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A recombinant *Chlamydia trachomatis* major outer membrane protein binds to heparan sulfate receptors on epithelial cells

(chlamydiae/pathogenesis/cytoadhesin/glycosaminoglycan receptor)

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ABSTRACT **Chlamydial attachment to columnar conjunctival or urogenital epithelial cells is an initial and critical step in the pathogenesis of chlamydial mucosal infections. The chlamydial major outer membrane protein (MOMP) has been implicated as a putative chlamydial cytoadhesin; however, direct evidence supporting this hypothesis has not been reported. The function of MOMP as a cytoadhesin was directly investigated by expressing the protein as a fusion with the *Escherichia coli* maltose binding protein (MBP–MOMP) and studying its interaction with human epithelial cells. The recombinant MBP–MOMP bound specifically to HeLa cells at 4°C but was not internalized after shifting the temperature to 37°C. The MBP–MOMP competitively inhibited the infectivity of viable chlamydiae for epithelial cells, indicating that the MOMP and intact chlamydiae bind the same host receptor. Heparan sulfate markedly reduced binding of the MBP–MOMP to cells, whereas chondroitin sulfate had no effect on binding. Enzymatic treatment of cells with heparitinase but not chondroitinase inhibited the binding of MBP–MOMP. These same treatments were also shown to reduce the infectivity of chlamydiae for epithelial cells. Mutant cell lines defective in heparan sulfate synthesis but not chondroitin sulfate synthesis showed a marked reduction in the binding of MBP–MOMP and were also less susceptible to infection by chlamydiae. Collectively, these findings provide strong evidence that the MOMP functions as a chlamydial cytoadhesin and that heparan sulfate proteoglycans are the host-cell receptors to which the MOMP binds.**

Chlamydia trachomatis is a prokaryotic obligate intracellular parasite. Chlamydial infections are a major cause of sexually transmitted disease (1) and a leading cause of preventable blindness (2). The initial event in chlamydial infection is the attachment of the organism to epithelial cells. Identification of chlamydial surface components that mediate attachment to host cell and the receptors that chlamydial ligands bind is key to understanding the pathogenesis of chlamydial infection. Definition of the chlamydial cytoadhesin should provide a rational target for development of much needed immunological or chemotherapeutic intervention strategies for the prevention of chlamydial infection.

Ligands that function in the interaction of bacterial pathogens with eukaryotic cells have been identified largely through the use of genetic studies (3). Genetic systems that allow for mutation or deletion of genes have not been developed for chlamydiae; therefore, studies to identify chlamydial cytoadhesins have utilized indirect assays. These studies have implicated the major outer membrane protein (MOMP) (4) or its associated glycan moiety (5), as well as other chlamydial outer membrane or surface components (6–10), as potential chlamydial cytoadhesins.

Heparan sulfate (HS) is a ubiquitous cell surface linear heteropolysaccharide composed of alternating uronic acid and glucosamine residues that are variably N- and O-sulfonated. The negatively charged sulfonated carbohydrate residues, termed glycosaminoglycans (GAGs), are usually covalently anchored to the plasma membrane via a transmembrane core protein or glycosylphosphatidylinositol (11). The ectodomains of HS proteoglycans bind to a large spectrum of cellular ligands including matrix components, growth factors, lipolytic enzymes, protease inhibitors, and transcriptional regulators (11). HS proteoglycans also function as receptors for a variety of infectious agents. Viruses that utilize HS receptors include herpes simplex (12), cytomegalovirus (13), pseudorabies (14), varicella zoster (15), and retroviruses (16). The protozoan parasite *Trypanosoma cruzi* (17) and bacterial mucosal pathogen *Neisseria gonorrhoeae* (18) have also been shown to bind HS proteoglycan receptors. Thus, it is evident that a diverse group of infectious agents has evolved common strategies that utilize HS proteoglycans as receptors in their interaction with eukaryotic host cells. Herein we present evidence that the chlamydial MOMP functions as a ligand in the attachment of chlamydiae to epithelial cells and that the receptor to which the MOMP binds is HS.

MATERIALS AND METHODS

Cell Lines and Chlamydial Strains. The *C. trachomatis* strain MoPn was grown in human cervical epithelial cells (HeLa 229) and elementary bodies (EBs) were purified as described (19). Chinese hamster ovary (CHO-K1) cells and the mutant CHO lines *psgD*-606, *psgE*-677, and *psgA*-745 were provided by J. Esko (University of Alabama at Birmingham). The phenotypic characteristics of these cell lines has been described (20–22).

