseminate systematically by the hematogenous route.

ACKNOWLEDGMENT

This work was supported by Public Health Service grant EY00219 from the National Eye Institute.

REFERENCES


28. Suppan, A. F., and C.-C. Kuo. 1991. Evidence that the major outer membr...


