INFECTION AND IMMUNITY, May 1985, p. 546–551
0019-9567/85/050546-06$02.00/0

Vol. 48, No. 2

Effect of Proteolytic Cleavage of Surface-Exposed Proteins on Infectivity of *Chlamydia trachomatis*

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Received 3 December 1984/Accepted 31 January 1985

The proteolytic cleavage of *Chlamydia trachomatis* LGV-434 surface proteins and resultant effects on infectivity and association with cultured human epithelial (HeLa) cells have been examined. Of several proteases examined, trypsin, chymotrypsin, and thermolysin extensively cleaved the chlamydial major outer membrane protein (MOMP). Two proteases, trypsin and thermolysin, cleaved the MOMP to the extent that monomeric MOMP was not detectable by immunoblotting with monospecific polyclonal antibodies. In the case of thermolysin, not even antigenic fragments were detected. Surprisingly, infectivity toward HeLa cells was not diminished. In addition, the association of intrinsically ^{14}C-radiolabeled elementary bodies (EBs) with HeLa cells or their dissociation by proteinase K was not measurably affected by prior trypsinization of the EBs. Trypsinization of lactoperoxidase surface-iodinated elementary bodies demonstrated that most of the ^{125}I-labeled surface proteins were cleaved. In all cases, however, a number of proteolytic cleavage fragments remained associated with the EB surface after surface proteolysis. When trypsinized EBs were electrophoresed under nonreducing conditions and immunoblotted with either polyclonal or type-specific monoclonal MOMP antibodies, MOMP was found in a large oligomeric form that failed to enter the polyacrylamide stacking gel. Additionally, trypsinized viable EBs bound radioiodinated type-specific MOMP monoclonal antibody as efficiently as did the control nontrypsinized organisms. Taken together, the findings indicate that although the MOMP is highly susceptible to surface proteolysis, the supramolecular structure of the protein on the EB surface is apparently maintained by disulfide interactions. Thus, if surface-exposed chlamydial proteins are involved in the initial interaction of chlamydiae with eucaryotic cells, the functional domains of these proteins which mediate this interaction must be resistant to proteolysis and remain associated with the EB surface.

Chlamydiae are obligate intracellular bacteria that replicate within phagosomal vesicles of eucaryotic cells. The developmental cycle of chlamydiae is complex, involving an extracellular infectious cell type, the elementary body (EB), and a metabolically active but noninfectious reticulate body that multiplies by binary fission (3, 15, 29). Chlamydiae resemble gram-negative bacteria in their cell wall structure but differ from them in that chlamydiae lack demonstrable peptidoglycan (1, 17, 24, 36). It is believed that the structure conferring rigidity to the EB cell wall is a network of disulfide cross-linked outer membrane proteins (2, 18, 19, 27). A predominant component of this structure is the chlamydial major outer membrane protein (MOMP). This protein has been estimated to make up as much as 60% of the total outer membrane protein (8). In extracts of freshly purified EBs, MOMP is found in monomeric, dimeric, and oligomeric forms. On the EB surface, a proportion of the MOMP also appears to be linked via disulfide bonds to form a large supramolecular structure dissociable with reducing agents (2, 18, 19, 27). The MOMP from reticulate bodies also appears in monomeric, dimeric, and oligomeric forms, although cross-linking is less extensive than that of EBs (18, 19). Reductive cleavage of this disulfide-mediated supramolecular structure therefore appears to precede differentiation and growth to the reticulate body stage (18, 19).

In addition to its structural role, the MOMP appears also to be an exposed surface antigen with different antigenic domains conferring serotype, serogroup, and species reactivities (8, 34). The predominance alone of this surface-exposed protein (8) and neutralization of infectivity by polyclonal rabbit anti-MOMP antisera (10) has led to speculation that this protein may be involved in the interaction of chlamydiae with host cells.

To explore possible roles of MOMP and other surface proteins, we have subjected intact, purified EBs to a variety of proteases and examined the effects of this surface proteolysis on the ability of chlamydiae to interact with eucaryotic cells. The conditions used resulted in extensive cleavage of surface-exposed chlamydial proteins but had surprisingly little effect on infectivity.

MATERIALS AND METHODS

Organisms. *Chlamydia trachomatis* LGV-434, serotype L2, was grown in suspension cultures of mouse L-929 cells, and EBs were purified as described previously (8). Intrinsic radiolabeling of EBs with ^{14}C-amino acids was also done as described previously (10). EBs were surface radiolabeled with Na^{125}I (ICN, Irvine, Calif.) by using lactoperoxidase and hydrogen peroxide (25).

Infectivity determinations. Inclusion-forming units (IFUs) were determined by the method of Furness et al. (16) as modified by Hackstadt et al. (18).

PAGE. Polyacrylamide gel electrophoresis (PAGE) has already been described (22), as have immunoblotting procedures (18).