Production of Maltose Binding Protein–MOMP (MBP–MOMP) Fusion Protein. Oligonucleotides (5′-CTGCCTGTGGGGAATCCTGC-3′; 5′-GGCGTCGACTTAGAAGCGGAATTGTGCATTTACGTG-3′) were designed that allowed amplification of the *ompI* sequence coding for the predicted mature MOMP polypeptide (aa 23–387) for fusion with the *Escherichia coli malE* gene. Purified *Sal*I-digested PCR product was cloned into *Xmn*I/*Sal*I-digested pMAL-c2 vector (New England Biolabs) and transformed into *E. coli* DH5α. A clone expressing the fusion protein (DH5α{pMMM3})was identified by colony blot, using mAb 33b as probe. Both the MBP–MOMP fusion protein

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Abbreviations: MOMP, major outer membrane protein; MBP–MOMP, maltose binding protein–MOMP; EB, elementary body; GAG, glycosaminoglycan; HS, heparan sulfate; IFA, indirect fluorescent antibody; SEM, scanning electron microscopy; IFU, inclusion forming unit(s); CS, chondroitin sulfate; 2-ME, 2-mercaptoethanol.

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and the control MBP (produced by DH5α{pMAL-c2}) were purified from lysates of *E. coli* as described (23).

Indirect Fluorescent Antibody (IFA) Staining. HeLa 229 cells were grown on glass coverslips in 24-well tissue culture plates for 24 hr at 37°C. The monolayers were washed three times with ice-cold Hanks' balanced salt solution containing 10 mM Hepes (HBSS) and then placed on ice for 30 min. MBP–MOMP or MBP was diluted in ice-cold 10 mM sodium phosphate/0.25 M sucrose/5 mM glutamic acid, pH 7.2 (SPG). A 200-μl volume of the proteins was inoculated onto monolayers and the plates were placed on ice on a rocker platform for 1 hr. The inoculum was removed, and the cells washed three times with ice-cold HBSS and were either examined as viable or fixed cells by IFA staining. Living cells were incubated with rabbit anti-MBP serum for 30 min on ice. The monolayers were washed five times with chilled HBSS, incubated for 30 min on ice with fluorescein isothiocyanate-conjugated goat anti-rabbit IgG, and washed an additional five times. Viable stained cells were fixed by sequential treatment with ice-cold 50% acetone (1 min), 100% acetone (5 min), and 50% acetone (1 min) and examined for fluorescence (24). When cells were fixed before IFA staining, they were washed as described above, fixed for 10 min with methanol, washed three times with PBS, and stained as described for living cells.

Scanning Electron Microscopy (SEM). MBP or MBP–MOMP was fixed on 5 × 7 mm silicon wafer chips with 0.5% glutaraldehyde in 0.1 M sodium cacodylate (pH 7.4) for 30 min at room temperature. HeLa cells grown on glass coverslips were incubated with MBP or MBP–MOMP as described above for IFA staining. The chips or HeLa cells were washed with PBS and incubated with rabbit anti-MBP, anti-MOMP, or normal rabbit sera diluted 1:100 in PBS containing 2% bovine serum albumin (BSA) at 37°C for 1 hr. Specimens were washed with PBS and incubated at 37°C for 1 hr with goat anti-rabbit IgG conjugated with 15-nm gold (BB International, Cardeff, U.K.). The cells were fixed with 2.5% glutaraldehyde in 0.1 M sodium cacodylate at 4°C for 16 hr. Specimens were postfixed in 1% aqueous osmium tetroxide, dehydrated in a graded series of ethanol washes (50–100%), and dried in a Blazers critical point dryer. The preparations were mounted on aluminum studs, sputter-coated with chromium (110 Å), and viewed with a Hitachi S-4500 Cold Field Emmissison SEM (Hitachi) in either secondary or backscatter imaging modes.

Infectivity Competition Assays. A 200-μl volume of MBP–MOMP, MBP, or ultraviolet (UV)-inactivated EBs (0.24 J/cm^2) were inoculated onto HeLa cell monolayers for 1 hr at 4°C. The concentration of UV-inactivated EBs is expressed as inclusion forming units (IFU) equivalents, which were determined from the IFU concentrations of stock chlamydial preparations prior to inactivation. A 50-μl volume of viable EBs (1.5 × 10^6 IFU) was added to the wells and incubated for an additional 1 hr at 4°C. The monolayers were washed, fed with minimum essential medium supplemented with 10% fetal bovine serum (MEM-10), and incubated at 37°C for 30 hr. Cells were fixed and chlamydial IFUs were quantified after IFA staining. In this and all subsequent assays, incubation of EBs with cells were done at 4°C; a temperature at which EBs adhere to cells but are not internalized. Thus, although infectivity was the read-out, only the binding of intact EBs to cells was being measured experimentally.

