Adherence of Multiple Serovars of *Chlamydia trachomatis* to a Common Receptor on HeLa and McCoy Cells Is Mediated by Thermolabile Protein(s)

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(Received 12 June 1989; revised 17 August 1989; accepted 12 September 1989)

Several aspects of the adherence of purified elementary bodies (EB) of *Chlamydia trachomatis* to HeLa and to McCoy cells were examined using different techniques, including an ELISA. Serovar-specific, biotinylated monoclonal antibodies were used to detect cell-bound chlamydiae. In addition, purified chlamydiae were biotinylated and their adherence properties were studied. The assays were done at 4 °C to exclude the energy-dependent internalization of the cell-bound EB and host-cell membrane recycling that occur at 37 °C. Saturation kinetics were routinely observed at 4 °C, and the rate of adherence remained linear for approximately 60 min. Lineweaver–Burk analysis of the kinetics data showed that adherence of any one serovar was competitively inhibited by other serovars of *C. trachomatis*. This competition for the same receptor on the two alternative hosts, HeLa and McCoy, was also seen when the adherence assays were done at 37 °C in the presence of sodium azide, an energy poison that inhibits endocytosis of cell-bound chlamydiae. Chlamydiae exposed to 56 °C for 5 min, or treated with low doses of trypsin, failed to exhibit competitive inhibition, having suffered considerable loss of the ability to adhere to host-cells. These data suggest that heat- and trypsin-labile chlamydial moieties participate in the adherence reaction, and that oculo-genital serovars of *C. trachomatis*, including that of lymphogranuloma venereum, attach to the same receptor on the host-cell membrane.

INTRODUCTION

Chlamydia trachomatis is an obligate intracellular bacterial pathogen that must attach to the membrane of a susceptible eukaryotic cell in order to initiate its replication cycle. Little is known about the nature of the chlamydial surface-exposed ligand(s) that recognize specific receptors on the host-cell membrane. Heat-lability of attachment, as first noted by Kuo & Grayston (1976) and by Byrne & Moulder (1978), suggests the participation of a proteinaceous component in the infectious entity, the so-called elementary body (EB). In our studies (Bose & Smith, 1984; Bose & Goswami, 1986*a, b*), the rate of chlamydial adherence was assayed at 4 or 5 °C in order to exclude the post-adherence internalization of the cell-bound EB and the recycling of the host-receptor that normally occur at higher temperatures. While Kuo & Grayston (1976) noted that association at room-temperature of both LGV L2 and the B serovar of *C. trachomatis* with HeLa cells was drastically reduced when radioactively labelled EB were exposed to 56 °C for 30 min, Lee (1981) was unable to detect heat-lability of association of *C. trachomatis* serovar A with McCoy cells at 37 °C. We observed that only during incubation of the EB/host-cell reactants at 5 °C could heat-lability of adherence both of LGV and non-LGV serovars be discerned (Bose & Goswami, 1986*a*). Detection of the response to lectin wheat-germ agglutinin and to haptenic sugars also requires incubation at 5 °C (Bose & Goswami, 1986*a*).

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Abbreviations: EB, elementary bodies; HSC, HEPES/sucrose/cation buffer.

Reduced adherence to glycosylation-defective mutants of CHO cells is detectable only when the assays are done at 4 °C (Bose & Goswami, 1986b).

By enzyme-linked immunoassay (ELISA) utilizing serovar-specific monoclonal antibodies, biotinylated EB, and radiolabelled, purified EB, we investigated several aspects of the adherence properties of LGV L1 and two non-LGV serovars of *C. trachomatis* to the two alternative hosts, HeLa and McCoy cells. Under appropriate conditions of assay, saturation kinetics were readily observed with the serovars examined.

Some of the data reported here were first presented by Vretou *et al.* (1988a, b).

METHODS

Organisms. *C. trachomatis* serovars B, E and LGV440 (L1) were grown in HeLa and in McCoy cells (Bose & Goswami, 1986a). Chlamydia-infected cells were disrupted by sonication with glass beads (Vibra-Cell sonicator; 30% output, 6 mm microtip). Cellular debris was removed by centrifugation at 200 *g* for 10 min, and the supernatant was filtered through a 600 nm pore-size polycarbonate membrane (Nuclepore) to remove the chlamydial reticulate bodies (Bose & Paul, 1982). The EB were purified using a Percoll gradient procedure (Newhall *et al.*, 1982), and single-use aliquots were stored at -80 °C in HEPES/sucrose/cation buffer (HSC; 10 mM-HEPES, pH 7.3, 1 mM-MgCl₂, 1.5 mM-CaCl₂, 90 mM-NaCl, 0.2 M-sucrose).