Immunological reagents. Preparation and specificity of the immunoglobulin G fraction of hyperimmune rabbit polyclonal antisera against purified sodium dodecyl sulfate (SDS)-denatured *C. trachomatis* (L2 serotype) MOMP have been described previously (11). The monoclonal antibody L2-1-6, which recognizes a genus-specific epitope on the lipopolysaccharide of chlamydiae, has been described previously (7).

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Monoclonal antibodies L2-1-45 and L2-1-10 were similarly prepared and recognized type- and species-specific epitopes, respectively, located on the MOMP of *C. trachomatis* LGV-434 (H. D. Caldwell, manuscript in preparation). A control monoclonal antibody (C53) reactive with a surface protein of *Borrelia hermsii* was kindly provided by Alan Barbour, Rocky Mountain Laboratories.

Binding of monoclonal antibodies to surface-proteolysed EBs. Monoclonal antibodies were radioiodinated by the Iodogen (Pierce Chemical Co., Rockford, Ill.) procedure (14). Control or trypsinized EBs were incubated for 30 min at 37°C in 50 mM sodium phosphate–150 mM NaCl (pH 7.4), containing 3% bovine serum albumin (PBSA) and 2.0 μg (5.2×10^4 to 7.2×10^4 cpm) of the control of chlamydia-specific ^{125}I-monoclonal antibodies. The EBs were pelleted in a microcentrifuge and washed twice with PBSA before determination of associated radioactivity with a Beckman Gamma 4000 (Beckman Instruments, Inc., Fullerton, Calif.).

Surface proteolysis of EBs. Purified *C. trachomatis* LGV-434 EBs (approximately 100 to 150 μg of protein in 200 μl of 10 mM sodium phosphate–15 mM NaCl [pH 7.4] [PBS]) were pulsed with 10 μl of protease (1 mg/ml of 0.001 N HCl) four times at equal intervals over a 2-h incubation period at 37°C. Phenylmethylsulfonyl fluoride was added after 2 h, and the suspension was chilled to 4°C and pelleted in a Beckman microfuge 12 (Beckman Instruments). The pellets were resuspended in PBS and layered over 1 ml of 30% (vol/vol) Renografin (E. R. Squibb & Sons, Inc., Princeton, N.J.) and pelleted through the Renografin pad by centrifugation at 70,000 × *g* for 30 min. The pellet was carefully resuspended in 1 ml of 250 mM sucrose–10 mM sodium phosphate–5 mM glutamate buffer (pH 7.2) (SPG) and dissociated by vigorous vortexing (proteolysed EBs tended to aggregate more than control EBs). A portion was taken for determination of IFUs, and the remainder was pelleted in a Beckman Microfuge 12 and solubilized for PAGE.

Binding of ^{14}C-chlamydiae to HeLa cells. Association of control or trypsinized ^{14}C-chlamydiae with host cells was determined by a method similar to that used by Söderlund and Kihlström (33). Monolayers of HeLa 229 cells were seeded 24 h earlier at a density of 5×10^5 cells per ml (3 ml per well) in 6-well plastic tissue culture plates (Flow Laboratories, Inc., McLean, Va.). Control or trypsinized EBs were suspended in cold Hanks balanced salt solution, and the medium was aspirated from the monolayers. The HeLa cells were inoculated with 1 ml of the ^{14}C-EBs per well and incubated at 4°C for 2 h, after which the monolayers were washed rapidly three times with cold PBS, 1 ml of PBS was added to half the wells, and 1 ml of PBS plus 250 μg of proteinase K per ml (Boehringer Mannheim Biochemicals, Indianapolis, Ind.) was added to the remaining wells. The plates were held for an additional 30 min at 4°C. The monolayers were dislodged with a rubber policeman and transferred to a prechilled conical glass centrifuge tube. The wells were washed once with cold PBS, and the wash was pooled with the cells in the centrifuge tube. The cells and associated chlamydiae were pelleted by centrifugation at 250 × *g* for 5 min at 5°C, the pellet was washed once with 4 ml of cold PBS, 1 ml of 0.1 N NaOH was added to the pellet, and the cells and associated ^{14}C-chlamydiae were solubilized at 70°C for 45 min. The solubilized cells and chlamydiae were transferred to scintillation vials, and 7.5 ml of Aquasol (New England Nuclear Corp., Boston, Mass.) was added to each vial. Associated counts were determined by liquid scintillation spectroscopy, and proteinase K-sensitive and -resistant radioactivity was calculated.

