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A heat-labile protein of *Chlamydia trachomatis* binds to HeLa cells and inhibits the adherence of chlamydiae

(surface exposed/extrinsic iodination/trypsin sensitive/adhesin/common receptor)

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ABSTRACT **From highly purified elementary bodies (EBs) of *Chlamydia trachomatis*, we have identified a protein of 38 kDa that selectively binds to monolayer cultures of HeLa cells. This protein, which we have named the chlamydial cytadhesin (CCA), is present on the surface of the EBs of three *C. trachomatis* serovars (B, E, and L1) that were examined. Localization of the CCA at the surface was confirmed by its ability to be labeled when viable EBs were iodinated and by its absence in preparations from trypsin-treated EBs. Viable EBs, but not heated or trypsin-treated EBs, inhibited the binding of the CCA to HeLa cells, indicating competition for a common receptor on the host cell membrane. A dose-dependent inhibition of adherence of radioactive EBs to HeLa cells was effected by extracts containing the CCA. This inhibition occurred even with extracts prepared from the EB of heterologous serovars. However, no inhibition could be demonstrated with extracts prepared from heat-treated EBs. Heat treatment of the extract resulted in the loss of ability of the CCA to bind to the host cells. HeLa cells preincubated with CCA-containing chlamydial extract showed reduced ability to bind labeled EBs and to develop cytoplasmic inclusions after infection. This protective activity was lost after exposure of the extract to heat. These findings indicate that the CCA is a thermolabile surface-exposed chlamydial adhesin; it may be useful in the development of vaccines for diseases caused by the pathogenic bacterium.**

Chlamydia trachomatis is an obligate intracellular bacterium and a pathogen of human beings. Studies of adherence of chlamydiae to eukaryotic cells have indicated a ligand–receptor type of interaction (1). Based on the inability of heat- and trypsin-treated elementary bodies (EBs) to competitively inhibit the adherence of chlamydiae, the participation of heat-labile and surface-exposed chlamydial protein(s) in the adherence reaction was suggested (1). Two proteins of ≈18 and ≈32 kDa, capable of binding to HeLa "membrane" preparations, have been implicated as chlamydial adhesins (2, 3). The functional roles of these two proteins have yet to be determined. More recently, the major outer membrane protein (MOMP) of 40 kDa has been proposed as an adhesin (4). The MOMP was implicated in the binding of chlamydiae to Syrian hamster kidney cells by nonspecific (electrostatic and hydrophobic) interactions. Thus, although the kinetic properties of chlamydial attachment to host cells has been studied in detail, little is known of the surface components of the bacterium mediating specific adherence.

We undertook this study to identify chlamydial proteins that bind to HeLa 229 monolayers under conditions that allow only the attachment step of the infection to occur. A nonionic detergent, *n*-octyl β-D-glucopyranoside (OGP) was used to extract highly purified EBs of three *C. trachomatis* serovars (B, E, and L1) propagated in HeLa or in McCoy cells to identify chlamydial proteins capable of binding to host cells in a specific manner. Only one protein, of 38 kDa, was found to exhibit cytadherence activity. Extracts containing this protein, the chlamydial cytadhesin (CCA), were also used to study inhibition of adherence of viable EBs to HeLa cells. The effects of exposure to heat and to trypsin on the cytadherence and protection activity of this protein were examined.

MATERIALS AND METHODS

Organisms. *C. trachomatis* serovars B, E, and LGV440 (L1) were grown in mycoplasma-free HeLa 229 or McCoy cells; the EBs were purified as described (1), and samples were stored at −80°C. Mixing experiments using mock-infected HeLa cells labeled with a mixture of [^{3}H]amino acids and unlabeled chlamydia-infected cells had shown that less than 0.02% of the host-derived protein copurified in the final EB preparation (1).

Radioactive Labeling of EBs. Metabolic labeling of the chlamydial proteins was effected with Tran^{35}S-label (ICN) in the presence of emetine, an irreversible inhibitor of eukaryotic protein synthesis, as described in detail (1, 5). Extrinsic labeling of the purified EBs with ^{125}I was performed using *N*-chloro-bezenesulfonamide-derivatized beads (Iodo-Beads, Pierce). Briefly, to 100 μl of the purified EB suspension (protein content, 100–500 μg) in phosphate-buffered saline (PBS), two Iodo-Beads, and 250–350 μCi of Na^{125}I (1 Ci = 37 GBq) were added and incubated for 10 min on ice. Unreacted Na^{125}I was removed by centrifuging the reaction mixture through a desalting gel (Bio-Gel P-6DG, Bio-Rad) equilibrated with 2% (wt/vol) bovine serum albumin in PBS (6). The iodinated EBs were recovered by sedimentation. Adherence of these EBs to unfixed as well as to glutaraldehyde-fixed HeLa cells at 4°C was a saturable process (1).