MBP–MOMP Binding Assay. An antibody-protein A assay described by Zhu *et al.* (15) was used to quantitatively assess MBP–MOMP binding to HeLa cells. Briefly, cells were washed, chilled, and inoculated with 200 μl of diluted MBP–MOMP or MBP. The plates were incubated on ice for 1 hr. The monolayers were washed three times in ice-cold HBSS, and 200 μl of rabbit anti-MBP or normal rabbit serum (1:100 dilution) in HBSS containing 2% BSA was added to the wells. Plates were incubated at 4°C for 1 hr and the cells washed with ice-cold HBSS. A 200-μl volume of ^{125}I-labeled protein A (2 × 10^5 cpm) was added to the wells and the plates were incubated on ice for 1 hr. The cells were washed and lysed with 0.5 ml of 1% SDS. Radioactivity was determined by counting in a gamma counter (Beckman). The effect of temperature and reduction on MBP–MOMP binding was assayed after incubation of MBP–MOMP at 4, 37, or 56°C for 1 hr in the presence or absence of 10 mM dithiothreitol (DTT).

Inhibition Assays and Enzyme Treatment. MBP–MOMP was mixed with different concentrations of heparin, chondroitin sulfate C (Sigma), or HS IV (Celsus Laboratories, Cincinnati) and inoculated onto chilled cells. The cells were incubated at 4°C for 1 hr and MBP–MOMP binding was assayed as described above. Chlamydial EBs were diluted in SPG containing different concentrations of heparin, HS, or chondroitin sulfate (CS), and inoculated onto chilled cells (1.5 × 10^6 IFUs per well). After a 1-hr incubation at 4°C, cells were washed, fed, incubated at 37°C for 30 hr, and assayed for chlamydial IFUs. HeLa cells and chlamydial EBs were treated with heparinase III (heparitinase I) or chondroitinase ABC (Sigma) in HBSS containing 0.1% BSA at 37°C for 3 hr. Enzyme-treated cells were washed three times, chilled, inoculated with MBP–MOMP or EBs, and incubated on ice for 1 hr. Enzyme-treated EBs were inoculated directly onto washed chilled cells and incubated at 4°C for 1 hr.

RESULTS

Characterization of Recombinant MOMP. MBP–MOMP was not cleaved to produce monomer MOMP after digestion with factor Xa protease; thus, all subsequent experiments were done using the intact fusion protein. MBP–MOMP was analyzed by SDS/PAGE to assess purity and to ascertain if the recombinant protein was disulfide cross-linked since it contains eight cysteine residues and exists naturally as a disulfide cross-linked homopolymer at the chlamydial cell surface (25, 26). Solubilization of MBP-MOMP in the presence of 2-mercaptoethanol (2-ME) showed a 80-kDa polypeptide consistent with the predicated mass of both MBP (42 kDa) and MOMP (39 kDa) (Fig. 1*A*, lane 2). In the absence of reduction, the recombinant protein migrated as a polypeptide with a mass greater than 250 kDa (Fig. 1*A*, lane 4), suggesting that the recombinant MOMP was disulfide cross-linked. Purified MBP migrated as a 50-kDa polypeptide (42-kDa MBP plus the 8-kDa LacZ α peptide) when reduced and as a 100-kDa polypeptide in the absence of reduction (Fig. 1*A*, lanes 1 and 3), the reasons for which are not clear since neither the MBP nor α peptide contain cysteine residues. Both MBP and MBP–MOMP were found to be insoluble after centrifugation at 100,000 × *g* (Fig. 1*A*, lanes 5–8), indicating that each exists as high molecular weight aggregates.

MBP and MBP–MOMP were examined by SEM to determine whether the proteins exhibited any identifiable structural characteristics. SEM showed that both purified MBP and MBP–MOMP exist as colloidal particles with a diameter of 30–40 nm that reacted specifically with monospecific antisera against MBP or MOMP, respectively (Fig. 1*B a–d*). The particles were not immunoreactive after staining with normal rabbit sera (data not shown). Thus, the aggregate nature responsible for the formation of the colloidal particles is a property of the MBP and not the MOMP. Nevertheless, the MOMP is accessible on the particle surface as shown by immunostaining and is present to at least some degree as a disulfide cross-linked oligomer.

Interaction of MBP–MOMP with Epithelial Cells. MBP–MOMP incubated at 4°C with HeLa cells and stained with anti-MBP serum showed a fine punctate fluorescent staining pattern that was uniformly distributed over the cell surface (Fig. 2*a*). By SEM the MBP–MOMP appeared as aggregates of particles that localized to distinct cellular focal contact points (Fig. 2*e*), which were clearly identified following im-