In order to determine the extent to which any host-derived protein co-purified with the final EB preparation, a mixing experiment was done using chlamydia-infected cells and uninfected host-cells labelled with a tritiated amino acids mixture. Less than 0.02% of the label appeared in the purified EB (Table 1). Electron microscope examination of the EB preparations showed that unless filtration of the low-speed supernatant through a 600 nm pore-size polycarbonate membrane (Bose & Paul, 1982) was included, the samples contained about 20% chlamydial reticulate bodies as had been noted by Newhall *et al.* (1982).

Biotinylation of the EB. Percoll-purified EB suspension was adjusted to a protein concentration of 1 mg ml⁻¹ and centrifuged in an Eppendorf microcentrifuge for 15 min at 4 °C. After carefully removing the supernatant, the EB were resuspended at the same concentration in 0.1 M-sodium bicarbonate containing 0.145 M-NaCl and 0.2 M-sucrose (pH 8.0). Sulfo-NHS-biotin (Pierce Chemical Co.) was dissolved in water at 1 mg ml⁻¹, and 5 µl was added per 100 µl of the EB suspension. After gentle mixing, the suspension was kept at 0 °C for 2 h. Biotinylation was stopped by adding 2 vols of serum-free minimum essential medium, and after 15 min the mixture was applied to a Sephadex G-25 Quicksep column (Isolab) pre-equilibrated with 0.5% (w/v) BSA in PBS (Bose & Goswami, 1986a). Fractions (100 to 150 µl) were collected and the presence of EB in the fractions was detected using a dot assay with a serovar-specific monoclonal antibody. In several experiments, purified radioactively labelled EB were co-chromatographed and served as a marker. Fractions 7, 8 and 9 containing the biotinylated EB were pooled. The EB were concentrated by centrifugation, resuspended in HSC buffer and stored at -80 °C.

Assay for chlamydial adherence to cell cultures. *A, Biotinylated monoclonal antibody-based assessment.* This technique was developed by Ridderhof & Barnes (1988), who used KB-8, the E- and L1-specific monoclonal antibody to assay chlamydial adherence to HeLa cultures in 96-well dishes. Briefly, the anti-B (DH-8) and anti-E (KB-8) immunoglobulins, kindly provided to us as ascites fluid by Dr Robert C. Barnes of the Centers for Disease Control, Atlanta, GA, USA, were purified using a Beckman recombinant protein A-agarose column, and after dialysis against 0.1 M-sodium bicarbonate, pH 8.0, biotinylation was effected with biotin succinimide. Following dialysis against PBS containing 0.02% sodium azide, the IgG was stabilized with 1 mg BSA ml⁻¹.

HeLa or McCoy cells were plated in 96-well cell culture dishes at 5×10^4 cells in 200 µl of growth medium per well and incubated at 37 °C. The next day the medium was carefully aspirated and the wells were filled with ice-cold growth medium. After 30 min at 0 °C, the monolayers were washed once with ice-cold PBS, and the assay was initiated in triplicate wells by addition of 25 µl per well of appropriately diluted, purified EB suspension in HSC buffer. Incubation was for 45 to 60 min at 4 °C with continuous rocking on a Belco rocker set at maximum speed. The adherence reaction was stopped by adding 200 µl ice-cold PBS to each well, and the monolayers were washed rapidly with ice-cold PBS at least three times. The cells were fixed with methanol. For the ELISA, methanol was removed and the fixed cells were washed three times with PBS, blocked for 1 h with 3% (w/v) BSA in PBS at 37 °C, and then incubated with biotinylated monoclonal antibody for 1 h at 37 °C. After washing five times with PBS containing 0.01% Tween 20, the biotinylated anti-chlamydial IgG bound to the EB attached to the host cells was detected quantitatively with streptavidin-conjugated β -galactosidase, using *o*-nitrophenyl galactopyranoside as the chromogen.

