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# Sulfated Polyanions Block *Chlamydia trachomatis* Infection of Cervix-Derived Human Epithelia

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**Using a cell line derived from the human cervix and a rapid fluorescence cytotoxicity assay, we have shown that *Chlamydia trachomatis* infection can be blocked by certain sulfated polysaccharides (carrageenan, pentosan polysulfate, fucoidan, and dextran sulfate) and glycosaminoglycans (heparin, heparan sulfate, and dermatan sulfate) but not by other glycosaminoglycans (chondroitin sulfate A or C, keratan sulfate, and hyaluronic acid). The most negatively charged molecules are the most effective at blocking infection. Results of infection at 4°C suggest that sulfated polyanions act by preventing the adherence of chlamydiae to target cells. These and additional blocking studies with enzymes suggest that a heparan sulfate-like glycosaminoglycan on the surface of elementary bodies is involved in the adherence of chlamydiae to target cells, probably through a nonspecific charge interaction or possibly a heparin-binding protein. We previously observed that the same sulfated polysaccharides inhibit transmission of human immunodeficiency virus in vitro and suggested that these compounds could be used in a vaginal formulation to inhibit infection by human immunodeficiency virus. The results of the present study suggest that the same type of formulation may inhibit sexual transmission of chlamydia.**

*Chlamydia trachomatis* is the most common sexually transmitted bacterial pathogen in the world. In developed countries, serovars D to K cause pelvic inflammatory disease, ectopic pregnancy, urethritis, and epididymitis (27), while in developing countries, blindness (serovars A to C) and lymphogranuloma venereum (LGV; serovars L1, L2, and L3) are prevalent (6). The latter cause more invasive genital tract infections which may proliferate in lymphoid tissue of the groin (7).

Our understanding of the basic mechanisms involved in human chlamydial infection is fragmentary, and strategies to prevent chlamydial infection are, unfortunately, even more inadequate. Despite considerable research, no candidate vaccine has yet been developed. Recently, there has been an emphasis on development of alternative modalities, other than vaccines, to prevent sexually transmitted diseases. One especially promising method is a vaginal formulation. In order to examine potential compounds for a formulation that will specifically inhibit sexually transmitted diseases, rapid and appropriate in vitro screening assays must be developed.

We describe here a chlamydial infection assay that is faster than those used in the past. Using this assay, compounds that are highly effective in blocking infection by chlamydiae, and are likely to be safe when applied topically to the vagina, have been identified. This study has also yielded some observations concerning fundamental aspects of chlamydial infection.

## MATERIALS AND METHODS

**Cells.** The human cervical epithelial cell line, ME180, and the mouse fibroblast cell line, McCoy, were purchased from the American Type Culture Collection (ATCC; Rockville, Md.). All cells were maintained in RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Whittaker BioProducts, Walkersville, Md.) and 20 μg of gentamicin per ml at 37°C in an atmosphere of 5% $CO_2$.

**Chlamydiae.** *C. trachomatis* serovar E strain BOUR and *C. trachomatis* LGV type II strain 434 ($L_2$/434) were purchased from ATCC. Chlamydiae were grown in McCoy cell monolayers seeded in 75-$cm^2$ T-flasks. All infections were performed at 37°C in an atmosphere of 5% $CO_2$. To harvest chlamydiae, infected cells were removed from the flask with a cell scraper (Becton Dickinson, Lincoln Park, N.J.), and the cell suspension was transferred to a 50-ml centrifuge tube (Corning Glass Works, Corning, N.Y.). To release intracellular organisms, the suspension was sonicated for 1 min, then centrifuged at 500 × *g* for 10 min to remove cellular debris. It has been demonstrated that this technique results in preparations containing primarily elementary bodies (EBs), as reticulate bodies are lysed by sonication (16). Since crude preparations have been shown to retain higher infectivity titers than gradient purified EBs, the supernatant was collected and frozen at −70°C. Titration experiments demonstrated that chlamydiae harvested and frozen by this method were slightly less infectious than freshly harvested EBs but were equally as infectious as EBs obtained from ATCC.

**Reagents.** The sulfated polysaccharides kappa, lambda, and iota carrageenans (C-1263, C-3889, and C-4014, respectively), dextran sulfate (D-6001), fucoidan (F-5631), and pentosan polysulfate (P-8275) and the glycosaminoglycans (GAGs) heparin (H-3393); heparan sulfate (H-7641); chondroitin sulfate A, B (dermatan sulfate), and C (C-8529, C-2413, and C-4384, respectively); keratan sulfate (K-3001); and hyaluronic acid (H-0902) were obtained from Sigma. Stock solutions were made at a concentration of 10 mg/ml in RPMI 1640 containing 10% FBS. Heparinase and heparitinase were obtained from Seikagaku America, Inc. (Rockville, Md.).

**Transmission electron microscopy.** ME180 cells were seeded on 24-well plates (Becton Dickinson) at a density of $2 \times 10^5$/well and were grown for 48 h at 37°C in an atmosphere of 5% $CO_2$. Cells were washed once in fresh RPMI 1640 and 100 μl of serovar L2 was inoculated onto the monolayers. Two methods were employed to examine entry. Infection was either carried out at 37°C with intermittent shaking for 20 min prior to fixation in 2.5% glutaraldehyde in phosphate buffer, or infection was carried out for 1 h at 4°C to allow chlamydial attachment but not entry. In the latter case, cells were subsequently warmed to 37°C in a water bath and then fixed at 2, 5, or 10 min. Fixed cells were processed and embedded in Epon for transmission electron microscopy as previously described (30). To observe the course of infection, infection was carried out for 1 h at 37°C with intermittent shaking. Cells were subsequently washed three times with phosphate-buffered saline (PBS) containing 0.1% bovine serum albumin (BSA) (PBS/BSA) to remove nonadherent chlamydiae and were incubated at 37°C in RPMI 1640 containing 10% FBS and 1 μg of cycloheximide per ml. Cells were fixed at 1, 3, 6, 9, 12, 16, 24, 48, or 72 h and processed for transmission electron microscopy as previously described (30).