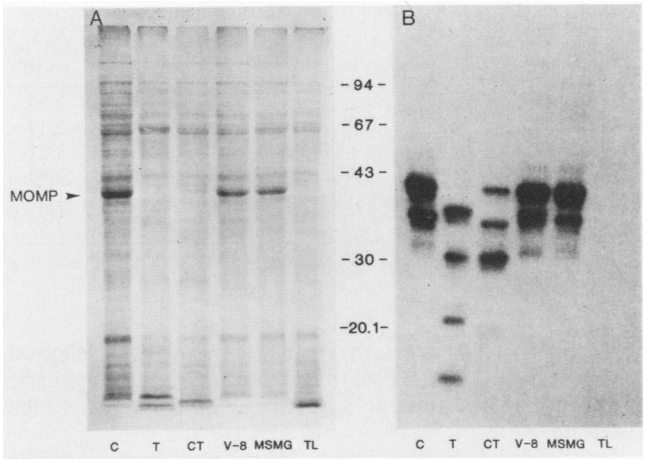

FIG. 1. Effect of protease treatment of intact *C. trachomatis* LGV-434 EBs. The EBs were treated with no protease (control [C]), trypsin (T), α-chymotrypsin (CT), staphylococcal V-8 protease (V-8), mouse submaxillary gland protease (MSMG), or thermolysin (TL), as described in the text. The EBs were washed and solubilized for electrophoresis in Laemmli buffer (22) plus 2-mercaptoethanol and applied in parallel to 12.5% acrylamide SDS-PAGE. Gels were either stained with CBB (A) or immunoblotted with rabbit polyclonal anti-*C. trachomatis*, L2 serotype, MOMP immunoglobulin G (B). The position and molecular weight of markers are indicated. Note that even in the control lane some cleavage of the MOMP is apparent. The fragments do not appear to comigrate on SDS-PAGE with any of the fragments generated by the proteases used here. It is not clear whether these fragments result from spontaneous breakdown of the MOMP, endogenous proteases, or exposure to host proteases during purification, but they do seem to vary in amount between chlamydial preparations.

RESULTS

Protease susceptibility of EBs. A battery of proteases was examined for cleavage of *C. trachomatis* (L2 serotype) EB surface-exposed protein, and effects on infectivity. A Coomassie brilliant blue (CBB)-stained gel and accompanying immunoblot of L2 EBs electrophoresed after surface proteolysis are shown in Fig. 1. Trypsin, chymotrypsin, and thermolysin all cleaved MOMP to the extent that its monomeric form is not apparent on CBB-stained gels. Staphylococcal V-8 protease and mouse submaxillary gland (MSMG) protease had little effect. On this 12.5% polyacrylamide gel, two CBB staining fragments are seen near the dye front in the trypsin-treated EB lane and one fragment from the chymotrypsin- and thermolysin-treated EBs. These fragments are not reactive with the anti-MOMP antibody by immunoblotting. In the immunoblot of a parallel gel with rabbit polyclonal anti-MOMP antibody, some fragments of MOMP are apparent even in the control EBs incubated in the absence of protease. EBs incubated in the presence of V-8 or MSMG protease are similar to the control, although one additional large fragment is seen in the V-8-treated EBs. While chymotrypsin treatment reduced amounts of monomeric MOMP to undetectable levels by CBB staining, some residual monomeric MOMP is detected by immunoblotting, as are two or three large fragments. No monomeric MOMP is seen after trypsin treatment, but four predominant polypeptides, reactive with anti-MOMP antibodies, remain associated with the EBs. Thermolysin treatment cleaved MOMP to such an extent that no immunoreactive species was detected.

TABLE 1. Effect of protease treatment on chlamydial infectivity[a]

Protease	IFU/ml ($\times 10^6$)
None	2.65
Trypsin	2.11
Chymotrypsin	2.81
V-8	2.41
MSMG	1.32
Thermolysin	2.63

[a] Infectivity of those preparations subjected to SDS-PAGE and immunoblotted in Fig. 1.

Although two proteases, trypsin and thermolysin, cleaved surface-exposed MOMP to the extent that no monomeric MOMP was detectable, no reduction in infectivity was seen (Table 1).

Trypsin concentration. Increasing ratios of trypsin to EBs result in fewer antigenic fragments and fragments of lower molecular weight than those seen in the less extensively cleaved samples (Fig. 2). At the highest trypsin concentration, five antigenic fragments are detected by immunoblotting with the polyclonal anti-MOMP antibodies. In this 15% polyacrylamide gel, up to five additional CBB staining fragments in the molecular weight range of 3,000 to 14,000 are resolved. The intensity of their CBB staining suggests that these fragments may be cleavage products of MOMP; however, only one of these low-molecular-weight (8,000) fragments is reactive by immunoblotting with anti-MOMP antibody. The lack of reactivity by immunoblotting is not due to failure of these peptides to transfer to or remain associated with the nitrocellulose paper, since intrinsically ^{14}C-amino acid-labeled chlamydial peptides after trypsinization transferred to and remained associated with the nitrocellulose paper during a mock immunoblot (data not shown). Attempts to demonstrate that these fragments were of MOMP origin by radioimmunoprecipitation of control and trypsinized ^{14}C-EBs were unsuccessful (data not shown) as a result of the coprecipitation of unrelated polypeptides or epitopes. Since we did not precipitate these polypeptides with the control anti-ovalbumin antisera, we presumed that the lack of specificity of radioimmunoprecipitations seen even with monoclonal antibodies (data not shown) was due to the formation of mixed micelles among the hydrophobic fragments of MOMP and other outer membrane proteins. The origin of these fragments therefore remains unknown. In nine separate trypsinizations in which cleavage of monomeric MOMP was virtually complete, the mean number of IFUs after proteolysis was 103.0% ± 20.0% ($\bar{x}$ ± standard error of the mean) of control samples.