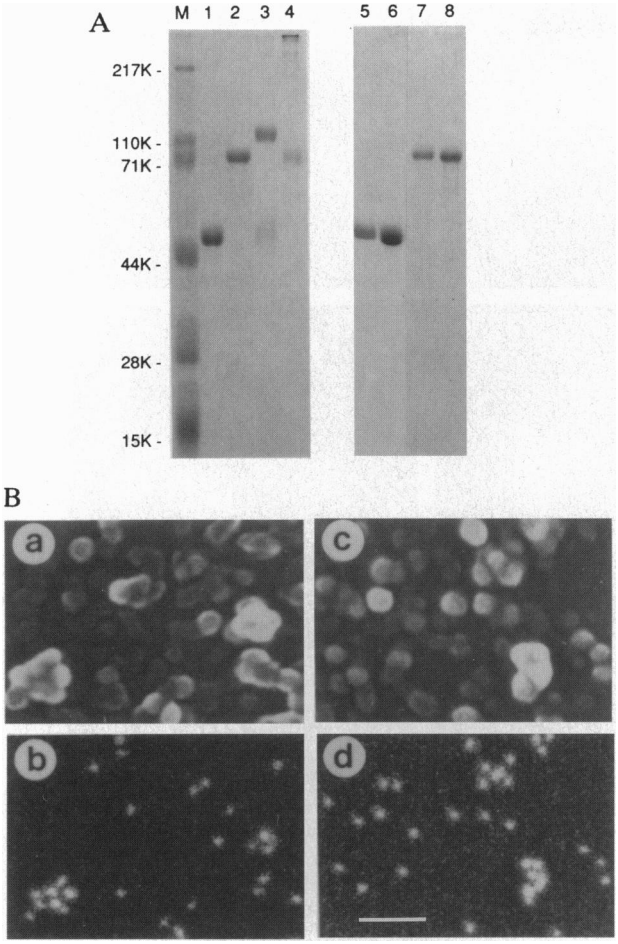

FIG. 1. Characterization of recombinant MBP–MOMP. (*A*) SDS/PAGE of purified MBP and MBP–MOMP solubilized in the presence and absence of 2-ME and after centrifugation at 100,000 × *g*. Lanes: 1, MBP with 2-ME; 2, MBP–MOMP with 2-ME, 3, MBP without 2-ME; 4, MBP–MOMP without 2-ME. Lanes 5–8 contain 100,000 × *g* supernatant and pelleted material. Lanes: 5, MBP supernatant; 6, MBP pellet; 7, MBP–MOMP supernatant; 8, MBP–MOMP pellet. (*B*) SEM of purified MBP and MBP–MOMP. (*a*) Secondary imaging of purified MBP–MOMP. (*b*) Backscatter imaging of *a* reacted with rabbit anti-MOMP serum and goat anti-rabbit IgG conjugated to colloidal gold. (*c*) Secondary imaging of purified MBP. (*d*) Backscatter imaging of *c* reacted with rabbit anti-MBP serum and goat anti-rabbit IgG conjugated to gold. (Bar = 100 nm.)

munogold staining (Fig. 2*f*). Thus, the punctate staining pattern observed by IFA staining reflects aggregates of cell surface MBP–MOMP particles not individual particles. To determine whether bound MOMP could be internalized, the temperature of the cells was shifted to 37°C and the fate of the MOMP was followed temporally by IFA staining. Methanol-fixed and viable cells were similarly stained and compared to differentiate internalized from surface-associated MBP–MOMP. The staining pattern for viable and fixed cells was similar at each time period; therefore, only the results of viable cells are shown. Twenty minutes after shifting the temperature, MOMP staining changed from a fine punctate pattern to an aggregate staining pattern (Fig. 2*b*). Surface aggregation of the MOMP was even more pronounced 3 hr after shifting the temperature (Fig. 2*c*). MBP particles were not found to bind to cells by either IFA staining or SEM (Fig. 2 *d* and *g*). A MalE fusion protein, termed MBP-IncA, that consists of MBP fused to the 39-kDa chlamydial inclusion membrane protein (27), was tested as an additional negative control and was found not to bind to cells. These findings clearly support a role for the specific binding of MOMP to eukaryotic cells; however, they indicate that the recombinant protein is not internalized after interaction with its receptor.

The Effect of Temperature on MBP–MOMP Binding. To further study the interactions of MBP–MOMP, an antibody-protein A assay was utilized to quantitatively measure MBP–MOMP binding to cells. MBP–MOMP bound to HeLa cells with dose-dependent kinetics (Fig. 3*A*). Saturable binding was achieved with MBP–MOMP at 100 μg/ml. The attachment of intact chlamydiae to eukaryotic cells is markedly affected by moderate heat treatment or exposure to reducing agents (25, 28, 29); therefore, MBP–MOMP was exposed to moderate heat treatment and reduction, and their effects on binding were studied. Incubation of MBP–MOMP at 37°C reduced binding by as much as 67% and exposure of MBP–MOMP to 56°C reduced binding to less than 15% of that observed for controls (Fig. 3*B*). The requirement of MOMP disulfide bonds for binding was examined by treatment of MBP–MOMP with 10 mM DTT at 4 and 37°C for 1 hr. SDS/PAGE of DTT-treated MBP–MOMP showed that the recombinant protein was reduced to its monomeric form (data not shown). This treatment did not affect MOMP binding to cells nor did the combination of reduction and heat treatment reduce binding to a greater degree than that observed for heat treatment alone (data not shown). Parallel infectivity experiments were also performed using heat-treated EBs. These experiments showed a similar thermolability for intact organisms (Fig. 3*B*) and suggest that a thermolabile conformation of the MOMP may be a prerequisite for its binding to cells.