B, Direct assay with biotinylated EB. When biotinylated EB were used, the cell-bound EB were detected with streptavidin-conjugated β -galactosidase. Identical results were obtained using either assay procedure.

C, Assay with radioactively labelled EB. Adherence assays with EB labelled during intracellular replication with a mixture of [³⁵S]methionine and [³⁵S]cysteine (Trans S; ICN Radiochemicals, Irvine, CA) were done in 24-well or 96-well multidishes as described earlier (Bose & Paul, 1982; Bose *et al.*, 1983; Bose & Goswami, 1986a).

Table 1. *Host-protein contribution to the EB of C. trachomatis serovar B purified by the modified Percoll procedure*

A tritiated amino acid mixture was used to label the proteins of uninfected HeLa 229 G1 cells. The labelled cells were mixed with unlabelled HeLa cells infected with serovar B, and the EB were purified essentially according to Newhall *et al.* (1982) (see Methods). The low-speed supernatant was filtered through a 600 nm pore-size polycarbonate membrane filter. Samples (10 μ l) were removed at each step, and hot-TCA-precipitable radioactivity was determined. A parallel extract of labelled, uninfected HeLa cells was simultaneously processed; the radioactivity appearing in the final pellet was 0.02% of the starting material, the same as in the purified EB (data not shown).

Fraction	Vol. (ml)	$10^{-5} \times$ Total radioactivity (c.p.m.)	(%)
Sonicate	12.0	287	(100)
Low-speed supernatant	11.5	187	(65)
30000 g pellet	2.0	19.8	(7)
Percoll-layer pellet	1.4	0.518	(0.18)
Pellet, through 30% sucrose cushion	0.5	0.07	(0.02)

RESULTS

In our previous studies on the adherence characteristics of *C. trachomatis* to monolayer cultures of HeLa, McCoy and CHO cells and the glycosylation-defective mutant cell lines of CHO (Bose & Paul, 1982; Bose *et al.*, 1983; Bose & Goswami, 1986*a, b*) we utilized metabolically radioactively labelled, highly purified preparations of chlamydial EB. Whenever possible, the data obtained with radioactive EB were confirmed by infectivity determinations. However, the experiments with highly radioactive preparations suffered from the fact that radioactive decay occurred in the ^{32}P - or ^{35}S -labelled material, resulting in the loss of infectivity, and the possible introduction of artifacts in these assays. We therefore sought to develop assays that did not require radioactive material, and could be used to screen monoclonal-antibody-producing hybridomas that affected chlamydial adherence. Use of either biotinylated monoclonal anti-chlamydial IgG or biotinylated EB enabled us to do the adherence assays in 96-well dishes, and to bypass the need for radioactively labelled chlamydiae. Comparison of the data obtained with the three different techniques convinced us that while detection of the cell-bound EB did not approach the sensitivity obtained with ^{32}P -labelled material (Bose & Smith, 1984), all three methods for the adherence assay provided equivalent data. Of the several serovar-B-specific monoclonal antibodies tested, only one, DH-8, was found to be capable of detecting cell-surface-bound EB. In the experiments with the serovar E, the monoclonal antibody utilized was KB-8. The epitopes recognized by these monoclonal antibodies were not heat-labile as measured by dot assay or ELISA (data not shown).

Adherence of C. trachomatis serovars B and E to HeLa and McCoy cells is mediated via heat-labile and heat-stable ligands

Adherence assays were done at 4 °C to distinguish the initial attachment of the EB to the host-cell membrane from the subsequent temperature-dependent endocytosis step. The rate of adherence of serovar E to McCoy cell monolayers remained linear for approximately 75 min, declining thereafter (Fig. 1), as reported previously with serovar K (Bose & Goswami, 1986*a*). Similar kinetics of adherence were obtained with serovar B and HeLa or McCoy cells (data not shown). Therefore, all adherence kinetic-studies reported here were done at 4 °C, with the incubation period not exceeding 60 min. Using ELISA-based detection of the cell-associated EB, exposure of the EB suspension to 56 °C for 10 min resulted in a 30 to 50% reduction in adherence rate (Fig. 1). Assays done at 37 °C indicated no reduction in the adherence rate of heated EB (data not shown), similar to the situation noted with serovars A (Lee, 1981), and K (Bose & Goswami, 1986*a*). Further incubation of the EB at 56 °C did not result in additional decrease in the chlamydial adherence-rate (data not shown), suggesting the participation of additional heat-stable chlamydial adhesin(s) in this reaction.

