Heparan Sulfate–like Glycosaminoglycan Is a Cellular Receptor for *Chlamydia pneumoniae*

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*Chlamydia pneumoniae* is an important human intracellular pathogen; however, the pathogenesis of *C. pneumoniae* infection is poorly understood, and the bacterial adherence mechanism to host cells is unknown. This study examined the role of glycosaminoglycans (GAGs) in the adhesion of *C. pneumoniae* to eukaryotic cells. Heparin and heparan sulfate were found to inhibit the attachment of *C. pneumoniae* to human epithelial cells. Reduction in infectivity resulted from the binding of heparin to the organism. Enzymatic removal of heparan sulfate moieties from the host cell surface led to a marked decrease in *C. pneumoniae* infectivity. Mutant CHO cell lines that were defective in heparan sulfate biosynthesis were less susceptible to *C. pneumoniae* infection than was the wild-type cell line. However, preincubation of the GAG-deficient CHO cells with exogenous heparin greatly increased infectivity.

*Chlamydia pneumoniae* is an obligate intracellular human pathogen with a unique biphasic developmental cycle. Clinical manifestations of *C. pneumoniae* infection are primarily diseases of the respiratory tract, such as pneumonia and bronchitis [1]. About 10% of cases of community-acquired pneumonia are associated with *C. pneumoniae* infection. In addition, there is growing evidence that *C. pneumoniae* may contribute to atherogenesis, as several studies have demonstrated the presence of the organism within atherosclerotic lesions [2–5].

The pathogenesis of *C. pneumoniae* infection is poorly understood. Virulence determinants of the organism have not yet been identified. The attachment of *C. pneumoniae* to the host cell is a critical primary event for the establishment of an infection, but the interaction of *C. pneumoniae* with host cell receptors has yet to be characterized. Specific receptors that determine the tissue tropism and the host range of *C. pneumoniae* have not been identified. Identification of the structures that take part in the adhesion process and uptake of *C. pneumoniae* will have important implications for the development of intervention and prevention strategies for *C. pneumoniae* infections.

Many bacteria, viruses, and protozoa use glycosaminoglycans (GAGs) as receptors for cell attachment [6, 7]. GAGs represent the polysaccharide chains of proteoglycans, which are found ubiquitously on the surface of eukaryotic cells. Proteoglycans consist of a protein core and ≥1 covalently linked GAG chain [8]. Heparan sulfate, chondroitin sulfate (CS) A, CSB, and CSC are the most common GAGs found in animal cells. Microorganisms most commonly use heparan sulfate moieties on the surface of the target cells as an adhesion receptor (e.g., *Neisseria gonorrhoeae*: [9]). There is evidence that, in contrast to many other GAG-binding microorganisms, *Chlamydia trachomatis* serovars L2 and B use a unique mode of heparan sulfate–dependent attachment. In these organisms, it appears that heparan sulfate is located on the surface of the bacteria and that it functions as a bridge between a chlamydial adhesin and a ligand on the host cell. Thus, a trimolecular mechanism of GAG-dependent cell attachment was proposed [10]. However, subsequent studies revealed that GAGs are being used for attachment by many chlamydial species in various ways [11].

The attachment of *C. pneumoniae* to its target cells is not understood, and host cell receptors for the organism or adhesins on the surface of *C. pneumoniae* have not been identified. In addition, it is not known whether *C. pneumoniae* uses GAGs for attachment. Therefore, we investigated the role of GAGs in the adhesion process of *C. pneumoniae*.

Materials and Methods

*Chlamydial strains and cell lines.* *C. pneumoniae* isolate GiD was isolated originally from a patient with bronchitis. The isolate was identified to be *C. pneumoniae* by staining of inclusions formed in cell culture with a species-specific monoclonal antibody and by sequence analysis of the omp1 gene [12]. Isolate GiD was propagated in HEP-2 cells (European Collection of Cell Cultures [ECACC]) with centrifugation [13]. The experiments with GiD outlined below were...
done without centrifugation. *C. trachomatis* L2/434/Bu [14] was grown in HeLa 229 cells (ECACC) [15].