Competitive Inhibition of Chlamydial Infectivity by MBP–MOMP. To evaluate the biological relevance of MBP–MOMP binding to host cells, we conducted infectivity inhibition assays with the recombinant protein and UV-inactivated chlamydiae. UV-inactivated chlamydiae retain their ability to attach to cells but are not infectious (30). There was a marked dose-dependent inhibition of infectivity by both UV-inactivated chlamydiae and MBP–MOMP (Fig. 4). Approximately equal levels of inhibition of infectivity (50–60% reduction) were achieved with MBP–MOMP and UV-inactivated EBs. These findings suggest that the MOMP and viable EBs compete for the same host cell receptor.

Inhibitors of MBP–MOMP Binding. Previous work by Zhang and Stephens (7) has shown that binding of chlamydiae to eukaryotic cells is inhibited by heparin or HS. It therefore was of interest to correlate these findings with the binding of MBP–MOMP to cells. Heparin and HS were potent competitive inhibitors of MBP–MOMP binding (Fig. 5*A*). Maximal inhibition of MBP–MOMP binding was observed with heparin or HS at as little as 1 μg/ml. In contrast, CS had no effect on the binding of MBP–MOMP to cells. Similarly, both heparin and HS were effective inhibitors of chlamydial infectivity, whereas CS was ineffective (Fig. 5*B*).

Effect of Enzyme Treatment on MBP–MOMP Binding. To determine whether inhibition of the binding of MBP–MOMP and chlamydial infectivity by heparin and HS was the result of GAG interaction with a MOMP ligand or HeLa cell components, intact EBs or HeLa cells were treated separately with heparitinase and chondroitinase. The binding of MBP–MOMP was markedly reduced after treatment of HeLa cells with heparitinase (Fig. 6*A*). Treatment of HeLa cells with chondroitinase had no effect on the binding of MBP–MOMP. Similarly, heparitinase treatment of HeLa cells also had a marked effect on chlamydial infectivity, whereas chondroitinase treatment had no such effect (Fig. 6*B*). Treatment of intact EBs with either enzyme did not have any effect on their infectivity for HeLa cells (Fig. 6*C*).

MBP–MOMP Binding to GAG-Deficient Cell Lines. To further examine the role of host cell surface HS proteoglycans as receptors for MBP–MOMP particles, we conducted MBP–MOMP binding and chlamydial infectivity studies using CHO-K1 cells and CHO cell mutants. MBP–MOMP exhibited