Trypsinization of surface-iodinated EBs. To examine the effects of proteolysis of surface-exposed proteins other than MOMP, *C. trachomatis* LGV-434 EBs were surface iodinated by the lactoperoxidase method and treated as controls or with trypsin as described above (Fig. 3). A number of ^{125}I-containing bands are apparent in the lane containing the control EBs. In the EBs treated with trypsin, however, most of ^{125}I-labeled surface proteins are cleaved from the surface or migrate on SDS-PAGE as small fragments near the dye front. A single ^{125}I-labeled polypeptide of about 15,000 daltons appears to be more resistant to cleavage by trypsin.

Trypsinization of [^{35}S]cysteine-labeled EBs. Disulfide-mediated cross-linking of chlamydial outer membrane proteins (2, 18, 19, 27) appears to confer structural rigidity to these gram-negative bacteria which lack demonstrable peptidoglycan (1, 17, 24, 36). In addition, disulfide exchange of chlamydial proteins with eucaryotic cell components has been proposed as a mechanism potentially involved in the interactions of chlamydiae with host cells (18). Because of the importance of disulfide bonding and its regulation in the life cycle of chlamydiae (18, 19), we examined the effects of trypsinization on EBs intrinsically labeled with [^{35}S]cysteine (Fig. 4). A number of [^{35}S]cysteine-containing bands remain associated with surface-proteolysed EBs. Again, the origin of most of these fragments is unknown, although the intensity of exposure suggests that many may be fragments of MOMP.

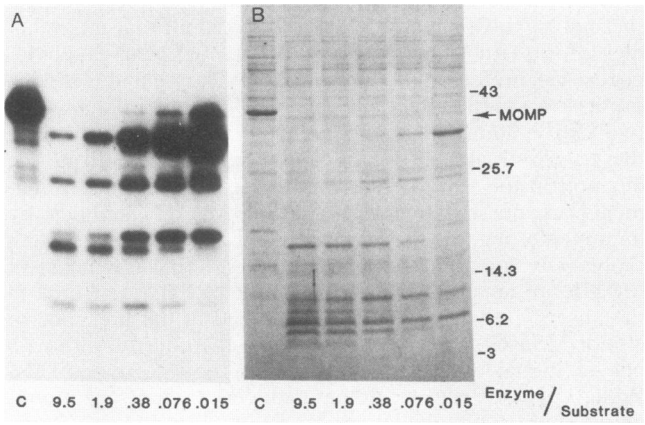

FIG. 2. Effect of trypsin concentration on cleavage of the *C. trachomatis* LGV-434 MOMP. Various enzyme-to-substrate protein ratios are shown. The acrylamide concentration in the gel depicted here was 15%; there is therefore considerably better resolution of the low-molecular-weight fragments. Antigenic fragments were detected by immunoblotting with rabbit polyclonal anti-*C. trachomatis*, L2, MOMP immunoglobulin G (A). A CBB-stained gel is also shown (B).

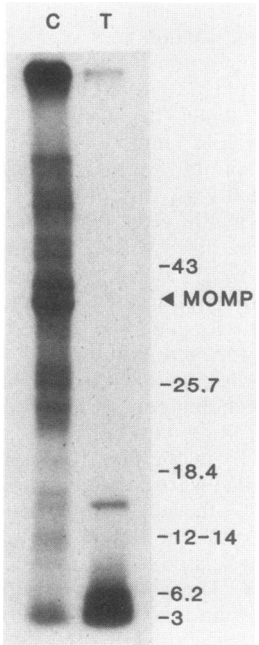

FIG. 3. Autoradiogram of *C. trachomatis* LGV-434 EBs surface iodinated by the lactoperoxidase method (24) and treated as controls or with trypsin.