Fig. 1

Fig. 2

Fig. 1. Kinetics of adherence of purified EB of serovar E to McCoy cell monolayers, before (□) and after (■) exposure to 56 °C for 10 min. The assay was done at 0 °C in a 96-well cell culture dish. Cell-bound *C. trachomatis* was detected by biotinylated monoclonal antibody KB-8. The experiment was repeated five times. The bars represent SD values from triplicate samples.

Fig. 2. Adherence of biotinylated EB of *C. trachomatis* serovar B to HeLa cell monolayers at 4 °C for 60 min as a function of chlamydial dose. Similar data were obtained in four experiments with different preparations of chlamydiae. The bars represent SD values from triplicate samples.

Fig. 3. Double-reciprocal plot of the adherence rates of *C. trachomatis* serovar B to McCoy cell monolayers, before (■) and after (□) heating at a temperature of 56 °C for 10 min, as a function of chlamydial dose. After incubation at 4 °C for 60 min, cell-bound EB were detected with biotinylated monoclonal antibody DH-8. The Figure was generated with the Analytical Graphics program for an Apple micro-computer, using linear regression to draw the lines. The experiment was repeated three times.

Saturation of chlamydial receptors on HeLa and McCoy cells

With increasing input of purified biotinylated EB of serovar B to HeLa cells, a Michaelis-Menten type of adherence kinetics with rectangular hyperbolae could be reproducibly obtained (Fig. 2). Saturation of adherence with increasing chlamydial dose was obtained with LGV L1 and serovars B and E as well using the monoclonal-antibody-based detection with both host-cell types (data not shown).

A double-reciprocal plot of the data with serovar B showed that the EB, after exposure to 56 °C for 10 min, exhibited a 50% reduction in the value for V and a doubling of the apparent K_m (Fig. 3), suggesting that heat-denatured EB may bind to alternate receptors on McCoy cells via modified ligands.

Adherence of one serovar is inhibited by other serovars in a competitive manner

We tested the effect of addition of heterologous serovars of *C. trachomatis* EB on the adherence of serovar B. Purified EB of serovars L1 and E were added along with varying doses of the test serovar B to monolayers of McCoy cells. Cell-bound serovar B chlamydiae were

Fig. 4. Double-reciprocal plot of adherence rates of EB of *C. trachomatis* serovar B to McCoy cell monolayers in the presence of purified EB of serovar E (□) or LGV440 (●) or in the absence (■) of both. Incubation with the McCoy cell monolayers in 96-well dishes was for 60 min at 4 °C. The ordinate represents the reciprocal of the adherence rates, as assayed in triplicate; the abscissa is the reciprocal of the input of serovar B. Cell-bound serovar B EB were detected using biotinylated monoclonal antibody DH-8. Essentially similar results were obtained in three different experiments. The Figure was computer-generated as in Fig. 3.

detected using the biotinylated monoclonal antibody DH-8. Fig. 4 is a double-reciprocal plot of the data from such an experiment, showing that both the LGV serovar L440 (L1) and the non-LGV serovar E competitively inhibited adherence of the trachoma serovar B to host-cells, with all three curves approaching the same values for V . The competitive nature of the inhibition suggests that the different serovars, including the LGV, bind to the same receptor on the host-cell membrane.

Adherence assays were also done at 37 °C in the presence of the energy poison sodium azide to prevent internalization of the cell-bound EB. Under these conditions, competitive inhibition of the adherence of [^{35} S]methionine/[^{35} S]cysteine-labelled EB of serovar E to HeLa cells by serovars B and L1 was found (Fig. 5). This indicates that the reactions studied at 4 °C were not qualitatively different from those at 37 °C provided the energy poison was used, and that at the higher temperature the serovars of *C. trachomatis* attached to the same receptor on both HeLa and McCoy cells. In the absence of azide, no inhibition by the other serovars was seen. In separate experiments, using radiolabelled L1, competitive inhibition of association by serovar E has been observed at 37 °C in azide-poisoned HeLa and McCoy cells (data not shown).