Extraction of Chlamydial Proteins. The pelleted EBs were resuspended in 100 μl of OGP buffer [2% (wt/vol) OGP in PBS, pH 7.5/10 mM DL-dithiothreitol/1 mM phenylmethylsulfonyl fluoride/aprotinin (10 μg/ml)/leupeptin (10 μg/ml)/*N*-α-tosyl-L-lysine chloromethyl ketone (TLCK; 10 μg/ml)]. The suspension was kept on ice for 1 hr and then sedimented in a microcentrifuge for 15 min at 4°C. The pellet was reextracted and the pooled supernatants (100 μl each) were dialyzed at 4°C for 2–3 hr against PBS. Protein concentrations in the extracts were determined with the Bio-Rad reagent, using bovine serum albumin as the standard. Samples of the dialyzed OPG extract (OGPE) were stored at −80°C.

Glutaraldehyde Fixation of HeLa Monolayers. HeLa cells were plated in 96-well cell culture dishes at 6 × 10^4 cells in 200 μl of growth medium and incubated overnight at 37°C to achieve confluence. The monolayers were fixed with gluta-

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Abbreviations: EB, elementary body; OGP, *n*-octyl β-D-glucopyranoside; OGPE, OGP buffer extract of the elementary bodies; CCA, chlamydial cytadhesin; MOMP, major outer membrane protein.

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raldehyde using a method similar to that employed for the detection of bacterial cytadhesins in *Mycoplasma pneumoniae* (7), *Treponema pallidum* (8), and *Trichomonas vaginalis* (9). Briefly, the medium from each culture well was carefully aspirated and the monolayers were washed once with ice-cold PBS; 100 μl of 2.5% (vol/vol) glutaraldehyde in PBS was then added to each well, including those without host cells. After 1 hr at 4°C, the wells were washed thrice with ice-cold PBS and incubated overnight at 4°C with 200 μl of 2% bovine serum albumin in PBS.

Binding of Chlamydial Proteins to HeLa Monolayers. Various amounts (5–25 μl) of the dialyzed OGPE of ^{125}I- or ^{35}S-labeled EBs were added in duplicate (final volume, 25 μl per well) to 96-well culture dishes containing fixed or unfixed HeLa monolayers. To detect nonspecific binding of the labeled components in the extract, various amounts of OGPE were also added in duplicate to wells without the host cells. When unfixed HeLa cell monolayers were used, the OGPE was diluted to prevent detachment of the host cells. After incubation at 4°C for 1 hr with gentle rocking, the wells were washed thrice with ice-cold PBS. The monolayer in each well was solubilized with 25 μl of Laemmli buffer (10) at room temperature, and labeled chlamydial proteins were resolved by SDS/PAGE after boiling the samples (10). Radioactive proteins in the gels were detected by fluorography or by autoradiography, depending on the isotope.

Inhibition of Adherence of ^{125}I-Labeled EBs to HeLa Cells by OGPE. Various amounts of OGPE were added in triplicate (final volume, 25 μl per well) to fixed or unfixed HeLa monolayers in 96-well culture plates. Dialyzed OGP buffer controls without the chlamydial proteins were included. The plates were incubated at 4°C for 1 hr with gentle rocking followed by the addition of 5 μl of ^{125}I-labeled purified EBs ($35–50 \times 10^3$ cpm) to each well. The input of the labeled EBs was selected after determining saturation kinetics of adherence (1) of each preparation of the iodinated EBs in the presence of dialyzed OGP buffer. After 1 hr at 4°C to allow attachment of the labeled EBs, cell-bound radioactive EBs were assayed (1, 5).

Inhibition of Binding of the 38-kDa Protein to HeLa Cells by Viable EB. Percoll-purified, viable EBs (120 μg of protein) were mixed with ^{125}I-labeled OGPE and incubated with fixed HeLa monolayers at 4°C for 1 hr with gentle rocking. Cell-bound radioactive chlamydial proteins were analyzed by SDS/PAGE and autoradiography as described in the preceding sections.

Effect of Trypsin on the Activities of the CCA. An ^{125}I-labeled or unlabeled Percoll-purified EB suspension (200 μg protein) was incubated with either 20 μg or 100 μg of trypsin in PBS at 37°C for 2 hr. Trypsin-treated EBs were sedimented, washed twice with ice-cold PBS, and extracted with OGP buffer. Dialyzed extracts were used for the cell-binding and the EB-adherence-inhibition experiments.