**Infection assay.** ME180 cells were seeded at $4 \times 10^4$/well on 96-well, flat-bottomed plates (Becton Dickinson) 24 to 48 h postinfection and were grown at 37°C in an atmosphere of 5% $CO_2$. The monolayers were washed once in fresh RPMI 1640 prior to inoculation with 25 μl of crude chlamydia stock. After 1 h of incubation at 37°C with intermittent shaking, monolayers were washed three times in PBS/BSA to remove nonadherent chlamydiae and then incubated for 4

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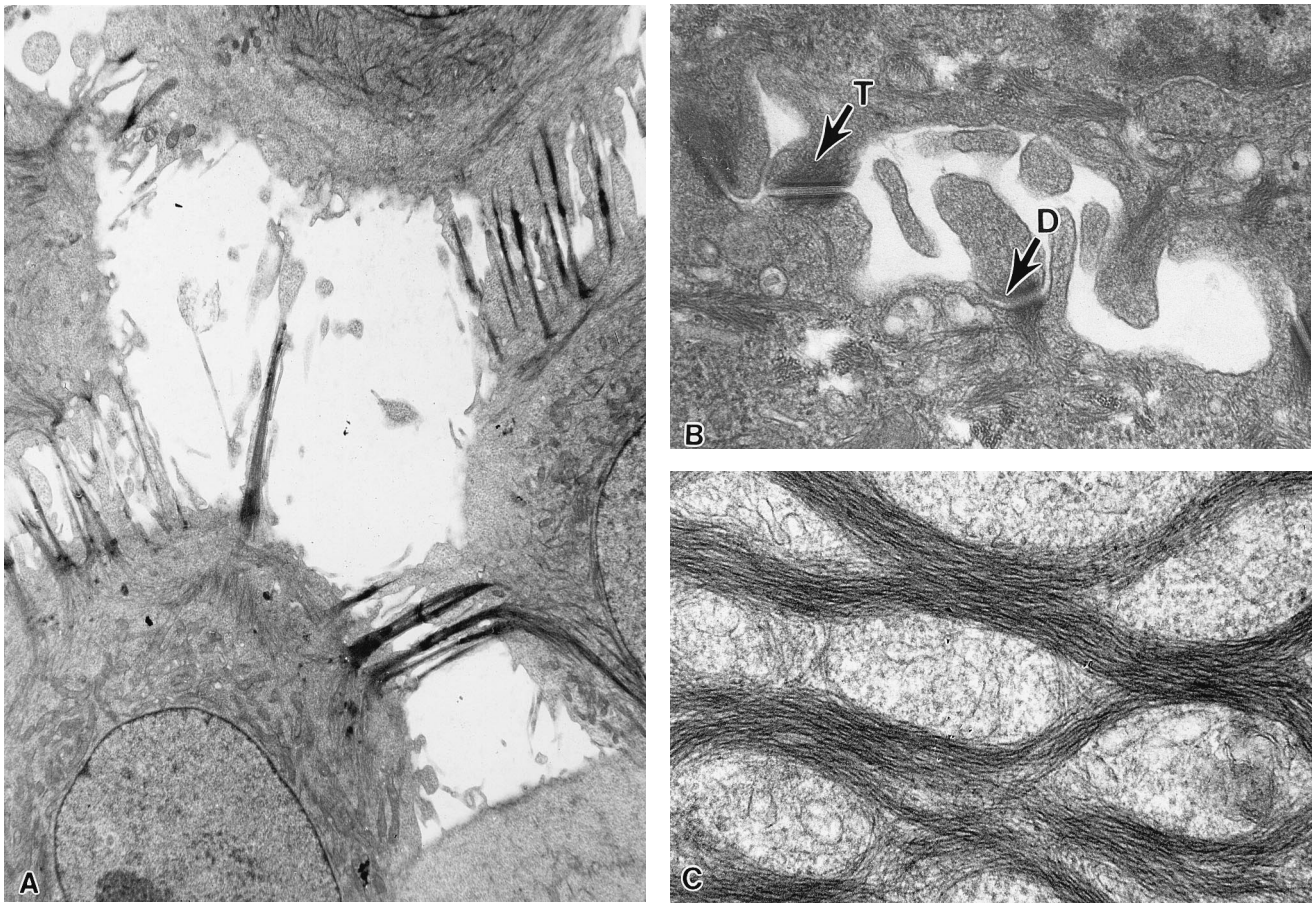


FIG. 1. Morphological features of ME180 cells show characteristics of a cervical epithelium. (A) Processes extending between adjacent cells contain electron-dense tonofilaments. Magnification, ×5,500. (B) Desmosomes (D) and tonofilaments (T) in the region of association between adjacent ME180 cells. Magnification, ×26,000. (C) Epidermal filaments in the cytoplasm of an ME180 cell. Magnification, ×26,000.

to 5 days at 37°C in RPMI 1640 supplemented with 10% FBS and 1 μg of cycloheximide per ml. For infection and adhesion inhibition experiments, twofold or fivefold serial titrations were made for all compounds (i.e., sulfated polysaccharides, GAGs, enzymes) in sterile 96-well U-bottomed plates (Becton Dickinson) and were then mixed with equal volumes of crude chlamydia stock. Twenty-five microliters of the mixture was inoculated onto monolayers, and infection was performed as described above. The adhesion inhibition experiments were performed by a modification of the above procedure. Prior to inoculation, monolayers were washed once in cold RPMI 1640 and then kept on ice. Following inoculation, cells were incubated at 4°C instead of 37°C, to inhibit chlamydial entry. Subsequent washes were performed with cold PBS/BSA.

