Chlamydia trachomatis OmcB protein is a surface-exposed glycosaminoglycan-dependent adhesin

Sanaa Fadel and Adrian Eley

Correspondence
Adrian Eley
a.r.eley@sheffield.ac.uk

Henry Wellcome Laboratories for Medical Research, Division of Genomic Medicine,
University of Sheffield Medical School, Beech Hill Road, Sheffield S10 2RX, UK

The OmcB protein of *Chlamydia trachomatis* is a cysteine-rich outer membrane polypeptide with important functional, structural and antigenic properties. The entire gene encoding the OmcB protein from *C. trachomatis* serovar LGV1 was cloned and expressed in *Escherichia coli* and the full-length protein used to raise polyclonal antibodies. Recombinant OmcB was used to show that OmcB is a surface-exposed protein that functions as a chlamydial adhesin. Infectivity inhibition assays carried out using HeLa cells with serovar LGV1 in the presence of purified anti-OmcB serum showed inhibition of infectivity, suggesting that some of the OmcB was surface exposed. Moreover, using recombinant OmcB in infectivity inhibition assays resulted in 70 % inhibition of infectivity, confirming that OmcB plays a role as an adhesin in *C. trachomatis*. Furthermore, recombinant OmcB protein bound to the surface of HeLa and Hec1B cells, but binding to glycosaminoglycan (GAG)-deficient cells (*pgsA*-745 and *pgsD*-677) was markedly reduced, indicating that OmcB binds to GAG-like receptors on host cells.

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INTRODUCTION

In both industrialized and developing countries, *Chlamydia trachomatis* infection remains one of the most important sexually transmitted diseases. With approximately 90 million cases annually, infection with *C. trachomatis* is the most prevalent sexually transmitted bacterial disease in the world (Gonzales *et al.*, 2004). As 70 % of these infections are asymptomatic, they can cause major complications such as acute pelvic inflammatory disease, ectopic pregnancy, infertility or infant pneumonia if left untreated. This organism is also the agent of the blinding eye disease, trachoma, which currently affects about 150 million people worldwide (Mabey *et al.*, 2003). Moreover, it is well established that *C. trachomatis* can cause conjunctivitis and pneumonitis in neonates and infants as a result of vertical transmission (Oroz *et al.*, 2001). In addition, *C. trachomatis* has been found to be a likely risk factor for cervical cancer (Luostarinen *et al.*, 2004).

As *C. trachomatis* is an obligate intracellular pathogen, it must initially attach to and enter the apical surfaces of epithelial host cells. Therefore, attachment to the host cell is the critical primary event for establishing an infection. A number of chlamydial ligands have been identified and characterized. These include the major outer membrane protein (MOMP) (Su *et al.*, 1990; Swanson & Kuo, 1994),

heat-shock protein 70 (Raulston *et al.*, 2002), the polymorphic outer membrane proteins and the thermolabile 34 kDa membrane protein (Hackstadt, 1999), as well as OmcB (also known as Omp2) protein (Stephens *et al.*, 2001), which remains controversial. Few epithelial plasma membrane components have been proposed as receptors (Vretou *et al.*, 1989; Su *et al.*, 1996; Mamelak *et al.*, 2001; Fadel & Eley, 2004).

OmcB is the second most abundant outer membrane protein in chlamydia. The *omcB* gene comprises 1641 bp and encodes a protein of 60 kDa. The translated amino acid sequence reveals a relatively basic protein containing 24 cysteine residues (Allen & Stephens, 1989). OmcB is highly conserved among chlamydia species (Newhall, 1987; Wagels *et al.*, 1994) and has a biologically significant role as a structural protein; it is involved in the conversion of the replicating reticulate body to the infectious elementary body (EB) (Mygind *et al.*, 1998) and is thought to contribute to cell-wall rigidity and osmotic stability of the EB (Newhall, 1987). Moreover, OmcB has been found to be a major immunogen in chlamydial infections (Mygind *et al.*, 1998), inducing antibody responses in both humans and animals (Wagels *et al.*, 1994). Not surprisingly, as it is a primary focus of the host immune response, OmcB is a potential candidate for a chlamydial vaccine (Eko *et al.*, 2004; Penttilä *et al.*, 2004).