Effect of Heat on the Activities of the CCA. Unlabeled or ^{125}I-labeled EBs in PBS (350 μg of protein) were incubated either at 60 or 75°C for 15 min. After sedimentation, the EB pellet was extracted with OGP buffer, and the extract was dialyzed and used for the cytadherence and EB-adherence-inhibition experiments. In addition, dialyzed OGPE prepared from untreated EBs was heated at 60 or 75°C for 15 min prior to use.

Inhibition of Chlamydial Adherence Effected by the CCA in the OGPE. Various amounts of ^{125}I-labeled OGPE from serovars B or L1 were analyzed by the binding assay for specific adherence of only the 38-kDa protein to fixed HeLa cells. At 1.5–3.5 μg of protein, only the 38-kDa protein was found to bind to HeLa cells. For the inhibition of chlamydial adherence, unlabeled OGPE (1.5–3.5 μg of protein) from the EBs of serovar B was incubated in triplicate with fixed HeLa cells at 4°C for 1 hr. After washing thrice with ice-cold PBS, labeled EBs were added, and adherence in 30 min was assayed.

Inhibition of Chlamydial Infectivity by CCA-Containing OGPE. For the inhibition of chlamydial infectivity, unlabeled OGPE (1.5–3.5 μg of protein) from serovar L1 was incubated in triplicate with unfixed HeLa cells at 4°C for 1 hr. Viable chlamydiae were then added to the pretreated cells. After 30 min at 4°C, the monolayers were fed with growth medium containing cycloheximide (1 μg/ml) and incubated at 37°C for 48 hr; the inclusion-forming units were detected with the anti-chlamydial monoclonal antibody KB-8 (1) and fluorescein isothiocyanate-conjugated anti-mouse antibody (Zymed). The 96-well plate was inverted and examined by fluorescence microscopy (×40 objective, Leitz).

RESULTS

Binding of the 38-kDa Chlamydial Protein to HeLa Monolayers. The highly purified ^{125}I-labeled EBs of *C. trachomatis* serovar E and the corresponding OGPE contained a 38-kDa protein (Fig. 1*A*, lanes 1 and 2). Incubation of the OGPE with HeLa cells fixed with glutaraldehyde (to prevent detachment during incubation with the detergent extract) revealed that the 38-kDa chlamydial protein bound to the cells in a dose-dependent manner (Fig. 1*A*, lanes 4 and 5). When a high concentration of the extract was used, additional chlamydial proteins also bound to the cells (Fig. 1*A*, lane 6). However, many of these proteins, especially the MOMP, also bound to the cell-free plastic surface of the culture dish (Fig. 1*A*, lane 3). The 38-kDa protein, on the other hand, did not bind to the plastic surface of the cell culture dish (Fig. 1*A*, lane 3, and *C*, lane 2). Similar results (i.e., exclusive cytadherence of the 38-kDa protein) were obtained using unfixed HeLa cells incubated with diluted OGPE from the EBs of serovar B (Fig. 1*B*, lanes 2 and 3). Binding of the MOMP to the plastic surface, seen with the OGPE of serovar E (Fig. 1*A*, lane 3), was detected with the preparation from serovar B as well (Fig. 1*B*, lane 4). *C. trachomatis* serovar L1 was labeled metabolically during intracellular growth with [^{35}S]cysteine plus [^{35}S]methionine in the presence of the eukaryotic protein synthesis inhibitor emetine. EBs were purified and extracted with OGP buffer, and the cytadherence of the labeled chlamydial proteins in the extract was measured. Fig. 1*C* shows that the 38-kDa protein was present in the extract (lane 1) and bound specifically to HeLa monolayers (lane 3). It should be noted that the low molecular mass components seen in the autoradiographs of the ^{125}I-labeled samples (Fig. 1 *A* and *B*) were absent in the ^{35}S-labeled OGPE (Fig. 1*C*).

Mock-infected HeLa cells were subjected to the purification procedure utilized for the chlamydiae (1), and the final pellet was iodinated and extracted with OGP buffer. The dialyzed extract was incubated with glutaraldehyde-fixed HeLa monolayers. Autoradiographs of SDS/PAGE gels of the detergent extract contained a single radioactive band comigrating with bovine serum albumin. Analysis by SDS/PAGE and autoradiography showed that the extract did not contain any material capable of binding to the HeLa cells.

