

Sulfated Carbohydrate Compounds Prevent Microbial Adherence by Sexually Transmitted Disease Pathogens

BETSY C. HEROLD,^{1*} ALICIA SISTON,¹ JAMES BREMER,² RISA KIRKPATRICK,³
GEORGE WILBANKS,² PETER FUGEDI,⁴ CSABA PETO,⁴ AND MORRIS COOPER³

Section of Pediatric Infectious Diseases, University of Chicago,¹ and Departments of Obstetrics and Gynecology and Microbiology, Rush University,² Chicago, and Department of Medical Microbiology and Immunology, Southern Illinois University School of Medicine, Springfield,³ Illinois, and Carbohydrate Chemistry Section, Glycomed, Inc., Alameda, California 94501⁴

Received 28 May 1997/Returned for modification 22 July 1997/Accepted 30 September 1997

Heparan sulfate (HS) serves as a receptor for adherence of herpes simplex viruses, *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and, indirectly, human immunodeficiency virus. Using primary human culture systems, we identified sulfated carbohydrate compounds that resemble HS and competitively inhibit infection by these pathogens. These compounds are candidates for intravaginal formulations for the prevention of sexually transmitted diseases.

Sexually transmitted diseases (STDs) such as gonorrhea, chlamydia, genital herpes, and AIDS are a major health problem of epidemic proportions worldwide. The World Health Organization estimates that 125 million new cases of major bacterial and viral STDs occur each year (2). In the United States, inner-city minority populations experience the most severe STD rates and a growing AIDS epidemic. The situation in the developing world is even more alarming. The World Bank estimates that for adults between 15 and 44 years of age, STDs, not including human immunodeficiency virus (HIV) infection, are the second leading cause of healthy life lost in women (34, 43).

Several different approaches will be required to combat this problem, including improved patient education, increased use of barrier methods, vaccine development, and development of new antimicrobial drugs. The development of female-controlled methods for STD prevention, including methods that are not necessarily spermicidal, is crucial. Women are especially at risk because many STDs are asymptomatic and the morbidity associated with untreated disease is high. Most currently available vaginal formulations are contraceptive and use a detergent, such as nonoxynol-9 (N9), as both the antimicrobial and the spermicidal agent. N9 inactivates enveloped viruses, such as herpes simplex virus (HSV), and other microorganisms, including *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and HIV (2, 9, 14, 22, 49). However, N9 is relatively toxic to cervical cells in vitro, and there have been conflicting reports regarding its toxicity in vivo. Concerns about N9 include an increased incidence of vulvar ulcers and vaginitis associated with its use (23, 52).

One strategy we are pursuing is the development of compounds designed for intravaginal use that would prevent microbial adhesion and entry and thus would prevent infection. Therefore, an understanding of the requirements for the entry of microbes into target cells is required. Recent studies suggest that adherence to cell surfaces for such diverse pathogens as *N. gonorrhoeae*, *C. trachomatis*, and HSV involves a common cell surface molecule, heparan sulfate (HS) glycosaminoglycans

(GAGs) (37). These observations suggest that polysulfated carbohydrates that mimic HS could potentially interfere with microbial entry. The potential advantages of these compounds include their broad-spectrum activity and simple chemical structure, which should allow production costs to be low. Moreover, toxicities are likely to be minimal. These studies, therefore, were designed to develop in vitro models of STD infection and test polysulfated carbohydrates for their ability to inhibit viral or bacterial adhesion.

In previous studies, it has been shown that HS serves as an initial receptor for the binding of HSV types 1 and 2 (HSV-1 and -2) to epithelial-cell surfaces (18, 19, 38, 40, 50). However, the studies were conducted with permanent non-cervical epithelial cell lines, such as HEP-2 or Vero cells. To determine whether HS moieties also serve as a receptor for the binding of HSV to primary cultures, we compared the ability of the GAGs heparin (an analog of HS), chondroitin sulfate, and dermatan sulfate to inhibit viral entry using primary human ectocervical, endocervical, and vaginal mucosal cells. Primary cultures were derived from specimens obtained from hysterectomies performed for "benign" conditions such as uterine leiomyomas and endometriosis, as previously described (41, 46, 47). Patients ranged in age from 30 to 54 years (mean, 43 ± 7 years). Primary cells in 96-well dishes were inoculated with HSV-1(17)(dUTPase/LAT) at a multiplicity of infection of 5 to 10 PFU/cell in the presence or absence of 100 µg of each GAG (Sigma)/ml. HSV-1(17)(dUTPase/LAT), which behaves like wild-type HSV-1(17) with respect to binding and infectivity, has been genetically engineered and expresses the β-galactosidase gene under control of the viral immediate-early gene promoter, dUTPase (a gift of E. Wagner, University of California, Irvine) (36, 39). After a 5-h period at 37°C, the β-galactosidase expression from infected cells was quantified as previously described (36). Only heparin, not chondroitin sulfate or dermatan sulfate, inhibited viral early gene expression (Fig. 1a). Similar results were obtained when experiments were conducted by using plaque assays or binding assays with both HSV-1 and HSV-2 (data not shown).