There has been considerable debate regarding the precise localization and role of the OmcB protein in *C. trachomatis* adherence to host cells. Topological studies have indicated that OmcB is localized at the inner surface of the outer

Abbreviations: COMC, chlamydial outer membrane complex; EB, elementary body; HS, heparan sulphate; MOMP, major outer membrane protein; MS/MS, mass spectrometry coupled to microcapillary liquid chromatography.

membrane (Everett & Hatch, 1995; Mygind *et al.*, 1998). It is thought not to be surface exposed (Watson *et al.*, 1994; Everett & Hatch, 1995), but it is considered to form a supramolecular lattice in the periplasm (Newhall, 1987; Sardinia *et al.*, 1988). However, a few reports have suggested that it plays a role in attachment and invasion of the host cell (Ting *et al.*, 1995; Stephens *et al.*, 2001). For accurate characterization of the localization and adherence of OmcB, it needs to be obtained free from other *C. trachomatis* proteins. However, the purification of antigens from *C. trachomatis* is very difficult, the main obstacles being its intracellular nature and the difficulty of cultivation of *C. trachomatis* in large quantities. Another complication is that even highly purified EB preparations are likely to contain damaged outer membrane fractions and unstable intermediate forms.

To overcome these obstacles, we isolated and sequenced the gene for this protein from *C. trachomatis* serovar LGV1 and cloned it into an *Escherichia coli* expression vector. The OmcB protein expressed in *E. coli* was used to define the location and the role in adherence of this highly debated 60 kDa protein.

METHODS

Cloning of *omcB*. Template DNA for the PCR was obtained from *C. trachomatis* serovar LGV1, which had been cultured from EBs and purified according to the method of Caldwell *et al.* (1981). The primers used (MWG Biotech) were OMP-RBS-FOR (5'-ATAGAATTCGAATAGGAGATCCTATGAACA-3') and OMP-HIS-STOP (5'-TCAGTGATGGTGATGGTGATGATAGATGTGTG-TATCTCTGTAT-3'). These were used to amplify the entire *C. trachomatis omcB* gene, including the ribosome-binding site. Amplification was performed by PCR using 2.5 U *Taq* DNA polymerase of the Expand High Fidelity PCR system (Roche). The PCR product was cloned into the appropriate sites of the pJONEX4 vector (Sayers & Eckstein, 1991), according to standard techniques (Sambrook *et al.*, 1989), for expression of the OmcB protein under the control of the bacteriophage λ P_L promoter. Protein expression from pJONEX4 was good. Therefore, the *omcB* gene was subcloned into the pET28a vector (Novagen), after digestion with the appropriate restriction enzymes. The ligation product was used to transform DH5 α *E. coli* cells. The colonies were screened for a plasmid containing the correct insert and this was used to transform BL21(DE3)RIPL *E. coli* cells by electroporation. This allowed protein expression from the T7 promoter. The construct *omcB*-pET28 was designed to produce full-length OmcB with a C-terminal His-tagged extension. The identity of positive clones was confirmed by nucleotide sequencing of the entire insert in both orientations.