Using very low inputs of the test serovar in the presence of the inhibitory serovars, evidence for co-operativity of adherence (Bose & Smith, 1984) has been repeatedly obtained. Under these conditions, the heterologous serovars produced a 2- to 3-fold stimulation of adherence of the biotinylated test serovar (data not shown).

Heat-labile, trypsin-sensitive chlamydial adhesin

When suspensions of EB of serovars E or LGV440 were exposed to 56 °C for 5 min, no inhibition of adherence of serovar B to the host-cell monolayer was seen (Table 2), indicating that the heat-denatured chlamydiae were unable to exhibit interfering activity, even though the heated EB exhibited some residual adherence ability (Figs 1 and 3). In addition, exposure of the purified EB of serovar E or LGV440 to 1 µg trypsin per 10 µg EB protein for 2 h at 37 °C resulted in a significant decrease in inhibitory activity of the two serovars on adherence of serovar B to McCoy cells. These results indicated that competitive inhibition by the heterologous serovars was mediated by thermolabile protein(s) on the chlamydiae. Trypsin- and heat-treatment of the purified EB resulted in a significant decrease in their ability to exhibit co-operativity-related stimulation of adherence at the low input, discussed in the preceding paragraph (data not shown). It has been shown that infectivity, i.e. inclusion-forming activity of serovars B and C was inhibited by addition of heat-inactivated B or C, while that of L2 was relatively unaffected (Kuo *et al.*, 1973). In that study, heated L2 had no effect on the infectivity of serovars B, C or L2.

Fig. 5. Double-reciprocal plot of adherence rates of [^{35}S]methionine/[^{35}S]cysteine labelled serovar E to HeLa cells in the presence of purified EB of serovar B (○) or LI (●), or in the absence (■) of both. Incubation was for 60 min at 37 °C in the presence of 5 mM-sodium azide. The ordinate represents the reciprocal of the adherence rates, expressed as cell-bound EB per well, assayed in triplicate; the abscissa is the reciprocal of the input of labelled EB of serovar E. The Figure was computer-generated as in Figs 3 and 4. The experiment was repeated three times.

Table 2. *Loss of inhibition of adherence of C. trachomatis serovar B to McCoy cells by serovars E and LGV440 following heat and trypsin treatment*

Cell-adherent serovar B was detected using monoclonal antibody DH-8, following incubation at 4 °C for 45 min, as described in Methods. The A_{405} of the control was 0.678. Purified EB of serovar E and LGV440 were treated with 1 µg trypsin per 10 µg chlamydial protein for 2 h at 37 °C prior to their addition to monolayer cultures of McCoy cells. This experiment was repeated four times.

Addition	Adherence rate of serovar B (% control)
None	100
Serovar E	55
56 °C, 5 min	99
15 min	102
Trypsin-treated	81
LGV440	46
56 °C, 5 min	100
15 min	103
Trypsin-treated	77

The adherence ability of trypsin-treated EB of the LGV serovar L1 was examined. The epitope recognized by monoclonal antibody KB-8 was found to be trypsin-sensitive. Therefore, the experiment was done with radioactively labelled EB. In three separate experiments adherence of the [^{35}S]methionine/[^{35}S]cysteine-labelled L1 *C. trachomatis* exposed to 1 µg trypsin per 10 µg EB protein for 60 min at 37 °C was inhibited by more than 95% when the assays were done at 4 °C (data not shown). No inhibition was detected when the adherence assays were done at 37 °C, confirming results with the L2 serovar (Bose & Paul, 1982; Hackstadt & Caldwell, 1985; Su *et al.*, 1988). A double-reciprocal plot of the data obtained at 4 °C with multiple inputs of untreated and trypsin-treated L1 revealed that, in addition to the decrease in V , the apparent K_m of adherence by treated EB was 100-fold higher (data not shown).