Chlamydial infection was detected by a cytotoxicity assay developed in this laboratory (23). Briefly, ME180 monolayers were washed three times in PBS to remove lysed, infected cells and then incubated for 35 min at 37°C in the presence of 5% $CO_2$ with a fluorogenic ester (BCECF-AM) which enters the live, uninfected cells remaining on the plate. The fluorescence emitted was read in a Cytofluor 2300 (Millipore) (excitation, 485 nm; emission, 530 nm; sensitivity, 4). It was determined, by morphological analysis, that the developmental cycle of both serovars L2 and E is slightly slower in ME180 cells than in other cell lines. Therefore, incubation was typically carried out for 1 or 2 days longer than the maturation times of the respective serovars to ensure that lysis of infected cells occurred prior to labeling with fluorochrome, as the presence of infected cells on the monolayer might affect the results.

**Inclusion assay.** Infection was carried out as described above with ME180 cells grown on 96-well, flat-bottomed plates. Forty-eight hours later, cells were fixed with methanol and stained with Giemsa. Three hundred cells per well were scored as either containing or not containing an inclusion by using an inverted microscope with a 20× objective. Datum points represent the percentage of inclusion-bearing cells.

**Enzyme analysis.** Enzyme treatment was performed by the method of Zhang et al. (34) with the following modifications: (i) ME180 cells were used rather than HeLa cells; (ii) cells were grown on 96-well plates rather than on glass coverslips; and (iii) infection was quantitated by the cytotoxicity method rather than by counting inclusions.

## RESULTS

**Ultrastructure of ME180 cells.** The ME180 cell line is derived from a squamous cell carcinoma of the human cervix. The cells are remarkable in having retained many of the morphological characteristics of squamous cervical epithelia (33). When viewed in the phase-contrast microscope, numerous tiny, phase-dense structures are observed between the cells, making the cells look as though they were stitched together. In the electron microscope, these structures are seen as processes extending from adjacent cells (Fig. 1A). Well-developed desmosomes are observed in the place where processes from adjacent cells come into contact. As in epithelial cells in vivo, an extensive array of tonofilaments emanate from the desmosomes (Fig. 1A and B). These cells are also characterized by an extensive network of epidermal filaments (Fig. 1C). By morphological criteria, ME180 cells are polar when grown on plastic. Microvilli on the base are strikingly different than on the apical surface, the network of epidermal filaments is situated only in the most basal region of the cell and the centrosome is always basal to the nucleus.

To study the ultrastructural aspects of chlamydial serovar L2 entry into ME180 cells and the cellular response to infection,