Expression of OmcB protein. BL21(DE3)RIPL expressing the C-terminal His-tagged OmcB was grown to mid-stationary phase at 37 °C in Luria-Bertani medium supplemented with 35 μ g chloramphenicol ml⁻¹, 25 μ g kanamycin ml⁻¹ and 0.4% glucose. Expression was induced by adding 0.7 mM IPTG followed by incubation overnight at room temperature in a rotary shaker. Cells were recovered by centrifugation at 13 000 g for 20 min at 4 °C and frozen at -80 °C. Proteins were examined from both the soluble fraction and the cell pellet. For large-scale purification, cells were grown in a 2 l fermenter. The bacteria were lysed and solubilized under denaturing conditions using 8 M urea in the presence of 2 mM DTT. The protein was purified using the QIAexpress purification method according to the

manufacturer's instructions (Qiagen). Eluate fractions were collected and analysed for the 60 kDa OmcB protein. The expressed protein was detected and identified by immunoblotting using a commercial anti-*C. trachomatis* antibody (Biogenesis) and anti-His tag antibody (Amersham Biosciences), in addition to tandem mass spectrometry coupled to microcapillary liquid chromatography (MS/MS) analysis.

The eluted protein fractions were combined and extensively dialysed against low-salt buffer (20 mM potassium phosphate, 2 mM EDTA, 10% glycerol, 10 mM DTT, 100 mM NaCl, pH 7.4) at room temperature. The soluble dialysate was further purified on a heparin column (HiTrap heparin HP column; Amersham Biosciences) using an automated gradient elution system (GradiFrac; Amersham Biosciences), followed by concentration of the protein solution using a Vivaspin concentrator with a 30 kDa cut-off (Sartorius). The final protein concentration was determined using the Bradford assay (Bradford, 1976). The purified protein was stored at -80 °C in 20 mM potassium phosphate buffer and 30% glycerol.

Production of rabbit anti-OmcB serum. Rabbits were immunized three times with 500 μ g each of the purified OmcB protein. For each rabbit, 500 μ g antigen in a maximum volume of 250 μ l neutral phosphate buffer was mixed with an equal volume of Freund's adjuvant. Immunization was as follows: a first injection with complete Freund's adjuvant, a second injection at day 22 with incomplete Freund's adjuvant and a third at day 43 with incomplete Freund's adjuvant. Sera were collected 10 days after the third injection and tested using immunoblotting. IgG was purified from the whole anti-OmcB serum by passage through a protein G column.

To determine the titre of the antiserum, the purified recombinant OmcB protein, as well as total proteins from purified *C. trachomatis* EBs and chlamydial outer membrane complex (COMC) preparations, were separated by SDS-PAGE and electroblotted on to nitrocellulose membranes as described by Towbin *et al.* (1979). The membranes were incubated for 2 h with sera from the immunized rabbits (diluted 1:1000–1:10 000), as well as with pre-immune sera, and then with a peroxidase-conjugated anti-rabbit IgG (diluted 1:3000; Sigma). After washing, blots were developed using 4-chloro-1-naphthol.

Localization of OmcB protein on the surface of *C. trachomatis* and *E. coli*

Proteinase lysis of the *E. coli* cell surface. The accessibility of OmcB to proteinase K was used to localize the recombinant protein to a specific cellular compartment of *E. coli*. The technique was essentially that used by Dascher *et al.* (1993) to study translocation of recombinant MOMP. OmcB protein was induced in *E. coli* containing the *omcB*-pET28 plasmid. The cells were harvested by centrifugation and resuspended in buffer S [100 mM Tris/HCl (pH 8.2), 0.05 M sucrose, 5 mM EDTA] plus 1 mg lysozyme ml⁻¹ to convert the cells to spheroplasts. After incubation on ice for 5 min, conversion of the cells to spheroplasts was monitored by dark-field microscopy. MgSO₄ (final concentration 18 mM) was added to stabilize the spheroplasts. These were then centrifuged for 40 s and the supernatant was taken as the periplasmic fraction. To remove any periplasmic proteins trapped in the pellet, the spheroplasts were resuspended in buffer T [50 mM Tris/HCl (pH 8.2), 0.25 M sucrose, 10 mM MgSO₄] and centrifuged a second time. The supernatant was discarded. Osmotic lysis was achieved by suspension of half of the spheroplasts in 0.5 ml 50 mM Tris/HCl (pH 8.2) and 2.5 mM EDTA. When the cells had been lysed, MgSO₄ was added to a final concentration of 20 mM. Whole cells, spheroplasts and cell lysates were then suspended in buffer T and each was divided into two equal portions. The first portions served as controls and the second portions were treated with proteinase K (final concentration 25 μ g ml⁻¹). The suspensions were incubated on ice for 20 min,

after which the protease inhibitor PMSF was added to a final concentration of 1 mM. Samples were analysed by SDS-PAGE and immunoblotted using an anti-*Chlamydia* antibody.