Inhibition of Adherence of ^{125}I-Labeled EBs to HeLa Cells by OGPE. HeLa cell monolayers were preincubated at 4°C for 1 hr with two doses of OGPE from the purified EBs of serovar E, and the adherence of ^{125}I-labeled purified EBs of the same serovar was assayed. A 37% inhibition of adherence of labeled EBs to HeLa cells was obtained with OGPE containing 1.75 μg of protein, whereas a sample containing 3.5 μg of protein gave 72% inhibition. Inhibition of adherence of ^{125}I-labeled EBs by OGPE was also examined using homologous and heterologous serovars of *C. trachomatis*. The adherence of the EBs of heterologous serovars was inhibited by OGPE prepared from the three serovars (Table 1), con-

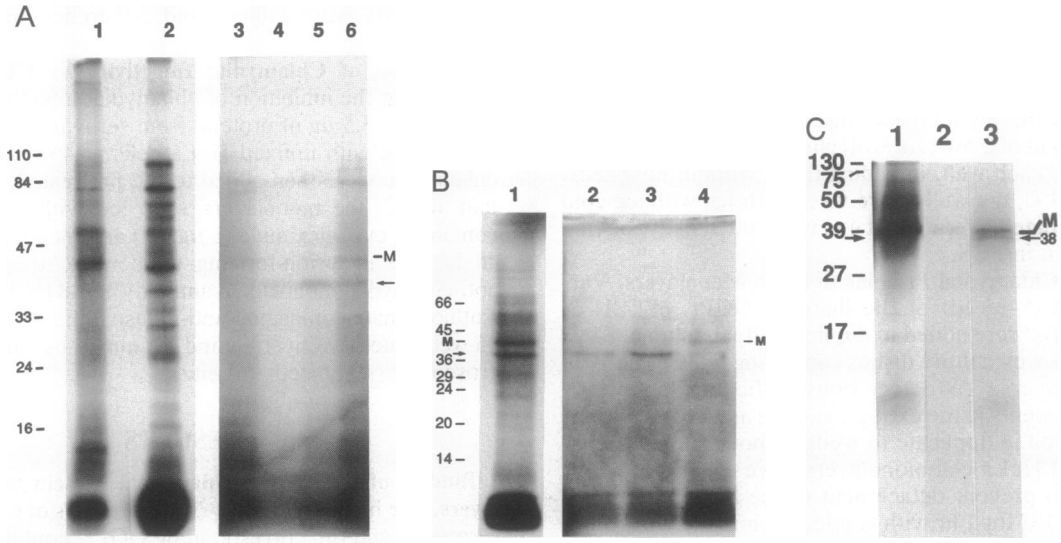

FIG. 1. (*A*) Autoradiograph of a 10–20% gradient gel (SDS/PAGE). Lanes: 1, total lysate of highly purified ^{125}I-labeled EBs of *C. trachomatis* serovar E; 2, OGPE from the EBs; 3, proteins in the OGPE that bound to the plastic surface of the 96-well cell culture dish; 4–6, the proteins that bound to glutaraldehyde-fixed HeLa cells. The amount of OGPE protein per well was 5.25 μg (lanes 3 and 6), 1.75 (lane 4), and 3.5 μg (lane 5). Molecular mass standards in kDa are shown on the left. The position of the 38-kDa protein is shown with an arrow, and the position of the MOMP is shown with the letter M. (*B*) Autoradiograph of a 12.5% gel (SDS/PAGE) of ^{125}I-labeled OGPE from EBs of serovar B incubated with unfixed HeLa cells. Lanes: 1, protein profile of the OGPE; 2 and 3, labeled proteins in OGPE (1.75 and 2.8 μg of protein, respectively) that bound to unfixed HeLa cells; 4, labeled proteins in OGPE (3.5 μg of protein) that bound to the plastic surface of the 96-well culture dish. Molecular size markers in kDa are shown on the left. The 38-kDa protein is shown by an arrow and the MOMP is indicated by M. (*C*) Fluorograph of a 15% gel (SDS/PAGE) of OGPE of ^{35}S-labeled EBs of serovar L1, incubated with HeLa cells. Lanes: 1, protein profile of the OGPE; 2, labeled proteins bound to the plastic surface of the culture dish; 3, cell-bound labeled protein in the OGPE. Protein content of the OGPE used for adherence assay represented in lanes 2 and 3 was 2.3 μg per well. Molecular mass markers are on the left, the MOMP is shown by M, and the 38-kDa protein is shown by arrows.

firming our previous data showing sharing of a common receptor by different serovars of *C. trachomatis* (1).