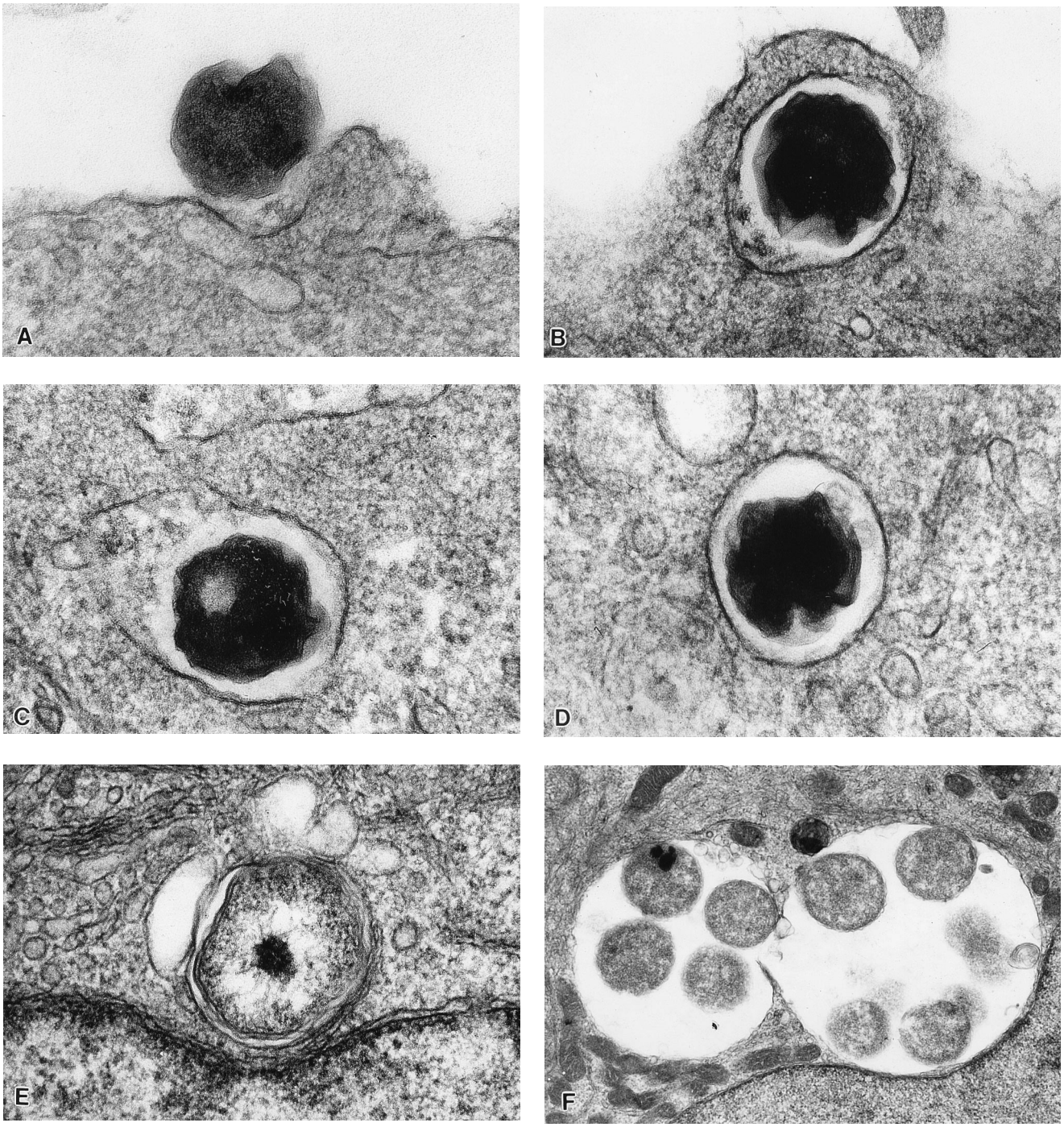


FIG. 2. Ultrastructure of L2 infection in ME180 cells is similar to infection in other cell lines. (A) EB on the surface of an ME180 cell 5 min after the addition of chlamydiae. No clathrin is observed. Magnification, ×85,000. (B to D) EB in a smooth vesicle immediately below the cell surface 5 min after the addition of chlamydiae. Magnification, ×85,000. (E) Chlamydial RB 9 h postinfection. Magnification, ×75,000. (F) Fusion of two vesicles containing reticulate bodies 12 h after addition of chlamydiae. Magnification, ×40,000.

epithelial cells were fixed at various times after addition of chlamydiae. At 5 min, several hundred elementary bodies were associated with the plasma membrane or within involutions of the plasmalemma in vesicles just below the cell surface. In all cases the membrane appeared smooth (Fig. 2A to D). This is in contrast to previous studies of McCoy and HeLa cells in which clathrin has been observed during uptake (8, 18, 26).

It takes some time for the elementary bodies to differentiate into reticulate bodies (Rbs). At 9 h, mature reticulate bodies were observed (Fig. 2E), and by 12 h many vesicles contained two or four reticulate bodies; fusion of these vesicles was also observed (Fig. 2F). At 24 h postinfection, infected cells contained a single vesicle which was roughly the size of the cell nucleus and contained all stages of the chlamydial life cycle. In

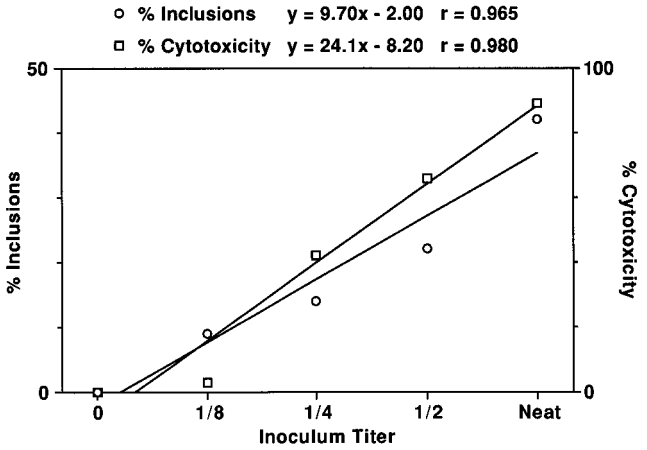


FIG. 3. Comparison of inclusion assay and fluorescence cytotoxicity assay with serovar E shows a direct relationship. The left *y* axis represents the percentage of inclusion-bearing cells 2 days postinfection. The right *y* axis represents the percentage of cytotoxicity 4 to 5 days postinfection.

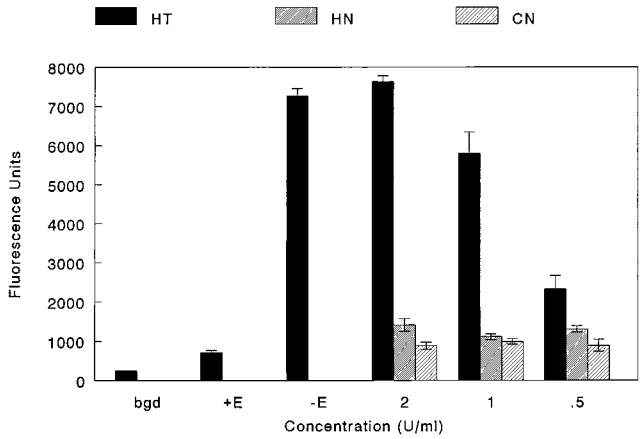


FIG. 5. Heparitinase treatment of serovar E blocks infection of ME180 cells. Chlamydiae were pretreated with twofold serial titrations of heparitinase (HT), heparinase (HN), or chondroitinase (CN) for 1 h prior to inoculation onto ME180 cells.

other cell lines studied, L2 generally fills vesicles at this stage; however, we have observed that L2 grows more slowly in ME180 cells.

**Infection assay.** Since infection results in cell death, we chose to use a cytotoxicity assay. This fluorescence assay has been shown to be accurate and reliable (13, 20, 23, 32). To determine the accuracy of this assay as compared to inclusion counting, twofold serial titrations of serovar E (Fig. 3) crude chlamydial stock were inoculated onto ME180 cell monolayers in 96-well plates. The percentage of cytotoxicity ($r = 0.98$) and the percentage of inclusions ($r = 0.97$) were found to be directly proportional to the inoculum titer (Fig. 3). Thus, cytotoxicity evidently reflects infectivity.

**Blocking experiments.** Heparin and heparan sulfate were both effective in blocking serovar E infection at 37°C (Fig. 4). Heparin was equally as effective in blocking serovar L2 infection. Serovar E infection was completely blocked by heparin at 30 μg/ml, whereas 300 μg of heparan sulfate per ml was required to block the infection (Fig. 4). At 4°C, heparan sulfate completely blocked infection at about 150 μg/ml, a twofold lower concentration than that required to block infection at 37°C. Zhang et al. (34) showed that 80 μg/ml of heparan sulfate was required to block attachment of serovar L2 to HeLa cells at 4°C.