Infectivity inhibition assays using anti-OmcB serum. Inocula of *C. trachomatis* serovar LGV1 were adjusted so that not more than ~50 inclusions were present in each field at $\times 400$ magnification ($\sim 5 \times 10^5$ infectious units) in 100 μ l PBS. The inocula were mixed with serial dilutions of the purified IgG of anti-OmcB serum (1:10–1:500). Inocula mixed with pre-immune serum and inocula without serum were used as negative controls. Anti-*Chlamydia* antibody was used as a positive control. These were incubated for 1 h at 37 °C and then used to infect confluent HeLa and Hec1B cell monolayers in 24-well tissue culture trays. The cells were incubated for 1 h at 37 °C in 5% CO₂ and then washed with PBS three times before adding 1 ml tissue culture medium supplemented with cycloheximide (2 μ g ml⁻¹) to each well. After incubation at 37 °C in 5% CO₂ for 48 h, infected monolayers were fixed, stained and counted by direct immunofluorescence microscopy using the *C. trachomatis* culture confirmation test (Trinity Biotech).

Examination of the binding of recombinant OmcB to eukaryotic cells

Adherence assay. The method used was similar to that of Thornley *et al.* (1996). *E. coli* cells containing the *omcB*-pET28 plasmid were induced to produce OmcB protein and harvested by centrifugation, washed twice with PBS (pH 7.4) and resuspended in an appropriate amount of Eagle's minimal essential medium. One millilitre of this bacterial suspension (OD₆₀₀ 0.14) was inoculated on to HeLa and Hec1B cell monolayers and incubated for 90 min at 37 °C with 5% CO₂. After incubation, non-adherent bacteria were removed by washing three times with PBS. The cell layer and adherent bacteria were then fixed with a 90% methanol/acetic acid solution (3:1) for 5 min. After washing with distilled water, the remaining cells were stained with 10% Giemsa for 20 min, mounted and viewed by light microscopy. Negative controls included BL21 cells and BL21 containing pET28 vector (with no insert), in addition to BL21 expressing a periplasmic protein, ComH. The *comH*-pET28 plasmid was a kind gift of K. Kositidis (University of Sheffield, UK). To check that the binding of OmcB to HeLa/Hec1B cells was specific, it was examined in the presence of anti-OmcB serum.

Examination of binding of *E. coli* producing OmcB to GAG-deficient cells. The degree of adherence was compared for GAG-deficient (*pgsA*-745 and *pgsD*-677) and wild-type (CHO-K1) cells to evaluate the importance of host-cell GAGs for OmcB binding. Cell monolayers were grown to semi-confluence in 24-well tissue culture trays on sterile coverslips. The production of OmcB was induced from *E. coli* containing the *omcB*-pET28 plasmid. The bacterial suspension (OD₆₀₀ 0.14) was inoculated on to the cell monolayers and incubated for 90 min at 37 °C with 5% CO₂ and the adherence assay was completed as described above, using the same controls.

Infectivity inhibition assays using recombinant OmcB. Confluent HeLa and Hec1B cell monolayers were prepared and subsequently infected with serovar LGV1 inoculum in 100 μ l PBS. Inocula were supplemented with serial dilutions of the purified recombinant OmcB and the assay was completed as described above.

RESULTS

Expression of OmcB protein in *E. coli*

OmcB protein could be expressed as a full-length 65 kDa protein using pET28 vector, in the His-tagged form.