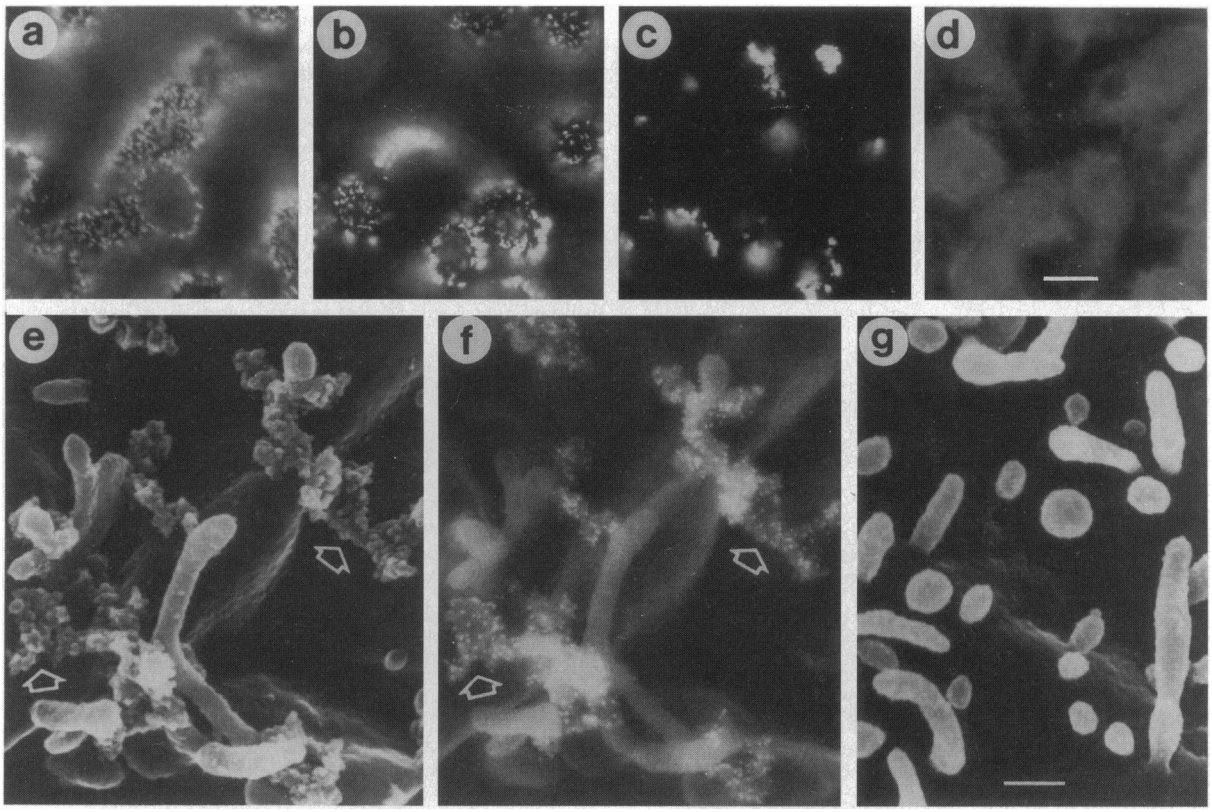

FIG. 2. Interactions of MBP–MOMP with HeLa cells. IFA staining and SEM of HeLa cells inoculated with MBP–MOMP and stained with rabbit anti-MBP serum. (*a–d*) IFA staining of viable cells. (*a*) Staining after incubation at 4°C for 1 hr. (*b*) Twenty minutes after shifting temperature to 37°C. (*c*) Three hours after shifting temperature to 37°C. (*d*) Staining after incubation of cells with MBP only. (Bar = 10 μm.) (*e–g*) SEM of HeLa cells incubated with MBP–MOMP or MBP at 4°C for 1 hr. (*e*) Secondary imaging of HeLa cells showing MBP–MOMP particles bound as aggregates at focal contact points on the cell surface. (*f*) Backscatter imaging of *e* after staining with rabbit anti-MOMP antiserum and goat anti-rabbit IgG conjugated to 15-nm colloidal gold. (*g*) Secondary imaging of HeLa cells inoculated with MBP. (Bar = 250 nm.) Arrows depict the aggregate binding of MBP–MOMP particles to the HeLa cell surface.

high-level binding to wild-type K1 cells (Fig. 7*A*). In contrast, MBP–MOMP bound poorly to the mutant cell lines 606 (undersulfonated HS), 677 (defective in HS synthesis but expressing 2–3 times more CS than wild-type CHO-K1 cells), and 745 (lacking any GAG chains due to a defect in the enzyme xylosyltransferase). The infectivity of EBs for the cell lines 606, 677, and 745 was reduced by 62, 67, and 75%, respectively, compared with the K1 parent cell line (Fig. 7*B*). Thus, there is a direct correlation between MBP–MOMP binding to and chlamydial infectivity for the same GAG mutant cell lines.

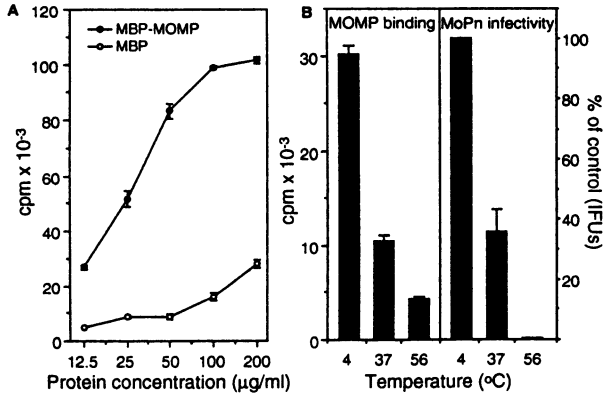

FIG. 3. MBP–MOMP binding assay and thermolability of MBP–MOMP binding and chlamydial infectivity. (*A*) The kinetics of MBP–MOMP binding to HeLa cells. (*B*) The effect of temperature on MBP–MOMP binding and chlamydial infectivity.

DISCUSSION

Identification of surface components that mediate chlamydial attachment and the host cell receptors to which they bind is critical to understand the pathogenesis of chlamydial infection. Herein we present data using a recombinant MOMP that provides strong evidence for MOMP functioning as a chlamydial cytoadhesin and that HS proteoglycans are the host cell receptors to which the MOMP binds. Our conclusions are based on the following observations: (*i*) the recombinant MOMP binds specifically to HeLa cells, (*ii*) MOMP binding competitively inhibits chlamydial infectivity, (*iii*) MOMP bind-

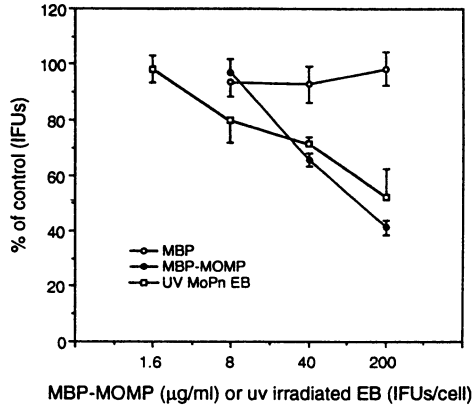

FIG. 4. Competitive inhibition of chlamydial infectivity by MBP–MOMP and UV-inactivated chlamydial EBs.