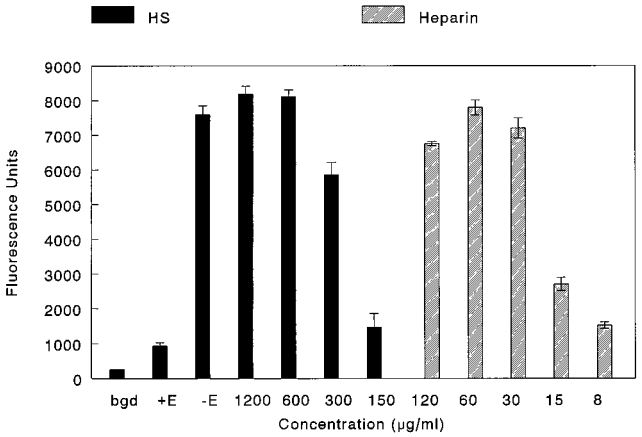


FIG. 4. Heparin and heparan sulfate (HS) block serovar E infection of ME180 cells as measured by the cytotoxicity assay. Twofold serial titrations of each compound were mixed with chlamydiae prior to inoculation onto ME180 cell monolayers at 37°C. In Fig. 4 through 7 chlamydial infection was measured by the cytotoxicity assay and datum points represent the mean of three wells (± standard deviation); bgd, background fluorescence of a confluent epithelial monolayer in the absence of fluorochrome label; +E and +LGV, fluorescence of monolayers infected with serovar E and LGV, respectively; −E and −LGV, fluorescence of uninfected monolayers.

To determine if a heparan sulfate-like GAG present on the surface of the EB is involved in adhesion and/or entry, a heparan sulfate lyase, heparitinase, was coincubated with serovar E prior to addition of chlamydiae to the cells. Heparitinase effectively blocked infection at 2 U/ml in a dose-dependent manner, whereas heparinase and chondroitinase were ineffective at the same concentration (Fig. 5). In similar experiments, Chen et al. (5) found that 2 U of heparitinase per ml was necessary to block serovar B infection of HeLa cells while Zhang et al. (34) found that only 0.5 U/ml was necessary to block serovar L2 infection.

Another way to test the specificity of blocking by heparin or heparan sulfate is to determine whether other polyanions can block infection. If the epithelial cell receptor is specific for heparin and heparan sulfate, one would expect other polyanions to be less effective in chlamydial blocking. These experiments are described below.

The sulfated polysaccharide carrageenan gives red seaweed its rubbery texture. Different types of carrageenans are extracted from different red seaweeds. Carrageenans extracted and purified from seaweed farmed in tropical seas are used extensively as thickeners in foods. Since they are "generally recognized as safe" (GRAS) compounds by the U.S. Food and Drug Administration, they may be possible candidates for a vaginal formulation to inhibit infection by sexually transmitted pathogens. Like heparin and heparan sulfate, carrageenans are cytotoxic to cells in culture only at very high concentrations. It was previously found that 10 mg/ml had no effect on viability when incubated with ME180 cells in RPMI 1640 medium supplemented with 10% heat-inactivated FBS for 90 min (unpublished observations).

All three carrageenans, iota, lambda, and kappa, were highly effective in blocking infection of both serovar E and L2. Lambda and iota carrageenan completely blocked infection at

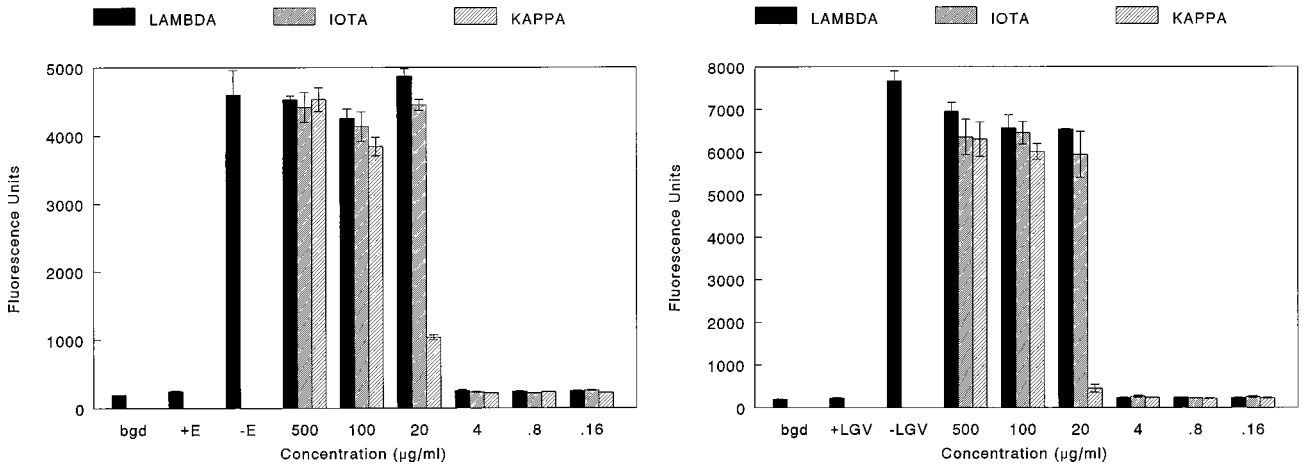


FIG. 6. Carrageenan blocks *C. trachomatis* infection of ME180 cells at 37°C. Fivefold serial titrations of each compound were mixed with serovar E (A) or L2 (B) prior to inoculation onto ME180 cell monolayers.

concentrations of 20 μg/ml at 37°C, while kappa was somewhat less effective (Fig. 6A and B).

It has been established that adherence of serovars E and L2 to target cells is not inhibited at 4°C during a 1-h adsorption period, whereas entry is (8, 21). At 4°C lambda, kappa, and iota carrageenans inhibited chlamydial infection of serovar E by approximately 50% at 4 μg/ml (Fig. 7A), while they were ineffective at 37°C at the same concentration (Fig. 6A). At 4°C, lambda and kappa carrageenans showed some inhibition of L2 at 4 μg/ml whereas iota carrageenan was ineffective (Fig. 7B). Kappa completely blocked infection of L2 at 20 μg/ml at 4°C but was ineffective at 37°C. Thus, carrageenans block infection of both serovars more effectively at 4°C than at 37°C. Since carrageenans block as or more effectively at 4°C than at 37°C, carrageenans inhibit chlamydial infection by blocking their attachment to target epithelia.