Expression of OmcB caused a reduction in *E. coli* cell viability, similar to that caused by expression of ComH protein (data not shown). Anti-*C. trachomatis* antibody was used for identification of the expressed protein. In addition, protein sequence obtained from MS/MS analysis confirmed the identity of the recombinant protein.

OmcB was purified using Ni-NTA agarose, which bound the six-His tag at the C terminus of the recombinant protein. The protein was refolded and concentrated using extensive dialysis and a heparin column. Anti-OmcB serum prepared by immunizing rabbits with the purified protein preparation reacted in Western blots at a high dilution (1:10 000) with the *C. trachomatis* EBs separated by SDS-PAGE, as well as with the recombinant protein preparation. Immunoblot analysis confirmed the specificity of this serum for OmcB with no detectable cross-reactivity with other COMC proteins (Fig. 1). Both single and double bands of OmcB could be seen, which depended on dialysis and refolding of the denatured purified protein.

Localization of OmcB protein on the surface of *C. trachomatis* and *E. coli*

Proteinase lysis of the *E. coli* cell surface. Cellular localization of recombinant OmcB expressed in *E. coli* was assessed by proteinase digestion. This technique relies on the selective susceptibility to proteinase K digestion of surface-exposed proteins in whole intact cells and of periplasmic and inner membrane proteins in spheroplasts. *E. coli* was induced to express OmcB and then converted to spheroplasts using a brief lysozyme treatment to allow access to the periplasmic space.

Spheroplasts treated with proteinase K showed a complete loss of the recombinant OmcB, whilst intact *E. coli* cells showed that only part of the OmcB protein was digested (Fig. 2). This suggested that part of the OmcB protein is

Fig. 1. Detection of OmcB protein by immunoblotting using anti-OmcB serum (diluted 1:10 000). Lanes: 1, cell lysate of *E. coli* without the *omcB* insert; 2, *E. coli* lysate expressing OmcB protein; 3, purified recombinant OmcB protein; 4, COMC prepared from *C. trachomatis* serovar LGV1. M, Low-range protein marker.

Fig. 2. Proteinase K digestion of intact cells, spheroplasts and the cytoplasmic fraction of *E. coli* expressing OmcB protein. Lanes contained controls (C) or cell extract lysed with 25 µg proteinase K ml⁻¹ for 20 min on ice (K) after which the protease inhibitor PMSF was added to stop the reaction. All cell fractions were resolved on SDS-PAGE and OmcB was detected by immunoblotting using anti-OmcB serum. M, Low-range protein marker.

located on the outer membrane, whilst the remainder is not. Immunoblot analysis of the periplasmic fraction indicated the presence of OmcB, suggesting that some of the protein might be located in the periplasmic space.

Infectivity inhibition assays using anti-OmcB serum.

Fig. 3 shows the results of infectivity inhibition experiments in which chlamydial EBs were incubated with anti-OmcB serum and then used to infect HeLa or Hec1B cells. Anti-OmcB was found to have an inhibitory effect on infectivity, which was statistically significant. Using a 1:100 dilution showed 39 and 36% inhibition using HeLa cells and Hec1B cells, respectively. Using a lower dilution did not lead to further inhibition of infectivity. For comparison, anti-*Chlamydia* antibody, which is immunogenic to all antigens of the EB, was used as a control.

Examination of the binding of recombinant OmcB to eukaryotic cells

Adherence assay. Binding was calculated on the basis of the number of adherent *E. coli* retained on the cell monolayer after washing. The mean number of adherent *E. coli* cells was counted per tissue culture cell and compared with the control. Fig. 4(a) shows that there was strong binding of *E. coli* cells expressing OmcB protein to HeLa cells. The HeLa cells showed stronger binding to OmcB than Hec1B cells; the mean number of adherent bacteria was found to be 47 for HeLa cells, compared with 33 for Hec1B cells. The negative controls (*E. coli* alone, *E. coli* containing the vector only and *E. coli* expressing ComH protein) showed only low levels of binding (Fig. 4b).