MOMP epitopes remaining surface associated after proteolysis. The preceding figures demonstrate that cleavage products of a number of chlamydial surface proteins remain associated with the EBs after trypsinization. Although intact monomeric MOMP is not apparent by immunoblotting in most cases after surface trypsinization, it is clear that certain antigenic domains remain associated with the EB surface. To examine the interaction of MOMP after trypsinization, EBs that had been treated as controls or with trypsin were solubilized in the absence of 2-mercaptoethanol, subjected to SDS-PAGE, and immunoblotted. Antigenic species were detected with rabbit polyclonal monospecific anti-MOMP antibodies or monoclonal antibodies reactive with type- or species-specific epitopes on the MOMP (Fig. 5). From these control or trypsinized EBs in which disulfide interactions of the MOMP have not been disrupted by reducing agents, MOMP is detected in large aggregates that fail to enter even the stacking gel. These large aggregates are characteristic of interactions of MOMP on the EB surface and were believed to make up portions of the disulfide-linked supramolecular structure which confers structural stability to the EBs (18). It is clear that even after trypsinization, MOMP fragments remain extensively cross-linked via disulfide bonds. It is further apparent that the type-specific epitope of the MOMP remains associated with these aggregates and is antigenic even after trypsinization. In contrast, either the species-specific epitope of the MOMP is cleaved from the surface or its antigenicity destroyed by trypsin.

Additional evidence for the association of the type-specific epitope of MOMP with the EB surface after trypsinization was obtained by examining the binding of ^{125}I-labeled monoclonal antibodies to control or trypsinized EBs (Table 2). Binding of the type-specific monoclonal antibody to trypsinized EBs was greater than 70% of control values. In contrast, binding of the species-specific monoclonal antibodies to trypsinized EBs was reduced to background levels.

Association of trypsinized EBs with cultured cells. Control of trypsinized *C. trachomatis* LGV-434 EBs intrinsically labeled with ^{14}C-amino acids were allowed to bind HeLa cell monolayers at 4°C. The monolayers were washed, and we attempted to release bound but not internalized chlamydiae by using proteinase K at 4°C, as has been described previously (33). As was also described, proteinase K removed only about 40% of the chlamydiae bound at 4°C (33). No difference in association of ^{14}C-EBs with HeLa cells or their release by proteinase K was observed whether the EBs were first trypsinized or not (Table 3).

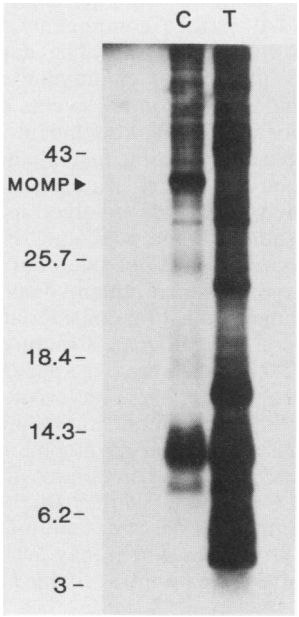

FIG. 4. Autoradiogram of *C. trachomatis* LGV-434 EBs intrinsically labeled with [^{35}S]cysteine and treated as controls or with trypsin.

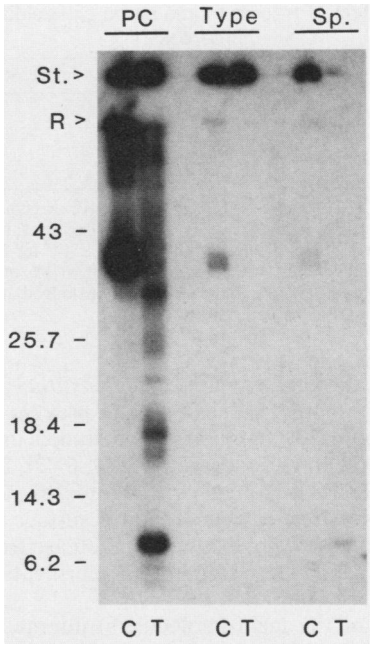

FIG. 5. Immunoblot analysis of MOMP interactions in the absence of reduction. Control or trypsinized *C. trachomatis* LGV-434 EBs were solubilized for SDS-PAGE in the absence of 2-mercaptoethanol, electrophoresed, and transferred to nitrocellulose, and antigenic species were detected by using polyclonal (PC) rabbit anti-MOMP IgG (11), monoclonal antibody L2-1-45 (type), or monoclonal antibody L2-1-10 (Sp.). Note that in these unreduced preparations much of MOMP apparently fails to enter even the stacking gel whether or not the EBs had been trypsin treated. The interface of the stacking gel (St.) and 15% acrylamide resolving gel (R) are indicated.

DISCUSSION

Processes critical to the intracellular life-style of chlamydiae are attachment to and ingestion by eucaryotic cells and the inhibition of phagosome-lysosome fusion (3, 15, 29). It is believed that the EB surface mediates both ingestion by eucaryotic cells and prevention of phagosome-lysosome fusion (13, 23). In contrast to rickettsiae (37), also obligate intracellular procaryotes, UV-killed EBs attach to and are ingested by host cells as efficiently as are infectious EBs (5,

TABLE 2. Binding of radioiodinated monoclonal antibodies to control or trypsinized *C. trachomatis* LGV-434 EBs

L2 EBs	Cpm bound[a] for:			
	C53 (control)	45 (type)	10 (Sp.)	6 (LPS)
Control	168 ± 13	18,928 ± 825	1,186 ± 7	3,445 ± 3
Trypsinized	212 ± 56	13,695 ± 149	190 ± 14	2,949 ± 117

[a] Mean ± standard error of the mean.