DISCUSSION

The results of our previous studies (Bose & Goswami, 1986*a, b*) on the adherence characteristics of *C. trachomatis* showed the necessity of distinguishing the initial attachment of the chlamydial EB from the subsequent ingestion process, which occurs at temperatures above

22 °C. A number of significant properties of chlamydial adherence to cell monolayers can be observed only when assays are done at 4–5 °C. For example, inhibition of adherence of HeLa-adapted *C. trachomatis* (Bose & Goswami, 1986*a*), and altered adherence in glycosylation-defective mutant CHO cell lines (Bose & Goswami, 1986*b*) can be detected at 5 °C but not at 37 °C. In contrast to the impaired association of EB of *C. psittaci*, *C. trachomatis* serovar L1 (Byrne, 1976; Byrne & Moulder, 1978), and *C. trachomatis* serovars L2 and B (Kuo & Grayston, 1976) exposed to 56 °C for 30 min, no inhibition can be detected with similarly treated serovar A (Lee, 1981). Exposure of purified EB of serovar K to 60 °C for 5 min causes a diminution of the rate of adherence, but only when the assays are done at 5 °C (Bose & Goswami, 1986*a*), or at 37 °C in the presence of sodium azide (unpublished observations). In addition, the adherence rate of heated EB of serovar A (Lee, 1981) and of K (Bose & Goswami, 1986*a*) is significantly greater than that of unheated EB at 37 °C. Kinetic analyses of data on attachment of unheated and heated EB of serovar B show that both V and the apparent K_m of the EB/cell attachment-reaction are altered (Fig. 3), suggesting that the chlamydial ligands may bind to alternate receptors on the host-cell membrane, presumably due to conformational changes due to the heat treatment.

Lectin-stimulated adherence of HeLa-adapted L1 to HeLa cells can be detected at 37 °C only when the monolayers are pretreated with sodium azide, and we concluded that under these conditions the cell-bound lectin remained externally disposed, providing additional binding sites for the EB (Bose & Goswami, 1986*a*). Soderlund & Kihlstrom (1983) noted that attachment of McCoy-adapted L1 to McCoy cells is inhibited by wheat-germ agglutinin at 4 °C but not at 37 °C. Lack of interference of the association of the L2 serovar and E to McCoy cells by *N*-acetylglucosamine and its oligomers has been recorded at 35 °C (Allan & Pearce, 1987). Therefore, considerable attention must be paid to assay conditions, especially temperature, when only the initial adherence process without the concomitant ingestion of the cell-associated EB and membrane recycling is examined. It may be that specificity of attachment is discernible only in the absence of internalization of the bound ligand, i.e. the EB. It should be noted that EB that are allowed to attach to host-cells at 4 or 5 °C remain fully functional in that upon subsequent incubation at 35 or 37 °C, unimpaired inclusion formation is detected. Data summarized in Fig. 5 show that when energy-dependent processes, e.g. membrane recycling, receptor regeneration and internalization of the cell-bound EB are prevented in the presence of sodium azide, competitive inhibition of the adherence of one serovar by other serovars can be readily seen at 37 °C.

Using serovars LGV L1, B and E, saturable adherence is reproducibly obtained (Figs 2 and 3). The inability to achieve saturable adherence of *C. psittaci* to L cells (Byrne, 1978) or of *C. trachomatis* LGV L1 to McCoy cells (Soderlund & Kihlstrom, 1983) can be attributed to the temperature at which assays were done, i.e. 37 °C rather than 4 °C. At 37 °C, adherence as well as ingestion of chlamydiae occur, in addition to host-membrane recycling and rapid regeneration of receptors as noted with *C. trachomatis* serovar L1 (Byrne & Moulder, 1978).

Serovars E and LGV L1 inhibited adherence of serovar B, and adherence of L1 was inhibited by serovar E, provided the energy-dependent ingestion of cell-bound chlamydiae was prevented. Graphical analysis of the kinetic data by Lineweaver–Burk method (Figs 4 and 5) indicates that the inhibition is competitive type. This suggests that the three different serovars, including LGV, attach to the same receptor on the host-cell membrane. The LGV serovars, in contrast to the non-LGV ones, are far more efficient in terms of infectivity and adherence. The greater efficiency of infection by the LGV serovars does not imply that they bind to a different receptor, rather it may reflect greater avidity. The data summarized in Table 2 show that on exposing an EB suspension to 56 °C for 5 min, the inhibitory activity of the L1 and E serovars was greatly diminished. These results are not in accord with those reported by Kuo *et al.* (1973) who found heat-inactivated chlamydiae to be potent inhibitors of infectivity. Presumably, post-internalization events, such as phagolysosomal fusion and activation of lysosomal degradative enzymes were affected by the denatured EB. The heated EB that do attach to the host-cells (Figs 1 and 3), therefore, appear to be unable to interfere with adherence of the test serovar. The heat-lability of interference would suggest that competitive inhibition of adherence by undenatured