In addition, we examined binding in the presence of anti-OmcB serum to determine the specificity of OmcB in the adherence process. Fig. 5 shows that addition of anti-OmcB serum resulted in inhibition of binding of OmcB to the cells

Fig. 3. Infectivity inhibition assay, using purified IgG from anti-OmcB serum. Anti-*Chlamydia* antibody was used as a positive control. Pre-immune rabbit serum was used as a negative control. The figure represents the results of six different experiments, shown as means $\pm$ SEM. Results were analysed statistically using a one-way paired ANOVA test. *, $P < 0.05$; ***, $P < 0.001$; white bars, HeLa cells; black bars, Hec1B cells.

by 68 and 76% to HeLa and Hec1B cells, respectively. Pre-immune serum had no inhibitory effect on binding of OmcB to HeLa/Hec1B cells when added in the same concentration as the anti-OmcB serum.

Examination of binding of *E. coli* expressing OmcB to GAG-deficient cells.

Fig. 6 summarizes the results of adherence experiments in which the ability of *E. coli* producing OmcB to bind GAG-deficient CHO-K1 cell lines was explored. The cell lines chosen were *pgsA*-745, deficient for all GAGs, and *pgsD*-677, deficient for heparan sulphate (HS) but able to produce chondroitin sulphate, and each was compared with CHO-K1 (wild-type) cells. The ability of serovar LGV to infect *pgsA*-745 and *pgsD*-677 cells was markedly reduced in comparison with that of the CHO-K1 wild-type cells. The negative controls showed only minimal binding to all of the cell lines tested.

Infectivity inhibition assays using recombinant OmcB.

Fig. 7 shows the result of infectivity inhibition experiments in which chlamydial EBs were incubated with HeLa and Hec1B cells in the presence of various concentrations of purified recombinant OmcB protein. OmcB was found to have a significant inhibitory effect on the infectivity of serovar LGV1 of *C. trachomatis*. Inhibition was dose dependent and the maximum inhibition obtained was 70% at 150 µg ml⁻¹. No significant difference was observed between the two cell lines. Purified ComH protein, when

Fig. 4. Determination of the binding of recombinant OmcB to HeLa cells using an adherence assay. *E. coli* cells were incubated with HeLa cells for 90 min at 37 °C, followed by staining with 10% Giemsa stain: (a) *E. coli* cells induced to express OmcB protein, using 0.7 mM IPTG overnight at room temperature, (b) *E. coli* cells expressing ComH protein, induced under the same conditions.

used at 150 $\mu\text{g ml}^{-1}$, showed only minimal inhibition of infectivity.

DISCUSSION

Identification of surface components that mediate chlamydial attachment and the host cell receptors to which they bind is critical in understanding the pathogenesis of chlamydial infection. Studying the role of OmcB protein in chlamydial adherence to the host cell ideally necessitates evaluation of the whole protein purified from other chlamydial proteins. A good approach for this is expression

of the protein in *E. coli* with subsequent evaluation of the translocation and function in an *E. coli* host in addition to the native chlamydia. Therefore, we cloned and expressed OmcB of *C. trachomatis* serovar LGV1 into an *E. coli* expression vector. Immunoblot analysis of the clones revealed a protein of approximately 65 kDa and MS/MS analysis showed that the protein was processed correctly in the vector. To assess the cellular localization of OmcB in *C. trachomatis* and *E. coli*, two approaches were chosen:

Fig. 5. Effect of addition of anti-OmcB serum on the adherence of *E. coli* expressing OmcB protein to HeLa (white bars) or Hec1B (black bars) cells. Negative controls included *E. coli* (BL21), *E. coli* with the vector only (pET28-BL21) and *E. coli* producing ComH protein (*comH*-pET28). The figure represents the results of six different experiments given as means $\pm$ SEM. Results were analysed statistically using a one-way paired ANOVA test. ***, $P < 0.001$. NS, Not significant.