CHO cell line CHO-K1 (ATCC CCL-61) and the mutant CHO cell lines pgsD-677 (ATCC CRL-2244) and pgsA-745 (ATCC CRL-2242) were obtained from American Type Culture Collection. CHO-pgsA-745 cells have a defect in xylosyltransferase activity and do not produce any GAGs. CHO-pgsD-677 cells have a defect in the enzymes N-acetylgalactosaminyltransferase and glucurono-

Chlamydial inclusions were counted at 182 Wuppermann et al. JID 2001;184 (15 July) 181 600 magnification in 10 fields of each of 2 coverslips.

To determine whether soluble heparin binds to *C. pneumoniae* or *C. trachomatis*, chlamydial organisms or the host cells were incubated with heparin before the infection. Purified *C. pneumoniae* and *C. trachomatis* L2 EBs were suspended in PBS (3 × 10^6 and 2 × 10^6 ifu/mL, respectively) containing 500 μg/mL of heparin (corresponding to 85 U) and were incubated for 1 h at 4°C. *C. pneumoniae* and heparinase-digested *C. pneumoniae* organisms were washed 3 times with 1 mL of ice-cold PBS and were overlaid with 1 mL of growth medium, supplemented with 1.2 μg/mL of cycloheximide. After incubation for 72 h at 37°C in 6% CO_2, the cells were fixed, and the inclusions were counted. For the attachment restoration assays, CHO cells were grown on glass coverslips, as described above, were washed twice with 1 mL of ice-cold PBS, and were inoculated with a heparin concentration of 500 μg/mL (corresponding to 85 U), and were incubated for 1 h at 4°C. CHO cells were washed 3 times with 1 mL of ice-cold PBS and then were inoculated with *C. pneumoniae*, as described above.

**Results**

*C. pneumoniae* infectivity is decreased by soluble GAGs. HEP-2 cells were infected with EBs of *C. pneumoniae* isolate GD in the presence of different soluble GAGs. Heparin and heparan sulfate reduced the infectivity of *C. pneumoniae* competitively, whereas CSA, CSB, CSC, and keratan sulfate had no significant inhibitory effect, compared with the control (figure 1). Heparin inhibited 90% of *C. pneumoniae* infectivity, whereas heparan sulfate treatment resulted in a 50% reduction in infectivity. The inhibitory effect of heparin was dose dependent, and maximum inhibition was observed at heparin concentrations of ~6–6000 μg/mL (figure 2). For comparison, the effect of heparin on the infectivity of *C. trachomatis* strain L2 was analyzed (figure 1). At a heparin concentration of 500 μg/mL, the infection was reduced to 2%.
HEp-2 cells were incubated with chlamydial EBs in the absence or presence of indicated soluble GAGs (final concentration, 500 μg/mL). Results are expressed as percentage of inclusion-forming units formed in the absence of soluble GAG (gray bars). For comparison, the effect of heparin (500 μg/mL) on Chlamydia trachomatis L2 infectivity was measured (white bars). Each bar shows data from 6 C. pneumoniae experiments (mean ± SD). Results of the C. trachomatis experiments (n = 2) are also shown, with distance of each observation from the mean.

To rule out the possibility that heparin had an effect on the multiplication of C. pneumoniae in HEp-2 cells, the cells were infected with C. pneumoniae and, 4 h after infection, the infected cells were incubated with 500 μg/mL heparin. Heparin had no effect on the multiplication of the organism and was not cytotoxic to HEp-2 cells (data not shown).

Attachment of C. pneumoniae to HEp-2 cells is inhibited by heparin. HEp-2 cells were incubated with chlamydial EBs in the absence or presence of 500 μg/mL heparin. FACS analysis showed that the distribution of autofluorescence detected for the HEp-2 cells alone (mean fluorescence intensity, 8; figure 3C) was shifted to significantly higher fluorescence levels for HEp-2 cells incubated with C. pneumoniae EBs (mean fluorescence intensity, 13; figure 3A). The addition of C. pneumoniae to HEp-2 cells in the presence of heparin shifted the fluorescence intensity back to wild-type levels (mean fluorescence intensity, 7; figure 3B). In control experiments, HEp-2 cells alone treated with heparin showed some reduction in fluorescence (figure 3D). The FACS data were confirmed by inspection of the samples by fluorescence microscopy (data not shown). The results demonstrate that the addition of C. pneumoniae EBs to HEp-2 in the presence of heparin inhibits the adhesion of the bacteria to host cells.