TABLE 3. Effect of trypsin treatment on chlamydial binding to HeLa cells[a]

EBs	Cpm bound for cells:	
	−Proteinase K	+Proteinase K
Control	1,040 ± 79[b]	594 ± 162
Trypsin	1,018 ± 46	668 ± 57

[a] A total of 8.5×10^4 to 8.8×10^4 cpm of control or trypsin-treated ^{14}C-amino acid intrinsically labeled EBs in Hanks balanced salt solution were added to monolayers of HeLa cells and maintained at 4°C for 2 h. Monolayers were washed and incubated for an additional 30 min at 4°C in the absence or presence of proteinase K (250 μg/ml). Cells were dislodged and washed, and associated radioactivity was determined.

[b] Mean ± standard error of the mean.

6). Indeed, purified cell envelopes of *C. psittaci* attach to and are ingested by mouse L cells (23) and prevent phagosome-lysosome fusion (13). A number of studies have demonstrated that attachment or infectivity (4, 6, 20, 23) of EBs is not susceptible to treatment with proteases, although the extent of proteolytic cleavage of the EB surface was minimal (23) or, in most cases, not examined. Protease treatment has, in fact, been included as a step in some purification protocols (33, 35).

The nature of the macromolecule(s) interacting with the eucaryotic host cell is not known. Attachment to host cells and prevention of phagosome-lysosome fusion by intact EBs (4–6, 20, 23) and purified cell envelopes (13, 23) is inhibited by mild heat treatment (56°C for 30 min). This sensitivity to heat suggests denaturation of a critical polypeptide, although masking of the chlamydial ligand by heat treatment remains a viable alternative. In attempts to identify essential surface-exposed proteins potentially involved in interactions of chlamydiae and host cells, we have examined the effects of exposure of intact EBs to a variety of proteases.

The studies described above demonstrate that proteases cleave a number of exposed proteins on the surface of *C. trachomatis* LGV 434 EBs but that this surface proteolysis does not impair infectivity for cultured human cells. It is unlikely that resynthesis of cleaved chlamydial protein has taken place to restore infectivity, since the metabolic activity of EBs is minimal (3, 18, 29), and the EBs after proteolysis were either plated immediately or frozen at −70°C until IFUs could be determined. These results speak against a role for surface-exposed, protease-sensitive domains of MOMP or other chlamydial surface proteins in the initial interaction of chlamydiae with host cells. If such proteins are involved in interaction of chlamydiae and host cells, the active domains of these proteins must remain functional and associated with the EB surface after surface proteolysis. A number of proteolytic cleavage products remained associated with the EBs, although the origin of most of these remains questionable since only one of several low-molecular-weight tryptic fragments reacted with anti-MOMP antibodies by immunoblotting. The mechanism by which these fragments remain associated with the EB surface was not studied in detail, although it seems reasonable to suspect that hydrophobic interactions of membrane-embedded polypeptides play a major role. In view of the known disulfide interaction of chlamydial outer membrane proteins (2, 18, 19, 27), an additional possibility might be that cysteine-containing peptides remain associated with the surface via disulfide bonding. Therefore, although we have shown that protease-sensitive regions of surface proteins are not required for infectivity, it is likely that, if chlamydial surface-exposed proteins are involved in host cell interaction, the active sites of these proteins may be protease resistant or nonantigenic, or both. Alternatively, polypeptides involved in interaction with the host may not be exposed or accessible on the EB surface until some association with the host has occurred. Teleologically, protease resistance and nonimmunogenicity of the protein domains involved in host cell interactions might be advantageous for a microorganism that is exposed to a variety of proteases in its natural habitat.

Another consideration, although hypothetical, is that proteolytic cleavage by either exogenous (host) or endogenous (chlamydial) protease may be actually required for exposure of an active site, and so the treatments described here may have only superceded a required function. Analogies might be drawn from the F-glycoprotein of paramyxoviruses, which is the mediator of virus penetration and virus-induced cell fusion (26, 30–32). In this system an inactive precursor (F) protein is activated by proteolytic cleavage to yield two membrane active disulfide-linked polypeptides (32).

A role for reductive cleavage or disulfide exchange in the differentiation of EBs to reticulate bodies seems likely (18, 19). On the basis of analogies with insulin-receptor interactions (12) and diphtheria toxin internalization (38), we have speculated that disulfide exchange may also play a role in interactions of chlamydiae and hosts. The data presented here do not rule out that possibility, since a number of cysteine-containing fragments remain associated with the EB surface although the origin of many of these cleavage products remains unknown.