Inhibition of Binding of the 38-kDa Protein in the OGPE to HeLa Cells by Viable EBs. OGPE from ^{125}I-labeled *C. trachomatis* serovar E and unlabeled viable EBs of serovar E were mixed and incubated with glutaraldehyde-fixed HeLa monolayers. In the presence of viable EBs, there was a significant inhibition of binding of the 38-kDa protein in the OGPE to host cells (Fig. 2, lanes 2 and 4). This indicated that viable EBs and the 38-kDa protein in the OGPE competed for the same receptor on host cells. No proteolytic activity, especially in the presence of the protease inhibitors in the OGP buffer, could be detected in the Percoll-purified unlabeled EB preparations used in this and other experiments. No decrease was seen in the trichloroacetic acid-precipitable radioactivity in the ^{125}I-labeled OGPE incubated with the purified EBs. Densitometry of the autoradiograph also failed to indicate any additional accumulation of cell-associated ^{125}I-labeled fast-moving bands in the samples incubated with the unlabeled purified EBs. Trypsin- or heat-treated EBs were unable to effect the inhibition of cytadherence of the 38-kDa protein in the OGPE (data not shown).

Table 1. Inhibition of adherence to HeLa cells of ^{125}I-labeled EBs of *C. trachomatis* by OGPE prepared from heterologous serovars

Exp.	Serovar of labeled EB	% inhibition of adherence: L1 extract	B extract	E extract
1	L1	60	54	61
2	B	75	71	71
3	E	64	66	53

OGPE (10 μl) from the EBs of serovars L1, B, or E was incubated with fixed HeLa cells for at 4°C. After 1 hr, ^{125}I-labeled EBs of homologous or heterologous serovars were added and incubation was continued for another hour. Cell-associated labeled EBs were assayed. Labeled EBs added per well were as follows: L1, 22 × 10^3 cpm; B, 48 × 10^3 cpm; and E, 47 × 10^3 cpm. Protein concentration of extracts were as follows: L1, 0.45 μg/μl; B, 0.5 μg/μl; E, 0.35 μg/μl.

Effect of Trypsin Treatment. The extract prepared from the Percoll-purified labeled EBs treated with trypsin did not contain any CCA (Fig. 3*A*, lane 3), suggesting surface location of this chlamydial protein. After an extensive trypsin treatment of the EBs, the OGPE from it contained rapidly migrating bands at or near the dye front (Fig. 3*A*, lane 4). Some of these peptides, presumably generated from the CCA, bound to cells (Fig. 3*B*, lane 5). The adherence of labeled EBs to HeLa cells was partially inhibited by the extracts from trypsin-treated EB, reflecting the persistence

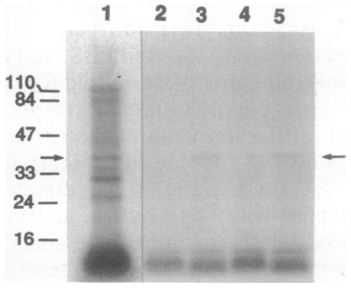

FIG. 2. Autoradiograph of a 12.5% gel (SDS/PAGE) showing the inhibition of binding of the 38-kDa protein in the OGPE from *C. trachomatis* serovar E by unlabeled chlamydiae. Lanes: 1, profile of the OGPE from ^{125}I-labeled EB; 3 and 5, binding of the 38-kDa protein to HeLa cells at protein concentrations of 1.7 and 2.8 μg; 2 and 4, inhibition of the binding in the presence of unlabeled EB of serovar E (120 μg of protein). Molecular mass markers (kDa) are shown on the left; arrows show the position of the 38-kDa protein. Densitometry of the autoradiograph showed that no additional accumulation of the fast-moving labeled material occurred in the samples containing EBs (lanes 2 and 4).