EB is mediated via thermolabile ligands, though adherence may additionally involve thermostable adhesin(s). Parasite-induced phagocytosis of *C. psittaci* and *C. trachomatis* by non-professional phagocytes such as L and HeLa cells was deduced by Byrne & Moulder (1978), based on the thermolability of the chlamydial ligands responsible for the ingestion of EB. Alternatively, the denatured EB may bind nonspecifically to different receptors on the host-cell membrane, probably via heat-modified ligands in view of the different apparent K_m of adherence (Fig. 3).

The 18 kDa eukaryotic-cell-binding polypeptide that has been isolated from serovar L2 of *C. trachomatis* (Hackstadt, 1986; Wenman & Meuser, 1986) does not appear to be the one that our studies would implicate in adherence or in interference of adherence of EB. The 18 kDa polypeptide adheres to isolated membrane preparations from HeLa cells after chlamydial samples are boiled in lysis buffer and resolved in SDS-polyacrylamide gels (Hackstadt, 1986; Wenman & Meuser, 1986). It may be that chlamydial adherence to the host-cell membrane is mediated by heat-labile as well as heat- and SDS-resistant ligands, possibly to alternative receptors.

Monoclonal antibodies that interfere with adherence of EB to host-cells have not been reported, although several do inhibit intracellular replication (Caldwell & Perry, 1982; Lucero & Kuo, 1985; Peeling *et al.*, 1984). The location of the chlamydial adhesin is such that it is accessible to the host-cell receptor via glycosylated residues on the cell membrane. However, immunoglobulins directed against the serovar-, species- or genus-specific epitopes are unable to interact with the ligand responsible for the attachment step, as was proposed by Zhang *et al.* (1987). It is significant that the treatment of EB of serovar B, but not L2, with trypsin resulted in the proteolysis of the externally disposed VDII domain in the outer-membrane protein complex, and resulted in a partial inhibition of association of trypsinized EB to HeLa cells at 37 °C (Su *et al.*, 1988). Our studies show that mild trypsinization of both LGV and non-LGV serovars results in almost complete inhibition of adherence to HeLa cells assayed at 4 °C. Such EB are also incapable of competitively inhibiting the adherence of untreated EB (Table 2). Trypsinization of the L2 serovar is innocuous both with respect to association with HeLa cells at 37 °C and infectivity (Bose & Paul, 1982; Hackstadt & Caldwell, 1985; Su *et al.*, 1988). Adherence exclusive of ingestion is greatly diminished due to trypsin treatment of purified EB, regardless of the serovar, and such chlamydiae are incapable of interfering with the adherence of heterologous serovars.

The importance of adhesin topology is also suggested by the lack of competition with EB heated at 56 °C for 5 min, a treatment presumably sufficient to alter the conformation of the adhesin in the outer-membrane complex. We suggest, therefore, that the chlamydial heat-labile adhesin may not be totally exposed and that its function is dependent on the native conformation of the chlamydial outer-membrane complex within which these moieties lie.

The data presented here indicate that chlamydial adherence may be a multi-factorial process involving heat-labile as well as heat-stable adhesins. A study of this process alone, without the subsequent energy-dependent internalization of cell-bound chlamydiae, reveals that only heat-labile proteinaceous adhesins of multiple serovars of *C. trachomatis* exhibit similar kinetic parameters and compete for the same receptors on the two alternative hosts. Heated or trypsin-treated chlamydiae show altered adherence characteristics, presumably due to modifications of the adhesins. With the occurrence of energy-dependent internalization, some of these parameters may become masked.

This work was supported by grants from the Edna McConnell Clark Foundation, the NATO Scientific Affairs Division, and the National Institutes of Health (AI-19575). We thank Professor L. J. Tolmach for valuable help in the preparation of the manuscript and Tana M. Wensel for her secretarial expertise.

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