Although it seems clear that exposure of EBs to proteolytic enzymes does not impair their infectivity toward cultured human epithelial cells, a role for these surface-exposed proteins may exist in the pathogenesis of chlamydial infection in the normal host. The ability to remain infectious after proteolytic cleavage of peptide epitopes suggests the intriguing possibility that chlamydial antigen-antibody complexes could be cleaved from the EB surface. Thus, the serologically dominant, surface-exposed, and protease-sensitive antigens may be expendable in regard to the actual internalization events and prevention of phagosome-lysosome fusion.

We began these studies as one approach toward identification of chlamydial surface components involved in the initial interaction with host cells. The data indicate that protease-sensitive domains of chlamydial membrane proteins are not essential for the initial events of interaction of chlamydiae and host, including attachment, internalization, inhibition of phagosome-lysosome fusion, and differentiation to the reticulate body. Whether protease-sensitive portions of outer membrane proteins are involved in the reorganization and condensation of the reticulate body to the EB remains in question. We have not ruled out polypeptides as the mediators of interaction of chlamydiae and host; however, other mechanisms must be considered.

Despite the diversity in antigenic structure (9) and genetic composition (21, 28) between the two species of *Chlamydia*, both species share a number of characteristics. These shared characteristics include site of replication, inhibition of phagosome-lysosome fusion, a similar developmental cycle (3, 29), and a common mechanism of maintaining structural stability in the absence of a peptidoglycan (2, 18, 19, 27) and some surface components (7). We are continuing attempts to identify common structures which may act as determinants of virulence in these obligate intracellular parasites.

ACKNOWLEDGMENTS

We thank Jim Simmons and Bob Cole for technical assistance and Susan Smaus for typing the manuscript. The helpful comments of

John Swanson, Paul Barstad, Greg McDonald, and the staff of the Laboratory of Microbial Structure and Function are greatly appreciated.