Having established that HS is important for HSV entry into primary human cervical cells, we next tested a series of carbohydrate compounds that structurally resemble HS for their ability to inhibit HSV binding and entry. The carbohydrate compounds selected for testing have low anticoagulant activity

* Corresponding author. Mailing address: Pediatric Infectious Diseases, University of Chicago, 5841 S. Maryland Ave., Chicago, IL 60637-1470. Phone: (773) 702-6176. Fax: (773) 702-1196. E-mail: bherold@midway.uchicago.edu.

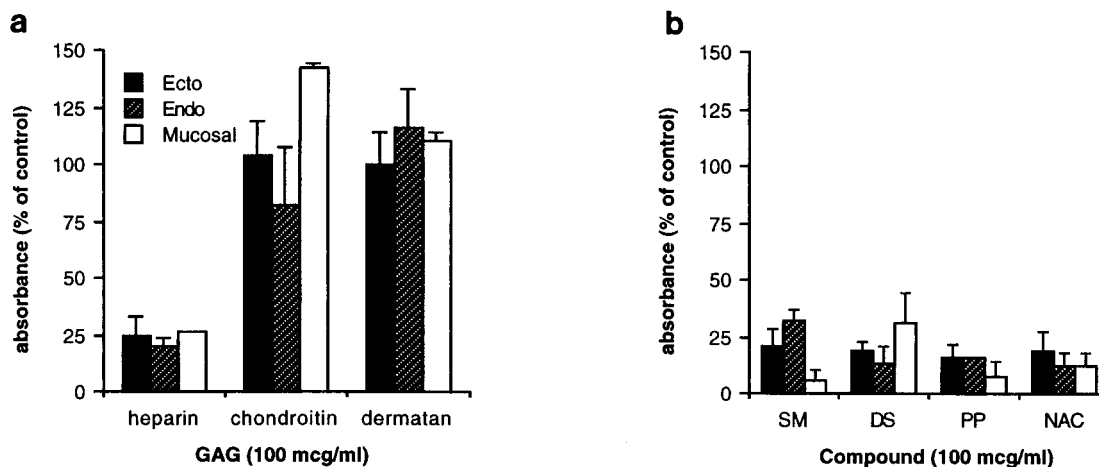


FIG. 1. Effects of GAGs (a) and polysulfated carbohydrate compounds (b) on HSV-1 infection of primary human ectocervical, endocervical, and vaginal mucosal cells. Primary human cells were seeded in 96-well dishes ($\sim 10^5$ cells/well) and allowed to attach overnight. Cells were exposed to HSV-1(17)(dUTPase/LAT) virus at a multiplicity of infection of 5 to 10 PFU/cell in the presence or absence of 100 μ g of heparin, chondroitin sulfate A, dermatan sulfate (a) or SM, DS, PP, or NAC (b)/ml. After a 5-h incubation period at 37°C, the inoculum was removed, and the cells were washed, fixed, permeabilized, and exposed to the substrate *o*-nitrophenyl- β -D-galactopyranoside (ONPG) for 1 h as previously described (36). The results are presented as β -galactosidase expression (absorbance at 410 nm) in the presence of 100 μ g of compound/ml as a percentage of β -galactosidase expression in the absence of compound. Each point is the mean of values obtained from two to three independent experiments performed in duplicate or triplicate; error bars indicate standard deviations. The inhibitory effects of heparin and the carbohydrate compounds on viral infection are significantly different from the effects of chondroitin sulfate and dermatan sulfate ($P < 0.01$).

and include non-anticoagulant heparin (NAC) (average molecular size, 10.6 kDa; Glycomed, Alameda, Calif.) (16, 17, 25), pentosan polysulfate (PP) (molecular size, 3.8 kDa; Sigma) (45), and dextran sulfate (DS) (molecular size, 8 kDa; Sigma) (25). We also tested a novel sulfated maltoheptaose derivative (*N*-acetyl- β -maltoheptaosylamine sulfate [SM]) (molecular size, 3.5 kDa; Glycomed). Details of the synthesis of SM will be published elsewhere. Briefly, maltoheptaose was treated with aqueous NH_4HCO_3 at room temperature to yield maltoheptaosylamine. Treatment of the crude amine with acetic anhydride in pyridine followed by purification afforded the anomerically pure peracetylated *N*-acetyl- β -maltoheptaosylamine derivative. Removal of the *O*-acetyl groups with sodium methoxide in methanol furnished *N*-acetyl- β -maltoheptaosylamine. This compound was sulfated with sulfur trioxide pyridine complex in *N,N*-dimethylformamide to produce the sulfated maltoheptaose derivative.