A number of other polysaccharides and GAGs were examined for their ability to inhibit infection. Some were effective in a similar dose to kappa carrageenan. These included fucoidan and pentosan polysulfate (Table 1). Dextran sulfate was the most effective compound, requiring a fivefold lower concentration than lambda and iota carrageenan to completely block infection at 37°C. The GAG dermatan sulfate also blocked infection, but it was found to be the least effective sulfated polyanion (Table 1). The other GAGs, chondroitin sulfate A and C, keratan sulfate, and hyaluronic acid, were ineffective at this concentration (Table 1).

## DISCUSSION

This study has shown that a number of sulfated polyanions inhibit chlamydial infection in an in vitro model that resembles the ectocervix in vivo. All of the sulfated polysaccharides tested blocked chlamydial infection, whereas most GAGs did not. The sulfated polyanions that block chlamydial infection generally have more than one sulfate group per disaccharide unit as compared to those that do not block infection, which are generally found to have one sulfate group per disaccharide unit, while hyaluronic acid has none. For example, of the carrageenans, lambda (three sulfates per disaccharide unit) is more effective than iota (two sulfates per disaccharide unit), which is more effective than kappa (one sulfate per disaccharide unit). Furthermore, highly charged heparin was 10 times more effective at blocking infection than the less negatively

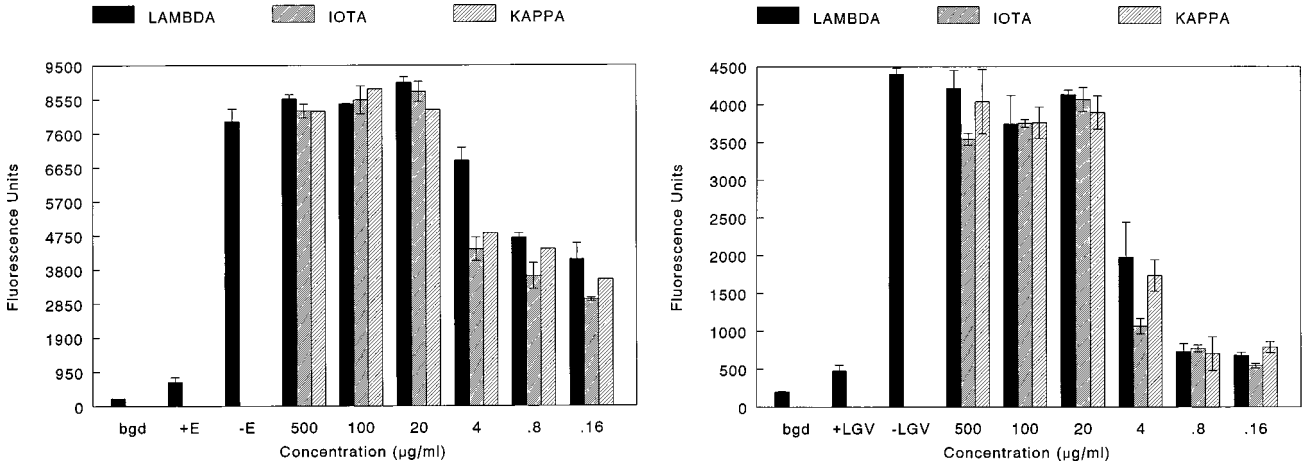


FIG. 7. Carrageenan blocks *C. trachomatis* infection of ME180 cells better at 4°C than at 37°C. Fivefold serial titrations of each compound were mixed with serovar E (A) or L2 (B) prior to inoculation onto ME180 cell monolayers.

TABLE 1. Effect of compounds on chlamydial infection[a]

| Compound | $IC_{50}$ (μg/ml) |
|---|---|
| Dextran sulfate | 1 < 4 |
| Lambda carrageenan | 4 < 20 |
| Iota carrageenan | 4 < 20 |
| Kappa carrageenan | 20 < 100 |
| Fucoidan | 20 |
| Pentosan polysulfate | 20 |
| Heparin | 15 < 30 |
| Heparan sulfate | 150 < 300 |
| Dermatan sulfate | 2,500 |
| Chondroitin sulfate A | >2,500 |
| Hyaluronic acid | >2,500 |
| Chondroitin sulfate C | No effect |
| Keratan sulfate | No effect |

[a] Effect of sulfated polysaccharides and GAGs on serovar E infection of ME180 cells at 37°C. $IC_{50}$, 50% inhibitory concentration range.

charged intestine-derived heparan sulfate. Kidney-derived heparan sulfate had no inhibitory effects, presumably because it is less negatively charged than intestine-derived heparan sulfate (10). Thus, the ability of sulfated polyanions to block infection appears to be primarily a function of charge. The largest, most negatively charged molecules are the most effective blocking agents; molecular weight seems to be less relevant than charge, since pentosan polysulfate, a highly charged small molecule, is just as effective as the larger molecules such as kappa carrageenan and fucoidan.

It has previously been established that heparin, heparan sulfate, and dextran sulfate block the attachment of radiolabeled chlamydiae to host cells (2, 4). Thus, we compared the results of blocking experiments carried out in the cold with those done at 37°C, because adherence is not inhibited by the cold, while entry is. The 4°C experiments suggest that sulfated polyanions block infection by blocking the attachment of serovars E and L2 to epithelia. By inoculating at 4°C in the presence or absence of sulfated polyanions, it was shown that the most likely effect sulfated polyanions have on the infection process is to inhibit chlamydial attachment.