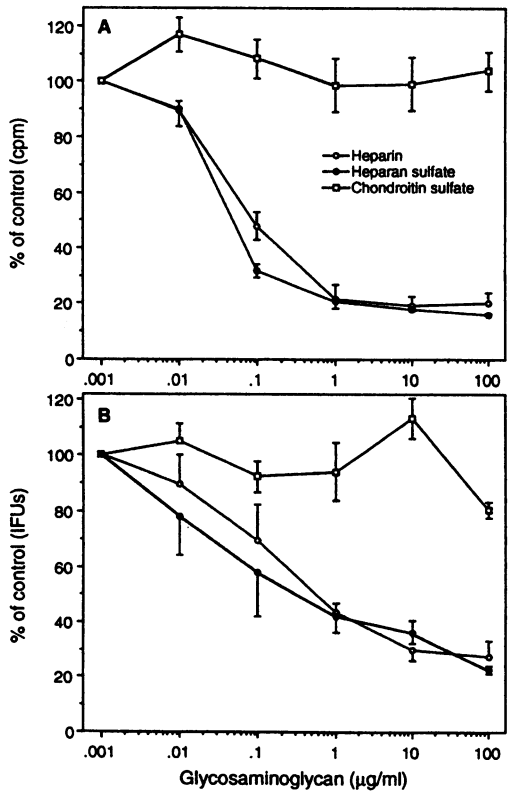

FIG. 5. Inhibition of MBP–MOMP binding and chlamydial infectivity by GAGs. (*A*) MBP–MOMP binding assays in the presence of different concentrations of heparin, HS, or CS. (*B*) Chlamydial infectivity assays using the same concentration of GAGs shown in *A*.

ing is inhibited by HS but not CS, (*iv*) enzymatic removal of host cell surface HS but not CS inhibits MOMP binding, and (*v*) MOMP does not bind mutant cell lines deficient in HS. These data are substantiated by the demonstration that intact chlamydiae display similar biological properties in their interaction with host cells.

We propose that the chlamydial MOMP functions as a cytoadhesin by initiating attachment of chlamydiae to host cell surface HS. This model is consistent with the strategies utilized by a number of other pathogenic microbes. Parasites that possess HS binding proteins that mediate attachment and entry into cells include a number of different viruses (12–16, 31), *Leishmania amazonensis* (32), *Plasmodium* (33), *Trypanosoma cruzi* (17), *Neisseria gonorrhoeae* (18), and *Bordetella pertussis* (34).

The model we suggest differs from that currently favored by Zhang and Stephens (7). They proposed that chlamydiae synthesize a HS-like GAG that is bound to the cell surface of the parasite. This chlamydial-specific GAG is thought to bridge a HS binding protein(s) on the bacterial surface to unidentified host cell surface receptor(s). In this work, we did not attempt to detect GAG synthesis by chlamydiae; however, it appears from our results that the chlamydial surface structure responsible for binding GAGs is the MOMP and that, at least *in vitro*, host cell surface HS is sufficient for chlamydial attachment.

Native MOMP is disulfide-linked and has only been purified previously in the presence of reducing agents and anionic detergents. Previous unsuccessful attempts to directly demonstrate biological function of purified MOMP in its interactions with host cells have utilized predominately nonnative protein. There are several potentially important characteristics of the recombinant MOMP described in this work that may have fortuitously contributed to its functional activity. The particulate structures formed by the MBP to which the MOMP was fused may have presented the MOMP as a multivalent ligand and facilitated its interaction with HS receptors. The disulfide cross-linking of MOMP domains on the MBP–MOMP fusion may also contribute to its function as a cytoadhesin. It cannot be concluded from this work that correct disulfide folding of the MOMP occurred; however, we suspect that this might be accurate and is at least in part responsible for the functional properties described here. Reduction of MOMP had no effect on its binding properties, which intuitively argues against this hypothesis. It is possible, however, that disulfide cross-linking of the recombinant MOMP was sufficient to allow proper folding of the protein into a structure that facilitates MOMP function and that this structure was maintained through noncovalent protein interactions after reduction. That the recombinant MOMP possessed some higher-ordered structure that was critical to its cytoadhesin function was suggested by the marked thermolability of the protein.