The results for HSV-1 entry in the presence or absence of 100 μ g of each of the carbohydrate compounds/ml are shown in Fig. 1b. All four compounds inhibited viral entry. The inhibition was at least as great when compounds were present only during initial attachment (4°C), suggesting that the carbohydrates inhibit infection by competing with cell surface HS for viral binding (data not shown). To examine the effects of the carbohydrate compounds on HSV-2, we used a plaque assay (19, 20). In pilot experiments we found that HSV-2(G) forms plaques on primary cells within 24 h. Representative results are shown in Table 1. PP and NAC were the most effective, but all four compounds inhibited at least 75% of viral infection at a concentration of 100 μ g/ml.

Having established that these compounds inhibit HSV-1 and HSV-2 infection, we tested their antimicrobial activity against several other STD pathogens known to interact with HS. Recent studies suggest that binding of *N. gonorrhoeae* to HS is required for pilus-independent tight attachment and entry into cultured human epithelial cells (11, 48). To determine the effect of compounds on *N. gonorrhoeae* adhesion, the human fallopian tube organ culture (HFTOC) model was employed (13). Tissue from HFTOC was infected with 10^5 CFU of *N.*

gonorrhoeae MS11/ml in the presence or absence of increasing concentrations of candidate compounds or heparin, and the number of viable gonococci in the tissue at 24 h was determined as previously described (13). The control wells containing HFTOCs with no compound are considered 100% infection. Results are summarized in Fig. 2. PP and DS were the most effective and completely inhibited *N. gonorrhoeae* infection of HFTOC at concentrations of ~ 100 and 500 μ g/ml, respectively. NAC and unmodified heparin inhibited about 70% of gonococcal infection of HFTOC at 500 μ g/ml, but SM had no demonstrable effect, even at 1,000 μ g/ml (data not shown).

C. trachomatis is thought to synthesize an HS-like molecule that facilitates bacterial invasion by binding to both the major

TABLE 1. Inhibitory and cytotoxic effects of carbohydrate compounds on HSV-2 infection of primary cervical cells

Compound	ED ^a (μ g/ml)		CD ^b (mg/ml)		SI ₅₀ ^c
	50%	90%	50%	90%	
PP	0.5	6	~ 10	> 10	5×10^5
NAC	0.4	6	> 10	> 10	$> 5 \times 10^5$
SM	25	~ 100	10	> 10	4×10^2
DS	6	~ 100	> 10	> 10	$> 1.7 \times 10^3$
N9	ND ^d	ND	$< 0.001\%$	0.1%	ND

^a The dose of polysulfated carbohydrate compound that inhibited 50 or 90% of HSV-2 plaque formation on primary human cervical or vaginal cells compared to plaques formed in the absence of carbohydrate compound was determined from a dose-response curve generated from an independent experiment conducted with an individual patient's cells in duplicate. Results are representative of 4 to 5 experiments conducted with ectocervical, endocervical, and vaginal mucosal cells from different patients. ED, effective dose.

^b The dose of compound that was cytotoxic for 50 or 90% of primary human cervical or vaginal cells by neutral red dye exclusion was determined from a dose-response curve generated from two independent experiments conducted with two individual patients' cells in duplicate. CD, cytotoxic dose.

^c Selectivity index (SI₅₀) is the ratio of CD₅₀ to ED₅₀.

^d The ED₅₀, ED₉₀, and SI₅₀ for N9 were not determined (ND). The concentration of N9 in currently available spermicidal products ranges from 1 to 6% (38).