It was also found that sulfated polyanions inhibited infection more effectively at 4°C than at 37°C. This could be explained, in part, by the fact that chlamydiae might escape blocking with low concentrations of sulfated polyanions at 37°C by continuously entering epithelia before nonadherent chlamydiae are washed away. At 4°C, adsorbed chlamydiae can not enter epithelia until the cells are warmed to 37°C; thus, if chlamydiae which dissociate bind to exogenous sulfated polyanions, they would be prevented from reattaching.

Previous reports of sulfated polysaccharide blocking chlamydial infection have concentrated on the functional role of target cell-surface heparan sulfate in infection (2, 14) or on comparisons between dextran sulfate and the polycation DEAE-dextran, which enhances infection (4, 15). Most recently, Zhang et al. (34) found that exogenous heparin, a commonly used analog of heparan sulfate, and heparan sulfate blocked chlamydial infection. They also found that infection was inhibited if chlamydiae were treated enzymatically to remove heparan sulfate. They concluded from their data that infection is mediated by binding of a heparan sulfate-like GAG, present on the surface of chlamydia, to a heparan sulfate receptor on the target cell. The fact that heparin binding proteins are known to be present on the surfaces of many cell types, including epithelial cells (1, 3, 10, 12, 17, 25), lends credence to this theory.

In the work described here, these results have been confirmed. Since Zhang et al. used serovar L2 and did not perform experiments at a physiological temperature, their data are difficult to compare to the work presented here; however, it is clear that higher concentrations of compounds are necessary to block infection at 37°C than at 4°C. More importantly, we found that sulfated polyanions other than heparin and heparan sulfate inhibit infection just as well or better. This finding is not necessarily contradictory, because it is known that the same sulfated polysaccharides used in this study to inhibit infection bind to heparin-binding proteins (e.g., carrageenans, dextran sulfate, and dermatan sulfate), while those that do not inhibit infection do not bind to these proteins (e.g., chondroitin sulfate and hyaluronic acid) (9–11).

A more probable explanation of our data is that blocking by sulfated polysaccharides, including heparin, is nonspecific. The mechanism of action of these molecules may be to bind to target cells, chlamydiae, or both, by virtue of the strong negative charge of the sulfated polyanion. Adherence of chlamydiae to the target cell would be prevented by charge repulsion. Our observation that uptake of chlamydiae into ME180 cells does not involve clathrin supports this hypothesis, because it suggests that uptake of chlamydiae may not be receptor mediated, although receptor-mediated endocytosis can occur in the absence of clathrin in some cases (31).

The two most commonly used cell lines for studying mechanisms of chlamydial infection have been McCoy and HeLa. McCoy cells are inappropriate models of human infection as they are neither human nor epithelium derived. Although HeLa cells were originally derived from a carcinoma of the human cervix, they bear little resemblance to human cervical epithelia. The human cell line ME180, although initiated from a squamous carcinoma (29), is more appropriate because it retains many of the morphological features of a normal human cervical epithelial cell line. However, the ME180 cell line is by no means the ideal model for chlamydial infection because chlamydiae generally infect columnar epithelial cells of the endocervix rather than squamous epithelia (19, 28).

Blocking experiments are generally quantitated by counting the percentage of cells that have a vesicle (inclusion) containing chlamydiae. This method is laborious as it involves counting individual cells. Since infection results in cell death, we chose to use a cytotoxicity assay. Other cytotoxicity assays, such as $^3$H-thymidine and dye reduction assays that use tetrazolium salts (XTT or MTT), could be used, but they are considerably more time-consuming than the fluorescence assay employed in these studies.

We previously presented evidence that human immunodeficiency virus (HIV) can target epithelial cells, as chlamydiae do, with HIV-infected mononuclear cells serving as the main vectors of infection (22, 30) and employed an in vitro model to determine which molecules might best block adhesion of HIV-infected lymphocytes to epithelia (23). Sulfated polyanions block HIV infection of the target epithelial cells (24) and blocking doses and the types of sulfated polyanions that were most effective were the same as found here for chlamydia. Thus, a vaginal formulation using a single sulfated polysaccharide as an active ingredient might be effective in inhibiting both HIV and chlamydial infection.