C. pneumoniae requires the presence of heparan sulfate on the host cell surface for infection. Chlamydial EBs or HEp-2 cells were treated before infection with heparinase, which cleaves glycosidic linkages in heparan sulfate chains, thus removing the GAG. Digestion of the host cells with heparinase before the addition of untreated bacteria led to a 90% reduction in the infectivity of C. pneumoniae, compared with untreated controls (P < .00001; figure 4A). In contrast, heparinase treatment of C. pneumoniae before incubation with untreated HEp-2 cells had only a very small effect on the infectivity of the pathogen (P = 0.04). This result demonstrates that C. pneumoniae requires heparan sulfate–like GAGs on the target cell surface for infection.

Preincubation with heparin reduces infectivity of C. pneumoniae but not of C. trachomatis L2. C. pneumoniae or host cells were incubated with heparin before infection. Heparin pretreatment of C. pneumoniae before infection resulted in a marked 95% reduction in infectivity, compared with the control (P < .00001; figure 4B). Preincubation of HEp-2 cells with heparin resulted in a small but significant reduction of the infectivity of C. pneumoniae (P = 0.03). Pretreatment with heparin had the opposite effect on C. trachomatis L2. C. trachomatis EB infectivity was reduced only when the host cells were treated with heparin before infection (P < .00001). Preincubation of C. trachomatis EBs with heparin resulted in a significant enhancement, rather than a loss, of infectivity (P = .002; figure 4C).

Cell lines lacking heparan sulfate are only weakly susceptible to infection with C. pneumoniae. CHO-K1 cells were ~4 times less susceptible to C. pneumoniae infection than were HEp-2 cells (data not shown). When CHO-K1 and the CHO-pgsA-745 and CHO-pgsD-677 cells defective in heparan sulfate biosynthesis were infected in parallel with C. pneumoniae, there was a 74%–90% reduction in infectivity for the pgsA and pgsD cells, compared with the wild-type K1 cells (figure 5). Thus,
heparan sulfate on the host cell surface is essential for Chlamydia pneumoniae infectivity, and overproduction of CS in pgsD cells cannot compensate for the lack of heparan sulfate.

C. pneumoniae infection of cell lines defective in heparan sulfate biosynthesis can be restored by the addition of exogenous heparin. Cells from the CHO wild-type and from mutant cell lines pgsA-745 and pgsD-677 were incubated with heparin before infection. The wild-type control cell line K1 showed a small but significant reduction in infectivity, compared with the untreated control (P = .01; figure 5). In contrast, the mutant cell line pgsA showed a 2.5-fold increase in infectivity (from 26% to 67%) after incubation with heparin (P < .00001). Similarly, mutant cell line pgsD showed a 5-fold increase in infectivity (from 11% to 56%) when pretreated with heparin (P = .002; figure 5). These data demonstrate that infectivity of GAG-deficient cell lines can be restored by exogenous heparin.

Binding of C. pneumoniae to GAG-coated plastic surfaces. In addition to studies that used HEp-2 cells, the adherence of C. pneumoniae to various GAGs was analyzed by an EIA. Microtiter plate wells were coated with heparin, CSA, or CSC. Significant binding of chlamydial EBs was observed in wells coated with heparin. In contrast, no net absorbance was found to wells coated with CSA or CSC (data not shown). Control experiments showed no binding of the immune serum to GAG-coated wells and no cross-reactivity of preimmune serum to chlamydial EBs. The results of this EIA indicated that there is a specific interaction between C. pneumoniae adhesin(s) and heparin-like GAGs on the eukaryotic cell.