Fig. 6. Examination of the binding of *E. coli* only (BL21) (white bars), *E. coli* cells induced to produce OmcB protein (*omcB*-pET28) (black bars) and *E. coli* cells induced to produce ComH protein (*comH*-pET28) (hatched bars) to GAG-deficient cells (*pgsA*-745 and *pgsD*-677), in comparison with wild-type CHO-K1, HeLa and Hec1B cells, using 0.7 mM IPTG overnight at room temperature. *pgsA*-745 and *pgsD*-677 cells are defective CHO-K1 cell lines. The figure represents the results of six different experiments given as means $\pm$ SEM. Results were analysed statistically using a one-way paired ANOVA test. ***, $P < 0.001$.

Fig. 7. Infectivity inhibition assay of *C. trachomatis* serovar LGV1 in the presence of recombinant OmcB protein. The assay was performed using HeLa (white bars) and Hec1B (black bars) cells. As an additional negative control, ComH was added at a concentration of 150 µg ml⁻¹. This figure represents the results of six different experiments, given as means ± SEM. Results were analysed statistically using a one-way paired ANOVA test. **, $P < 0.01$; ***, $P < 0.001$.

proteinase lysis of the cell compartments and an infectivity inhibition assay using anti-OmcB serum.

Results of proteinase lysis suggested that part of the recombinant OmcB protein was located on the outer membrane of *E. coli*, with the remainder located in the periplasmic space. To investigate the cellular localization of OmcB on *C. trachomatis*, we utilized an *in vitro* infection inhibition assay, similar to that used by others (Chen & Stephens, 1994; Herold *et al.*, 1997). We used two different cell types to represent the variety of epithelium found in the human female reproductive tract. The cell lines HeLa and Hec1B are derived from the human cervix and endometrium, respectively. The use of anti-OmcB led to an inhibitory effect on the infectivity of serovar LGV1, indicating that OmcB was accessible on the surface of chlamydial EBs. Although the effect was not dramatic, which could be due to redundant chlamydial adhesions, inhibition was dose dependent and was statistically significant.

There is a certain amount of controversy surrounding OmcB localization. It has been suggested that OmcB is located exclusively within the periplasm and is not surface exposed (Everett & Hatch, 1995). It has also been suggested that disulphide cross-linked polymers of OmcB are the functional equivalent of peptidoglycan in other Gram-negative bacteria, forming a disulphide cross-linked network with the periplasmic domains of OmcA and other membrane proteins, and that this may contribute to the considerable structural stability of EBs (Everett & Hatch, 1995). These workers also demonstrated the sensitivity of OmcB to trypsin treatment of EBs for serovar L2. Nevertheless, they concluded that the OmcB protein was not surface exposed and was not localized to the outer membrane.

In contrast, Ting *et al.* (1995) found that OmcB of *Chlamydia psittaci* was also susceptible to trypsin digestion of EBs, and proposed that this protein may be surface exposed. Moreover, Mygind *et al.* (1998) attempted to resolve the question of surface localization of OmcB in *C. trachomatis* by immunoelectron microscopy using OmcB-specific antibodies, but, as in previous studies (Collett *et al.*, 1989; Watson *et al.*, 1994), were unable to demonstrate that OmcB was accessible to antibodies using intact EBs.

Our findings provide conclusive evidence for the suggestion that part of OmcB is surface exposed (Stephens *et al.*, 2001). The previous failure of antibodies produced against the whole OmcB protein in binding intact EBs may be because of limited OmcB regions exposed on the surface or because these antibodies could not recognize the conformational epitopes of OmcB.