## ACKNOWLEDGMENTS

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## REFERENCES


1. **Bârzu, T., P. Molho, G. Tobelem, M. Petitou, and J. Caen.** 1985. Binding and endocytosis of heparin by human endothelial cells in culture. Biochim. Biophys. Acta **845:**196–203.
2. **Becker, Y., E. Hochberg, and Z. Zakay-Rones.** 1969. Interaction of trachoma elementary bodies with host cells. Isr. J. Med. Sci. **5:**121–124.
3. **Bilozur, M. E., and C. Biswas.** 1990. Identification of characterization of heparan sulfate-binding proteins from human lung carcinoma cells. J. Biol. Chem. **265:**19697–19703.
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. **Chen, J. C. R., and R. S. Stephens.** 1994. Trachoma and LGV biovars of *Chlamydia trachomatis* share the same glycosaminoglycan-dependent mechanism for infection of eukaryotic cells. Mol. Microbiol. **11:**501–507.
6. **Dawson, C. R., and J. Schacter.** 1985. Strategies for treatment and control of blinding trachoma: cost-effectiveness of topical or systemic antibiotics. Rev. Infect. Dis. **7:**768–773.
7. **Grayston, J. T., and S. P. Wang.** 1975. New knowledge of *Chlamydia* and the diseases they cause. J. Infect. Dis. **132:**87–105.
8. **Hodinka, R. L., C. H. Davis, J. Choong, and P. B. Wyrick.** 1988. Ultrastructural study of endocytosis of *Chlamydia trachomatis* by McCoy cells. Infect. Immun. **56:**1456–1463.
9. **Kindness, G., W. F. Long, and F. B. Williamson.** 1979. Enhancement of antithrombin III activity by carrageenan. Thromb. Res. **15:**49–60.
10. **Kjellen, L., A. Oldberg, and M. Hook.** 1980. Cell-surface heparan sulfate. J. Biol. Chem. **255:**10407–10413.
11. **Kjellen, L., A. Oldberg, K. Rubin, and M. Hook.** 1977. Binding of heparin and heparan sulfate to rat liver cells. Biochem. Biophys. Res. Commun. **74:**126–133.
12. **Kohnke-Godt, B., and H. J. Gabius.** 1991. Heparin-binding lectin from human placenta: further characterization of ligand binding and structural properties and its relationship to histones and heparin-binding growth factors. Biochemistry **30:**55–65.
13. **Kolber, M. A., P. R. Quinones, R. E. Gress, and P. A. Henkart.** 1988. Measurement of cytotoxicity by target cell release and retention of the fluorescent dye biscarboxyethyl-carboxyfluorescein (BCECF). J. Immunol. Methods **108:**255–264.
14. **Kuo, C. C., and J. T. Grayston.** 1976. Interaction of *Chlamydia trachomatis* with HeLa 229 cells. Infect. Immun. **13:**1103–1109.
15. **Kuo, C. C., S. P. Wang, and J. T. Grayston.** 1973. Effect of polycations, polyanions, and neuraminidase on the infectivity of trachoma-inclusion conjunctivitis and lymphogranuloma venereum organisms in HeLa cells: sialic acid residues as possible receptors for trachoma-inclusion conjunctivitis. Infect. Immun. **8:**74–79.
16. **Kuo, C. C., S. P. Wang, and J. T. Grayston.** 1977. Growth of trachoma organisms in HeLa 229 cell culture, p. 328–336. *In* D. Hobson and K. K. Holmes (ed.), Nongonococcal urethritis and related infections. American Society for Microbiology, Washington, D.C.
17. **Leung, L., K. Saigo, and D. Grant.** 1989. Heparin binds to human monocytes and modulates their procoagulant activities and secretory phenotypes. Effect of histidine-rich glycoprotein. Blood **73:**177–184.
18. **McGee, Z. A., G. L. Gorby, P. B. Wyrick, R. L. Hodinka, and L. H. Hoffman.** 1988. Parasite-directed endocytosis. Rev. Infect. Dis. **10:**S311–S316.
19. **Mitao, M., W. Reumann, B. Winkler, R. M. Richart, A. Fujiwara, and C. P. Crum.** 1984. Chlamydial cervicitis and cervical intraepithelial neoplasia: an immunohistochemical analysis. Gynecol. Oncol. **19:**90–97.
20. **Moore, P. L., I. C. MacCoubrey, and R. P. Haughland.** 1990. A rapid pH insensitive, two color fluorescence viability (cytotoxicity) assay. J. Cell Biol. **111:**58 (Abstr. 304).
21. **Moulder, J. W.** 1991. Interaction of chlamydiae and host cells in vitro. Microbiol. Rev. **55:**143–190.
22. **Pearce-Pratt, R., D. Malamud, and D. M. Phillips.** 1994. Role of the cytoskeleton in cell-to-cell transmission of human immunodeficiency virus. J. Virol. **68:**2898–2905.
23. **Pearce-Pratt, R., and D. M. Phillips.** 1993. Studies of adhesion of lymphocytic cells: implications for sexual transmission of human immunodeficiency virus. Biol. Reprod. **48:**431–445.
24. **Phillips, D. M., and X. Tan.** 1992. Mechanism of trophoblast infection by HIV. AIDS Res. Hum. Retroviruses **9:**1697–1705.
25. **Raboudi, N., J. Julian, L. H. Rohde, and D. D. Carson.** 1992. Identification of cell-surface heparin/heparan sulfate-binding proteins of a human uterine epithelial cell line (RL95). J. Biol. Chem. **267:**11930–11939.
26. **Reynolds, D. J., and J. H. Pearce.** 1991. Endocytic mechanisms utilized by chlamydiae and their influence on induction of productive infection. Infect. Immun. **59:**3033–3039.
27. **Schacter, J.** 1988. Overview of human diseases, p. 153–165. *In* A. L. Barron (ed.), Microbiology of *Chlamydia*. CRC Press, Boca Raton, Fla.
28. **Swanson, J., D. A. Eschenbach, E. R. Alexander, and K. K. Holmes.** 1995. Light and electron microscopic study of *Chlamydia trachomatis* infection of the uterine cervix. J. Infect. Dis. **131**(6)**:**678–687.
29. **Sykes, J. A., J. Whitesscarver, P. Jernstrom, J. F. Nolan, and P. Byatt.** 1970. Some properties of a new epithelial cell line of human origin. J. Natl. Cancer Inst. **45:**107–115.
30. **Tan, X., R. Pearce-Pratt, and D. M. Phillips.** 1993. Productive infection of a cervical epithelial cell line with human immunodeficiency virus: implications for sexual transmission. J. Virol. **67:**6447–6452.
31. **Tran, D., J. L. Carpentier, F. Sawano, P. Gorden, and L. Orci.** 1987. Ligands internalized through coated or noncoated invaginations follow a common intracellular pathway. Proc. Natl. Acad. Sci. **84:**7957–7961.
32. **Wierda, W. G., D. S. Mehr, and Y. B. Kim.** 1989. Comparison of fluorochrome-labeled and 51Cr-labeled targets for natural killer cytotoxicity assay. J. Immunol. Methods **122:**15–24.
33. **Wyrick, P. B., C. H. Davis, S. T. Knight, J. Choong, J. E. Raulston, and N. Schramm.** 1993. An in vitro human epithelial cell culture system for studying the pathogenesis of *Chlamydia trachomatis*. Sex. Transm. Dis. **5:**248–256.
34. **Zhang, J. P., and R. S. Stephens.** 1992. Mechanism of *C. trachomatis* attachment to eukaryotic host cell. Cell **69:**861–869.