LITERATURE CITED

1. **Barbour, A. G., K.-I. Amano, T. Hackstadt, L. Perry, and H. D. Caldwell.** 1982. *Chlamydia trachomatis* has penicillin-binding proteins but not detectable muramic acid. J. Bacteriol. **151**:420–428.
2. **Bavoil, P., A. Ohlin, and J. Schachter.** 1984. Role of disulfide bonding in outer membrane structure and permeability in *Chlamydia trachomatis*. Infect. Immun. **44**:479–485.
3. **Becker, Y.** 1978. The chlamydia: molecular biology of procaryotic obligate parasites of eucaryotes. Microbiol. Rev. **42**: 274–306.
4. **Bose, S. K., and R. G. Paul.** 1982. Purification of *Chlamydia trachomatis* lymphogranuloma venereum elementary bodies and their interaction with HeLa cells. J. Gen. Microbiol. **128**:1371–1379.
5. **Byrne, G. I.** 1976. Requirements for ingestion of *Chlamydia psittaci* by mouse fibroblasts (L cells). Infect. Immun. **14**:645–651.
6. **Byrne, G. I., and J. W. Moulder.** 1978. Parasite-specified phagocytosis of *Chlamydia psittaci* and *Chlamydia trachomatis* by L and HeLa cells. Infect. Immun. **19**:598–606.
7. **Caldwell, H. D., and P. J. Hitchcock.** 1984. Monoclonal antibody against a genus-specific antigen of *Chlamydia* species: location of the epitope on chlamydial lipopolysaccharide. Infect. Immun. **44**:306–314.
8. **Caldwell, H. D., J. Kromhout, and J. Schachter.** 1981. Purification and partial characterization of the major outer membrane protein of *Chlamydia trachomatis*. Infect. Immun. **31**:1161–1176.
9. **Caldwell, H. D., C. C. Kuo, and G. E. Kenny.** 1975. Antigenic analysis of chlamydiae by two-dimensional immunoelectrophoresis. I. Antigenic heterogeneity between *C. trachomatis* and *C. psittaci*. J. Immunol. **115**:963–968.
10. **Caldwell, H. D., and L. J. Perry.** 1982. Neutralization of *Chlamydia trachomatis* infectivity with antibodies to the major outer membrane protein. Infect. Immun. **38**:745–754.
11. **Caldwell, H. D., and J. Schachter.** 1982. Antigenic analysis of the major outer membrane protein of *Chlamydia* spp. Infect. Immun. **35**:1024–1031.
12. **Clark, S., and L. C. Harrison.** 1982. Insulin binding leads to the formation of covalent (—S—S—) hormone receptor complexes. J. Biol. Chem. **257**:12239–12244.
13. **Eissenberg, L. G., P. B. Wyrick, C. H. Davis, and J. W. Rumpp.** 1983. *Chlamydia psittaci* elementary body envelopes: ingestion and inhibition of phagolysosome fusion. Infect. Immun. **40**:741–751.
14. **Fraker, P. J., and J. C. Speck, Jr.** 1978. Protein and cell membrane iodinations with a sparingly soluble chloramide, 1,3,4,6-tetrachloro-3α,6α-diphenylglycoluril. Biochem. Biophys. Res. Commun. **80**:849–857.
15. **Friis, R.** 1972. Interaction of L cells and *Chlamydia psittaci*: entry of the parasite and host responses to its development. J. Bacteriol. **110**:706–721.
16. **Furness, G., D. M. Graham, and P. Reeve.** 1960. The titration of trachoma and inclusion blennorrhoea viruses in cell cultures. J. Gen. Microbiol. **23**:613–619.
17. **Garrett, A. J., M. J. Harrison, and G. P. Manire.** 1974. A search for the bacterial mucopeptide component, muramic acid, in *Chlamydia*. J. Gen. Microbiol. **80**:315–318.
18. **Hackstadt, T., W. J. Todd, and H. D. Caldwell.** 1985. Disulfide-mediated interactions of the chlamydial major outer membrane protein: role in the differentiation of chlamydiae? J. Bacteriol. **161**:25–31.
19. **Hatch, T. P., I. Allen, and J. H. Pearce.** 1984. Structural and polypeptide differences between envelopes of infective and reproductive life cycle forms of *Chlamydia* spp. J. Bacteriol. **157**:13–20.
20. **Hatch, T. P., D. W. Vance, Jr., and E. Al-Hossainy.** 1981. Attachment of *Chlamydia psittaci* to formaldehyde-fixed and unfixed L cells. J. Gen. Microbiol. **125**:273–283.
21. **Kingsbury, D. T., and E. Weiss.** 1968. Lack of deoxyribonucleic acid homology between species of the genus *Chlamydia*. J. Bacteriol. **96**:1421–1423.
22. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
23. **Levy, N. J., and J. W. Moulder.** 1982. Attachment of cell walls of *Chlamydia psittaci* to mouse fibroblasts (L cells). Infect. Immun. **37**:1059–1065.
24. **Manire, G. P., and A. Tamura.** 1967. Preparation and chemical composition of the cell walls of mature infectious dense forms of meningopneumonitis organisms. J. Bacteriol. **94**:1178–1183.
25. **Morrison, M.** 1974. The determination of the exposed proteins on membranes by the use of lactoperoxidase. Methods Enzymol. **32**:103–109.
26. **Nagai, Y., H. Ogura, and H.-D. Klenk.** 1976. Studies on the assembly of the envelope of Newcastle disease virus. Virology **69**:523–538.
27. **Newhall, W. J., V, and R. B. Jones.** 1983. Disulfide-linked oligomers of the major outer membrane protein of chlamydiae. J. Bacteriol. **154**:998–1001.
28. **Peterson, E. M., and L. M. de la Maza.** 1983. Characterization of *Chlamydia* DNA by restriction endonuclease cleavage. Infect. Immun. **41**:604–608.
29. **Schachter, J., and H. D. Caldwell.** 1980. Chlamydiae. Annu. Rev. Microbiol. **34**:285–309.
30. **Scheid, A., and P. W. Choppin.** 1974. Identification of biological activities of Paramyxovirus glycoproteins. Activation of cell fusion, hemolysis, and infectivity by proteolytic cleavage of an inactive precursor protein of Sendai virus. Virology **57**:475–490.
31. **Scheid, A., and P. W. Choppin.** 1976. Protease activation mutants of Sendai virus. Activation of biological properties by specific proteases. Virology **69**:265–277.
32. **Scheid, A., and P. W. Choppin.** 1977. Two disulfide-linked polypeptide chains constitute the active F protein of paramyxoviruses. Virology **80**:54–66.
33. **Söderlund, G., and E. Kihlström.** 1983. Effect of methylamine and monodansylcadaverine on the suceptibility of McCoy cells to *Chlamydia trachomatis* infection. Infect. Immun. **40**:534–541.
34. **Stephens, R. S., M. R. Tam., C. C. Kuo, and R. C. Nowinski.** 1982. Monoclonal antibodies to *Chlamydia trachomatis*: antibody specificities and antigen characterization. J. Immunol. **128**:1083–1089.
35. **Tamura, A., and N. Higashi.** 1963. Purification and chemical composition of meningopneumonitis virus. Virology **20**:596–604.
36. **Tamura, A., and G. P. Manire.** 1967. Preparation and chemical composition of the cell membranes of developmental reticulate forms of meningopneumonitis organisms. J. Bacteriol. **94**:1184–1188.
37. **Walker, T. S., and H. H. Winkler.** 1978. Penetration of cultured mouse fibroblasts (L cells) by *Rickettsia prowazeki*. Infect. Immun. **22**:200–208
38. **Wright, H. T., A. W. Marston, and D. J. Goldstein.** 1984. A functional role for cysteine disulfides in the transmembrane transport of diphtheria toxin. J. Biol. Chem. **259**:1649–1654.