Discussion

The first step in the life cycle of C. pneumoniae is the attachment of EBs to the surface of a host cell. Our findings strongly suggest that heparan sulfate–like GAGs on the host cell surface serve as a receptor for C. pneumoniae. The evidence for this model of C. pneumoniae attachment includes the following observations: (1) infectivity of C. pneumoniae could be competitively inhibited by soluble heparin and heparan sulfate, but not by other GAGs; (2) inhibition of C. pneumoniae infectivity by heparin was dose dependent; (3) enzymatic removal of surface heparan sulfate from the host cell resulted in a marked reduction in C. pneumoniae infectivity, whereas heparinase treatment of the organism itself had no effect; (4) preincubation of C. pneumoniae with heparin reduced the infectivity of the organism but pretreatment of host cells with heparin had no effect; (5) mutant CHO cell lines defective in heparan sulfate synthesis were much less susceptible to C. pneumoniae infection than was the wild-type cell line; and (6) chlamydial infectivity was restored for GAG-deficient cell lines by the addition of heparin.

Our findings of heparan sulfate–dependent C. pneumoniae attachment are different from the mechanism of adhesion proposed for most other chlamydial species. For example, for the Chlamydia trachomatis serovars L2 and B and for Chlamydia psittaci, the guinea pig inclusion conjunctivitis agent, a heparan sulfate–like GAG is located on the surface of the bacterium that binds to a corresponding receptor on the host cell surface [10, 20–22]. In the trachoma biovars of C. trachomatis, the infectivity is reduced drastically when the infection process is done in the presence of heparan sulfate, although the attachment rates are not affected. These results suggest a GAG-dependent and a GAG-independent mode of attachment for these C. trachomatis biovars [20, 22]. Finally, in yet another serovar of C. trachomatis, serovar E, attachment and infectivity are not dependent...
on heparan sulfate at all [23]. In contrast to all the mentioned C. trachomatis strains, a completely different mode of attachment has been suggested for the mouse pneumonitis biovar of C. trachomatis. In the murine biovar, MoPn, heparan sulfate on the host cell surface is sufficient for chlamydial attachment mediated by the bacterial major outer membrane protein (MOMP) [24]. Thus, this biovar uses an infection mechanism similar to the one described here for C. pneumoniae.

Our results do not exclude the presence of a heparan sulfate-like ligand on the surface of C. pneumoniae, but the experiments document that these GAGs would not be necessary for the attachment to the host cell. Indeed, it is not obvious from the recently completed C. pneumoniae and C. trachomatis genome sequences that chlamydiae possess the enzymatic machinery required for biosynthesis of GAG molecules [25, 26].

The observation that preincubation of C. pneumoniae with heparin nearly abolishes the infectivity of the organism, whereas heparin pretreatment of C. trachomatis L2 enhanced infectivity, also has been reported by other researchers [10]. These findings demonstrate that the heparan sulfate–dependent mechanism of infectivity fundamentally differs between these 2 chlamydial species. The available data seem to indicate that the mechanism of attachment is not conserved in the genus Chlamydia and that both GAG-dependent and -independent mechanisms of chlamydial attachment exist. It is possible that the differences in attachment may contribute to the differences in virulence seen in various chlamydia species (e.g., reflecting differences in cell tropism). In this context, it is intriguing that C. pneumoniae and C. trachomatis MoPn, which both cause respiratory tract infections, seem to use very similar attachment mechanisms. Furthermore, because GAGs are found on many different cell types, the observed mode of attachment may explain why C. pneumoniae can infect a wide variety of human cell lines.