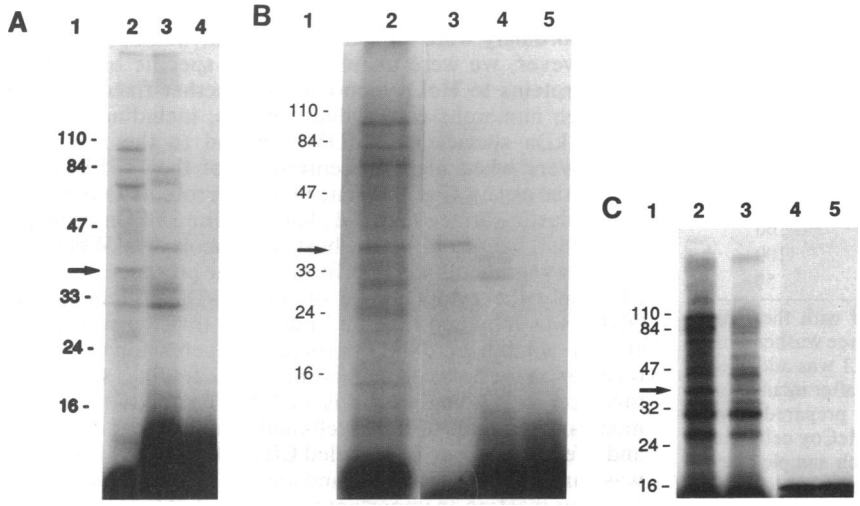

FIG. 3. Autoradiographs of SDS/PAGE gels showing the trypsin and heat lability of binding of the 38-kDa chlamydial protein to HeLa cells. Lanes 1 show the molecular mass markers in kDa. (*A*) Lanes: 2, OGPE of ^{125}I-labeled serovar E; 3 and 4, OGPE prepared from ^{125}I-labeled EB that were treated with 20 and 100 μg of trypsin. (*B*) Lanes: 2, OGPE; 3, iodinated proteins in the OGPE that bound to HeLa cells; 4 and 5, iodinated proteins from trypsin-treated (20 and 100 μg) EBs that bound to HeLa cells. (*C*) Lanes: 2 and 3, OGPE from untreated and heat-treated EBs; 4 and 5, lack of binding of the OGPE (1.7 and 3.5 μg of protein, respectively) from the heat-treated EBs. *A* and *B* were 10–20% gradient gels, and *C* was a 12.5% gel.

of the cell-binding ability of tryptic peptides produced from the CCA.

Effect of Heat Treatment. A labeled EB suspension was incubated at 60 or 75°C for 15 min and extracted with OGP; the OGPE was incubated with HeLa monolayers. After SDS/PAGE and autoradiography, no CCA could be detected in the host cells that had been incubated with such extracts (Fig. 3*C*, lanes 4 and 5), even though the CCA was present in the OGPE (Fig. 3*C*, lane 3). Thermal inactivation of cytadherence occurred after incubation of the EB suspension at both temperatures. In addition, after exposure of the OGPE to heat, inactivation of cytadherence of the CCA in the OGPE was also observed (Fig. 4, lanes 2 and 4).

We also examined the thermolability of inhibition of chlamydial adherence to HeLa cells by heat-treated OGPE. The extract after exposure to 60°C for 15 min failed to inhibit the adherence of labeled EB to HeLa cells (Table 2). In addition, data summarized in Table 2 show that after treatment of the EB with trypsin, the extract did not contain any adherence-inhibitory activity. These results confirm the cytadherence data.

Protection Effected by Pretreatment of HeLa Cells with OGPE. Glutaraldehyde-fixed HeLa cells were incubated with various amounts (1.5–3.5 μg) of OGPE prepared from purified EBs of *C. trachomatis* serovar B that had been propagated in McCoy cells, an alternative host of the bacterium. The amount of protein in the extract was selected so that only the CCA present therein bound to the host cells. After 1 hr at 4°C, the cells were washed and then the adherence ability of the pretreated host cells was assayed using ^{125}I-labeled EBs of serovar L1. An extract-dose-dependent inhibition was seen (Table 3), indicating that the cell-bound material from the OGPE remained capable of inhibiting the adherence of the EB.

To ascertain that the 38-kDa protein present in the detergent extracts from the purified EB indeed bound to the HeLa cells and not to an extracellular matrix produced by the monolayer cultures, infectivity measurements were performed with viable HeLa cells. A 40–50% inhibition of chlamydial infectivity occurred after pretreatment of the host cells with OGPE. At the doses used (1.5–3.5 μg of protein), only the CCA present in the OGPE bound to the HeLa cells. No protection was afforded by OGPE that had been exposed to heat (data not shown).

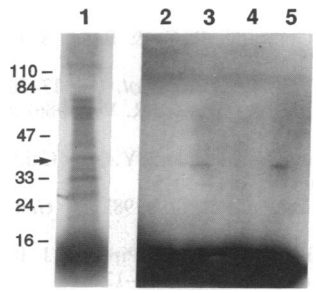

FIG. 4. Autoradiograph of a 12.5% gel (SDS/PAGE) of ^{125}I-labeled OGPE from EBs of serovar E heated at 60°C for 15 min and then incubated with fixed HeLa cells. Lanes: 1, protein profile of labeled OGPE; 2 and 4, lack of binding of the 38-kDa protein in the OGPE (1.7 and 2.8 μg of protein, respectively) after the heat treatment; 3 and 5, binding of the 38-kDa protein in the unheated OGPE (1.7 and 2.8 μg of protein, respectively) to HeLa cells. Molecular mass markers (kDa) are shown on the left and the 38-kDa protein is shown by an arrow.