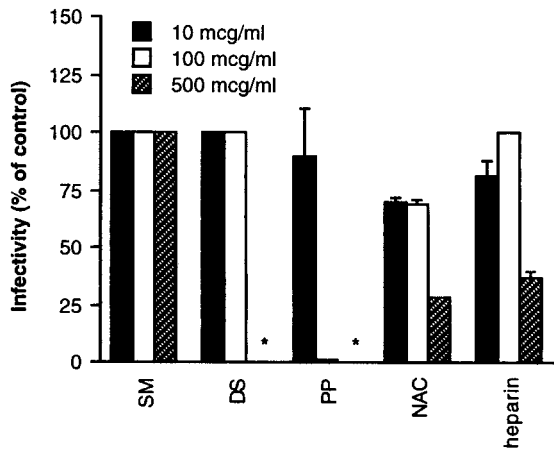


FIG. 2. Effects of polysulfated carbohydrate compounds on *N. gonorrhoeae* infection. Tissue from HFTOC was infected with 10^5 CFU of *N. gonorrhoeae*/ml in the presence or absence of increasing concentrations of carbohydrate compounds. After 24 to 48 h, the number of viable gonococci in the tissue was determined by plating homogenized tissue samples on gonococcal agar. The results are presented as gonococcal viability in the presence of carbohydrate as a percentage of gonococcal viability in the absence of carbohydrate compound. Each point is the mean of values obtained from three independent experiments performed in duplicate; error bars indicate standard errors. No viable gonococci remained in the tissue in the presence of 500 μ g of PP and DS/ml (*).

outer membrane protein on the chlamydial organism and a receptor on the epithelial-cell surface (10–12, 42, 44, 53). Hence, candidate carbohydrate compounds might effect chlamydial attachment by binding either to the chlamydial acceptor or the epithelial cell surface receptor. To determine the effect of carbohydrate compounds on *C. trachomatis* infection, HeLa cells were preincubated with various concentrations of test carbohydrate compounds and were then exposed to *C. trachomatis*, serotype E/UW-5/CX, and the number of infected cells was quantitated (8, 13, 24, 31, 32). Alternatively, chlamydial organisms were preincubated with the carbohydrate compounds prior to infecting HeLa cells. The results for both assays are shown in Fig. 3. PP, SM, and DS inhibited >90% of *C. trachomatis* infection when HeLa cells were pretreated with

the carbohydrates at a concentration of 1 μ g/ml; no inhibition was observed at a concentration of 0.1 μ g/ml (data not shown). NAC also inhibited chlamydial infection, but to a lesser extent. In contrast, pretreatment of *C. trachomatis* with carbohydrate compounds resulted in little inhibition of subsequent infection of HeLa cells. These results suggest that the carbohydrates compete with the chlamydial HS-like molecule for binding to the eucaryotic cell surface receptor.

Cell surface HS is believed to play a role in HIV infection of human T cells. Human T cells express HS and chondroitin sulfate GAGs on their surfaces. Treatment of T cells with heparatinase reduces HIV binding and infection, but treatment with chondroitinase does not (29, 30, 35). Moreover, when T cells are cultured in the presence of sodium chlorate, an inhibitor of ATP sulfurylase and thus an inhibitor of sulfation of GAGs, HIV binding and infection are impaired (29). These results suggest that HS participates in HIV infection by facilitating viral binding to T cells. Recent studies suggest that the HIV envelope may bind to both HS and CD4 to form a trimolecular complex and that it is the V3 region of the gp120-gp41 complex that mediates the envelope-HS interactions (35). Sulfated polysaccharides have previously been shown to inhibit HIV binding, replication, and formation of syncytia in vitro, presumably because of interactions of the polyanionic polysaccharide with positively charged amino acids concentrated in the V3 region of HIV gp120 (3, 5, 7, 21, 27). To determine whether the candidate carbohydrate compounds identified in these studies also inhibit HIV in vitro, syncytial assays were conducted. The results are summarized in Table 2. DS inhibited ~90% of HIV syncytial formation at concentrations of 0.1 μ g/ml. PP and SM inhibited >75% of syncytial formation at concentrations of 1 μ g/ml. The maximal inhibition observed with NAC was ~50% at a concentration of 0.1 μ g/ml; no further inhibition was observed even at concentrations as high as 100 μ g/ml.

To assess the potential cytotoxicity of the carbohydrate compounds for human primary cervical cells, cell viability was determined as the uptake of the dye neutral red (33). Briefly, cells were incubated for 16 to 18 h in media containing serial dilutions of test carbohydrate compounds. The medium was then removed, and cells were washed and then incubated for 1 h at