Heparin inhibited the infectivity of C. pneumoniae more effectively than heparan sulfate, possibly because heparin contains more negatively charged sulfate groups than does heparan sulfate. This could indicate that electrostatic interactions are involved in the binding of C. pneumoniae to eukaryotic cells [27]. Indeed, similar observations have been made for the attachment of N. gonorrhoeae to host cells [28]. However, in the present study, the mutant CHO cell line pgsD-677, which lacks heparan sulfate but overproduces highly sulfated CS, was much less susceptible to C. pneumoniae infection than was the wild-

![Figure 4](https://academic.oup.com/jid/article-abstract/184/2/181/1055870)

**Figure 4.** Effect of heparinase I and heparin treatment of Chlamydia or host cells. A, Elementary bodies (EBs) of Chlamydia pneumoniae or HEp-2 cells were incubated with heparinase I before infection. B and C, EBs of C. pneumoniae or of Chlamydia trachomatis or HEp-2 cells were incubated with heparin, were washed, and then were used for the infection experiment. Results are expressed as percentages of inclusion-forming units formed by untreated EBs in untreated HEp-2 cells (control, 100% infectivity). Each value represents mean ± SD (n = 4).

![Figure 5](https://academic.oup.com/jid/article-abstract/184/2/181/1055870)

**Figure 5.** Chlamydia pneumoniae infection of glycosaminoglycan (GAG)-deficient cell lines. Wild-type CHO-K1 cells and mutant cell lines pgsA-745 and pgsD-677 defective in GAG synthesis were inoculated without (black bars) and with (white bars) 500 µg/mL exogenous heparin before infection with C. pneumoniae elementary bodies. Infectivity was determined by counting the no. of inclusions; results are expressed as percentages of inclusion-forming units formed in CHO-K1 cells without heparin treatment (black bar, 100%). Each value represents mean ± SD (n = 6 for CHO-K1 and pgsA-745; n = 4 for pgsD-677).
type cell line. Thus, specific attachment of *C. pneumoniae* to heparan sulfate involves more than a simple electrostatic interaction. This is supported by the fact that *C. pneumoniae* EBs specifically bind to heparin-coated microtiter plates but not to CSA or CSC.

Although heparin or heparinase treatment drastically reduced the infectivity of *C. pneumoniae*, a significant level of residual infectivity (10%) was consistently observed. Similarly, a background level of *C. pneumoniae* infection also was found in heparan sulfate–deficient cell lines. These observations are of particular interest, because all attachment and infection experiments described here were done without centrifugation. Thus, the results indicate that *C. pneumoniae* attachment and entry can be mediated by other as yet unidentified receptors.

We speculate that the attachment of *C. pneumoniae* to heparan sulfate is only an initial interaction between the bacterial cell and the host cell and that further interactions are necessary for a successful infection. In several viruses that use heparan sulfate as a primary receptor, other coreceptors have been identified [29]. For example, in adeno-associated virus 2 infection, αVβ5 integrin and human fibroblast growth factor receptor 1 appear to be coreceptors for successful viral entry [30, 31]. The expression of these specific receptors in different cells and tissues might account for the tissue tropism and the spread of *C. pneumoniae*. GAG-binding proteins have been identified in several pathogens, including herpes simplex virus, *Listeria monocytogenes*, *Helicobacter pylori*, and *Mycobacterium tuberculosis* [32–35]. These GAG-binding proteins are promising candidates for the development of vaccines against these pathogens. In contrast, the surface structures of *C. pneumoniae* that mediate the attachment to the host cell are unknown [36]. In the mouse pneumonia (MoPn) biovar of *Chlamydia trachomatis*, the MOMP might function as an adhesin [37, 38] (e.g., recombinant MOMP binds to host cell surface heparan sulfate [24]). However, in vivo heparan sulfate could not inhibit the infection with the MoPn biovar, which suggests that alternate means of uptake could replace GAG-dependent means of entry [39]. Since there are structural and immunologic differences between the MOMPs of *C. pneumoniae* and *C. trachomatis* MoPn, it is not clear whether the MOMP of *C. pneumoniae* has a similar function [12, 40]. Other attractive *C. pneumoniae* candidate proteins relevant for GAG binding or for other aspects of the adhesion process are the recently identified 21 polymorphic membrane proteins [26, 41]. The identification of the surface structures of *C. pneumoniae* that bind to heparan sulfate and possibly to other host cell coreceptors will yield important insights into the pathogenesis of *C. pneumoniae* infections.

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References

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