DISCUSSION

Based on the kinetics of inhibition of adherence to monolayer cultures of HeLa cells of variously labeled chlamydiae of one serovar by unlabeled chlamydiae of heterologous serovars, it was proposed that the chlamydiae attach to the same receptor moieties on the host cells (1). By trypsin or heat treatment of the EB suspension, the adherence-inhibitory effect was lost (1). The data indicated the participation of thermolabile trypsin-sensitive surface components on the chlamydiae in the adherence reaction. Since the heated chlamydiae exhibited residual adherence, we proposed that multiple ligands, some heat-stable and others thermolabile, participated in the adherence step (1).

Several cytadhesins have been identified by mild extraction of pathogenic bacteria with nonionic detergents such as OGP, by using formaldehyde- or glutaraldehyde-fixed host cell cultures (7–9). Formaldehyde-fixed mouse L cells have been used to study the adherence of *Chlamydia psittaci* (11). In the present study, characteristic saturation kinetics of

Table 2. Loss of chlamydial adherence-inhibitory activity after heating of the OGPE or in OGPE prepared from trypsin-treated EBs

Treatment	^{125}I-labeled EBs bound	
	cpm	% of control
None	663 ± 69	100
OGPE	385 ± 16	58
Heated OGPE	616 ± 101	93
OGPE from trypsin-treated EB	515 ± 21	78

Labeled EBs of serovar B added was 34×10^3 cpm per well. OGPE from the EB of serovar E was heated at 60°C for 15 min. Data are mean ± SD.

Table 3. Sustained inhibition of chlamydial adherence by pretreatment of the host cells with OGPE

Addition	^{125}I-labeled EBs bound	
	cpm	% of control
Buffer (5 μl per well)	984 ± 18	100
Extract (5 μl per well)	804 ± 20	82
Buffer (8 μl per well)	1128 ± 114	100
Extract (8 μl per well)	750 ± 31	66
Buffer (10 μl per well)	1134 ± 140	100
Extract (10 μl per well)	670 ± 18	59

Glutaraldehyde-fixed HeLa cells were incubated with the indicated additions in triplicate for 60 min at 4°C. After three washes with PBS, 56×10^3 cpm of ^{125}I-labeled EBs of serovar L1 was added to each well. Cell-bound radioactivity was determined after incubation at 4°C for 30 min. Protein content of the OGPE prepared from purified EB of *C. trachomatis* serovar B grown in McCoy cells was 0.32 μg/μl. OGP-containing buffer controls for each sample were included in the assay since variable adherence was observed with the controls for the same concentration of ^{125}I-labeled EBs. Data for ^{125}I-labeled EBs bound are mean ± SD.

adherence of purified EBs extrinsically labeled with ^{125}I to glutaraldehyde-fixed HeLa monolayer cultures were routinely observed. By using both unfixed and fixed HeLa cells, we have identified a 38-kDa chlamydial protein present in OGPE that selectively binds to HeLa monolayers (Fig. 1 *A*, lanes 4 and 5, *B*, lanes 2 and 3, *C*, lane 3). This protein was present in EBs of all three *C. trachomatis* serovars, grown in either of the two host cells, HeLa or McCoy. Chlamydial EBs were labeled metabolically with [^{35}S]methionine plus [^{35}S]cysteine in the presence of emetine during intracellular replication of the bacterium, and the EBs were purified and extracted with OGP buffer. The ^{35}S-labeled 38-kDa protein bound to the host cells was detected by fluorography of the gel (Fig. 1*C*, lane 3), indicating the prokaryotic origin of this cytadhesin. Mock-infected HeLa cells, subjected to the purification procedure utilized (1), did not contain any cellular material that could be iodinated, confirming our demonstration of little, if any, contamination of the chlamydial EBs with host protein (1), nor did cells contain the 38-kDa host-binding protein or any other cytadhesin. In the purified EBs, differential iodination of this protein and its sensitivity to trypsin indicate surface localization of the 38-kDa protein (Fig. 3*A*, lane 3).