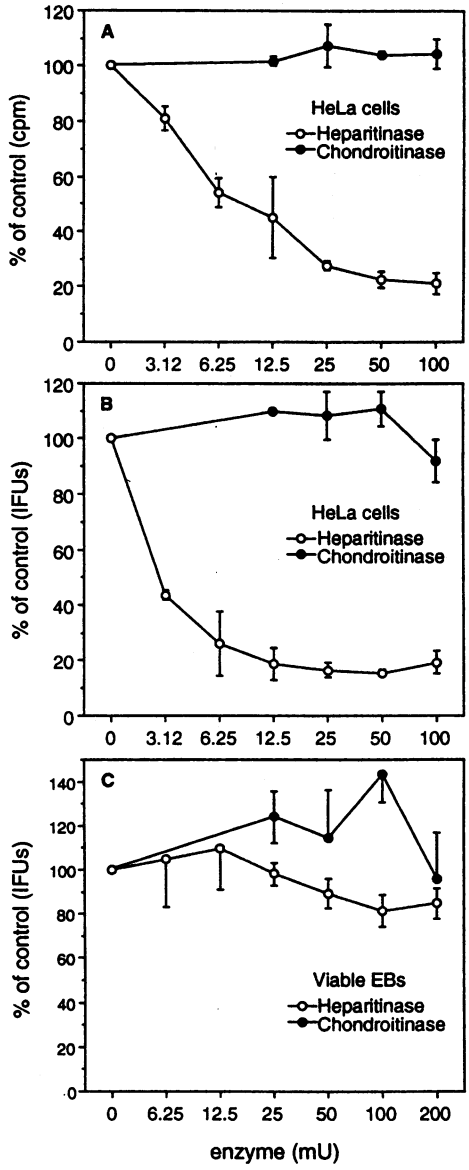

FIG. 6. Inhibition of MBP–MOMP binding and chlamydial infectivity after treatment with GAG lyases. (*A*) MBP–MOMP binding assays after treatment of HeLa cells with chondroitinase or heparitinase. (*B*) Chlamydial infectivity assays after treatment of HeLa cells with chondroitinase or heparitinase. (*C*) Chlamydial infectivity assays after treatment of EBs with chondroitinase and heparitinase.

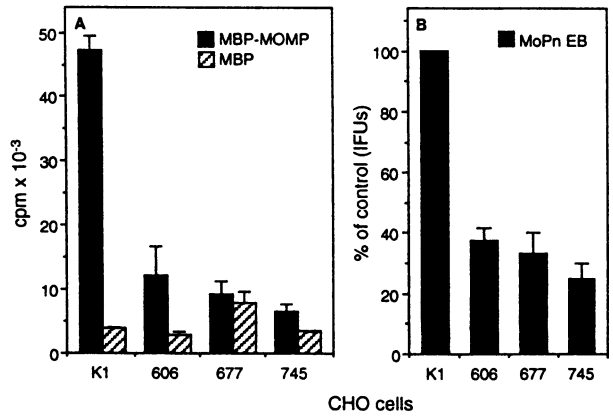

FIG. 7. MBP–MOMP binding to and chlamydial infectivity for CHO-K1 mutant cell lines. (*A*) Binding of MBP–MOMP to CHO-KI parent and mutant cell lines. (*B*) Chlamydial infectivity for CHO-K1 parent and mutant cell lines. Line 606, deficient in proteoglycan sulfonation; line 677, deficient in HS synthesis; line 745, deficient in proteoglycan synthesis.

We observed that at 4°C the MBP–MOMP particles bound to cells at focal contact points on the cell surface. After a shift in temperature, these foci further assimilated into larger cell surface aggregates. Interestingly, these results closely resemble those described for some types of HS proteoglycan receptors (24). Those studies have shown that antibody cross-linking of proteoglycan receptors at 4°C promotes a rapid temperature and energy-independent assimilation of receptors that appear as distinct cell surface clusters or foci. Moreover, these receptor–ligand foci were shown to assemble into large cell surface aggregates after shifting the temperature to 37°C. The parallels between those finding and the results described herein for MOMP binding are striking and provide further support for HS proteoglycans as the receptors to which the MOMP binds.

Our results showed that MOMP was not internalized after binding to cells, indicating that its primary function is in adherence of chlamydiae. Thus, MOMP may mediate only an initial reversible attachment step to promote other receptor–ligand interactions that may trigger the actual internalization event. Such a mechanism would be reminiscent of varicella zoster virus where attachment is mediated by GAGs but endocytosis is dependent upon the binding of mannose-6-phosphate receptors (15). It is also possible that separate domains of MOMP may function in the attachment and entry mechanism but that the conformation of the recombinant protein does not permit those interactions required to stimulate entry. Lastly, the recombinant MOMP may lack appropriate spacial organization and density of its binding domains that are necessary to induce internalization.

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