To examine the role of OmcB in adherence to host cells, anti-OmcB antibody was used to probe the recombinant protein in an adherence assay. *E. coli* cells expressing OmcB bound significantly to HeLa and Hec1B. Only minor binding was observed for *E. coli* not containing *omcB*, showing that expression of OmcB enabled *E. coli* to adhere strongly to tissue culture cells. To establish that the decreased viability and cell lysis of *E. coli* expressing OmcB protein was not responsible for adherence, we used ComH, a periplasmic protein (Smeets *et al.*, 2000), cloned using the same vector and expressed in the same *E. coli* strain, which also resulted in decreased viability of *E. coli* when expressed. ComH showed no adherence to either cell line. This is of particular note as ComH has a similar molecular mass and pI to OmcB, which means that the use of ComH as a negative control limits the possibility of OmcB adhering via non-specific electrostatic effects. To confirm that binding of *E. coli* expressing OmcB protein was specific, anti-OmcB antibody was added to the cells. The inhibition effect showed that this binding was specific and OmcB dependent. These findings clearly support a role for OmcB in specific binding to eukaryotic cells.

In a previous study using outer membrane fractions of *C. psittaci* in host-cell binding assays, Ting *et al.* (1995) found that OmcB bound HeLa cells in a dose-dependent fashion. Furthermore, Stephens *et al.* (2001) reported that OmcB prepared from COMC of serovar L2 bound heparin in a heparin column, whilst MOMP did not.

In order to explore the role of GAGs of the host cells as receptors for OmcB, we performed a second set of adherence assays using CHO cells known to be defective in key GAG-related genes. This approach has been used previously by us and other workers (Zhang & Stephens, 1992; Stephens *et al.*, 2000; Taraktchoglou *et al.*, 2001). For our experiments, we chose cell lines with clearly defined properties. *pgsA*-745 cells are defective in xylosyltransferase (the first sugar transfer in GAG chain synthesis) and therefore do not produce any GAGs (Esko *et al.*, 1985). The cell line *pgsD*-677 lacks both *N*-acetylglucosaminyltransferase and glucuronyltransferase

activities required for synthesis of HS, but they do produce chondroitin sulphate (Lidholt *et al.*, 1992). The results showed that *E. coli* expressing OmcB had a very low adherence to cells deficient in GAGs, especially those lacking HS (Fig. 6), in comparison with the wild-type CHO-K1 cells.

These results indicate that binding of OmcB to host cells is dependent on GAGs, and particularly on HS, and suggest that OmcB in serovar LGV1 uses host-cell GAGs to bind and infect cells. This approach is similar to the study by Su *et al.* (1996) who showed a marked reduction of binding of a recombinant MOMP to *pgsA*-745 and *pgsD*-677 cells. Our findings confirm other reports (Chen & Stephens, 1994; Davis & Wyrick, 1997; Taraktchoglou *et al.*, 2001) that LGV infection is dependent on an HS-related mechanism. They also support the evidence that these GAGs are on host cells and not on the surface of *Chlamydia* (Taraktchoglou *et al.*, 2001; Fadel & Eley, 2004).

To evaluate further the role of OmcB as an adhesin of *C. trachomatis*, we carried out infectivity inhibition assays in the presence of recombinant OmcB. The recombinant OmcB protein competed with the native OmcB of *Chlamydia* and led to a marked inhibition of infectivity (70 %), confirming that OmcB is an important chlamydial adhesin.

In conclusion, our experiments provide supportive evidence that OmcB protein is exposed on the surface of *C. trachomatis* and immunoaccessible to antibody binding. They also show that the recombinant OmcB protein is surface exposed in the *E. coli* host. We do not yet know whether the surface conformation and orientation of OmcB in *E. coli* are identical to those in native chlamydial EBs.

Our results also show that OmcB serves as an adhesin molecule for *C. trachomatis* and that it acts by binding a GAG-like compound(s), possibly HS. These conclusions are based on the observations that the recombinant OmcB bound specifically to HeLa and Hec1B cells, and that OmcB did not bind mutant cell lines deficient in GAGs. These findings support a role for OmcB in specific binding to eukaryotic cells, which in turn is responsible for mediating chlamydial infectivity.

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