Viable EBs inhibited the binding of the 38-kDa protein to the host cells (Fig. 2, lanes 2 and 4). In addition, the adherence of radioactive EBs to HeLa cells was inhibited by the OGPE containing the 38-kDa protein. We could demonstrate between 50 and 80% inhibition of adherence of the labeled EBs to HeLa monolayers by using OGPEs from homologous and heterologous serovars (Table 1), confirming our previous data showing the participation of a common receptor in the adherence of chlamydiae to host cells (1). Paucity of the 38-kDa protein in the EB has prevented us from purifying this protein and examining the adherence-inhibitory activity of isolated CCA. However, the absence of the 38-kDa protein in the extract from purified EBs treated with trypsin, and the failure of such preparations to effect the inhibition of adherence of labeled EB (Table 2), clearly indicates that the active component in the OGPE is the 38-kDa chlamydial protein. The cytadherence (Fig. 3) and the ability to inhibit chlamydial adherence to host cells (Table 2) were found to be thermolabile.

Two proteins of ≈18 and ≈32 kDa were resolved by SDS/PAGE from chlamydial samples that had been boiled in SDS-containing buffer; after electrophoretic transfer to nitrocellulose membranes, the proteins showed the ability to bind labeled membrane fragments isolated from HeLa cells and accordingly were implicated as chlamydial adhesins (2, 3). However, we were unable to detect specific binding of these proteins to HeLa monolayers, whether fixed or not. Although numerous chlamydial proteins, including the 18- and 32-kDa species and MOMP, bound to the host cell monolayers when high concentrations of the OGPE were used in the assays (Fig. 1*A*, lane 6), these proteins also bound to the plastic surface (Fig. 1*A*, lane 3). The 38-kDa protein, in contrast, bound only to the host cell monolayers. When the binding experiments were performed in the presence of viable EBs, only the cytadherence of the 38-kDa protein in the OGPE was inhibited (Fig. 2), indicating that the whole EB and the solubilized 38-kDa protein competed for the same receptor on the host cell membrane. These findings show that this receptor-binding protein is a CCA. The ability to bind to monolayer cultures of HeLa cells and to inhibit the adherence and infectivity of purified labeled EBs to the host cell cultures was found to be thermolabile and located on the surface of the EB, thus meeting an important requirement of the chlamydial adhesin (1).

The strategy we employed to identify the chlamydial protein with cell-binding ability is similar to strategies employed for identifying other microbial cytadhesins (7–9). In addition to its cytadherence property, the CCA inhibited the attachment as well as subsequent inclusion formation of viable chlamydiae. Viable chlamydiae, but not heated or trypsin-treated samples, inhibited specifically the cytadherence of the CCA. Similar activities have not been reported for the three other putative chlamydial adhesins. Chlamydial adherence is a multifactorial process mediated by heat-labile as well as heat-stable moieties present on the surface of the microorganism (1). We have demonstrated herein that the MOMP and the 38-kDa CCA, isolated under nondenaturing conditions from highly purified EBs, are capable of binding to cells, although only the CCA exhibits specific cytadherence.

This report discusses the identification and properties of a chlamydial protein present in highly purified EBs that is involved in adherence of EB to host cells and is capable of interfering with the attachment of viable chlamydiae. Paucity of this protein has prevented its purification. Cloning of the CCA gene and expression in a suitable system should yield quantities of this protein sufficient for further studies of chlamydial attachment to host cells. Investigation of the possibility of using chlamydial adhesin for prophylactic purposes should also be facilitated with such preparations.

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1. Vretou, E., Goswami, P. C. & Bose, S. K. (1989) *J. Gen. Microbiol.* **135,** 3229–3237.
2. Hackstadt, T. (1986) *J. Bacteriol.* **165,** 13–20.
3. Wenman, W. M. & Meuser, R. V. (1986) *J. Bacteriol.* **165,** 602–607.
4. Su, H., Watkins, N. G., Zhang, Y.-X. & Caldwell, H. D. (1990) *Infect. Immun.* **58,** 1017–1025.
5. Bose, S. K. & Paul, R. G. (1982) *J. Gen. Microbiol.* **128,** 1371–1379.
6. Tuszynski, G. P., Knight, L., Piperno, J. R. & Walsh, P. N. (1980) *Anal. Biochem.* **106,** 118–122.
7. Krause, D. C. & Baseman, J. B. (1982) *Infect. Immun.* **37,** 382–386.
8. Baseman, J. B. & Hayes, E. C. (1980) *J. Exp. Med.* **151,** 573–586.
9. Alderete, J. F. & Garza, G. E. (1988) *Infect. Immun.* **56,** 28–33.
10. Laemmli, U. K. (1970) *Nature (London)* **227,** 680–685.
11. Hatch, T. P., Vance, D. W. & Al-Hossainy, E. (1981) *J. Gen. Microbiol.* **125,** 273–283.