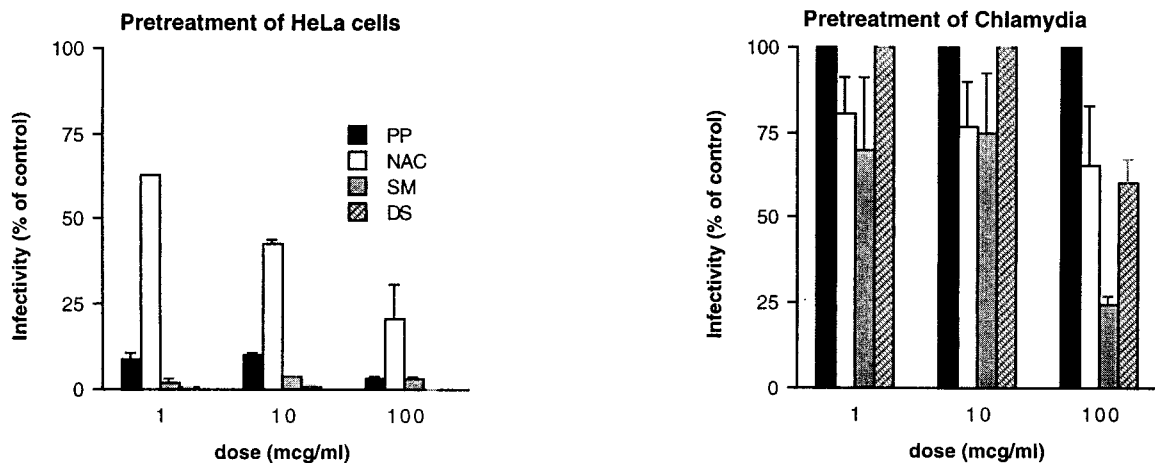


FIG. 3. Effects of polysulfated carbohydrate compounds on *C. trachomatis* infection. HeLa cells were preincubated with various concentrations of carbohydrate compounds and then exposed to *C. trachomatis*, and the number of infected cells was quantitated (left). Alternatively, chlamydial organisms were preincubated with the carbohydrate compounds prior to infection of HeLa cells (right). The results are presented as the number of inclusions formed in the presence of carbohydrate compound as a percentage of the number of inclusions formed in the absence of carbohydrate compound. Each point is the mean of values obtained from three independent experiments performed in duplicate; error bars indicate standard errors.

TABLE 2. Effect of polysulfated carbohydrate compounds on HIV^a infection of MT-2 cells^b

Compound	ED ^c (μg/ml)	
	50%	90%
PP	0.1	1
NAC	0.1	>100
SM	0.1	~100
DS	0.03	0.1

^a The HIV strain (G910-SI, AZT-R) was obtained from the National Institutes of Health AIDS Research and Reference Reagent Program (Rockville, Md.) and is identified as a stable HIV clone which will produce syncytia in MT-2 cells.

^b A 24th passage of the human T-cell line, MT-2, was obtained from the National Institutes of Health AIDS Research and Reference Reagent Program.

^c The concentration of polysulfated carbohydrate compound that inhibited 50 or 90% of syncytia formed compared to syncytia formed in the absence of any carbohydrate compound was determined from dose-response curves generated from two independent experiments performed in quadruplicate. ED, effective dose.

37°C with 50 μg of neutral red dye/ml. Cells were then rinsed, neutral red was eluted from the viable cells by the addition of 0.5 M HCl with 35% ethanol, and the absorbance was measured at a wavelength of 570 nm. The results are summarized in Table 1. None of the carbohydrate compounds exhibit cytotoxicity for primary human cervical cells at concentrations of 1 mg/ml in vitro, whereas N9 (obtained from Sigma) was significantly cytotoxic.

There is an urgent need for the implementation of new strategies to prevent the spread of STDs. Recent studies have shown that HS plays a key role in mediating the adherence of a diverse array of microbes to cell surfaces (37). However, these studies have been conducted primarily with permanent cell lines. We have extended these observations using primary genital-tract culture systems (primary human vaginal and cervical cells and HFTOC) and have developed an in vitro screening assay system to test the efficacy of HS analogs as competitive inhibitors of microbial adhesion or attachment. This system can be utilized to test a wide range of potential drug therapies. This culture system has distinct advantages over traditional permanent cell lines, such as the maintenance of certain differentiated characteristics. For example, primary ectocervical cultures express keratin 13, 10, and 14 and filaggrin, whereas primary endocervical cultures express keratin 18, 8, 9, and 19 (47). In contrast, permanent cell lines fail to express keratins. Thus, a primary culture system will be an important tool for identifying the microbial and cellular requirements for infection of cells of the female genital tract.

Using this model culture system, we identified polysulfated carbohydrate compounds that inhibit HSV infection by inhibiting viral binding. These compounds are not cytotoxic to primary cells and are effective against other STD pathogens including HIV, *N. gonorrhoeae*, and *C. trachomatis*. These compounds are quite potent and are effective at very low concentrations in vitro. For *C. trachomatis*, we found that the carbohydrates competed with the chlamydial organisms for the epithelial cell surface receptor. None of the carbohydrates enhanced chlamydial attachment even when the organisms were preincubated with the carbohydrates, a possibility suggested by the observation that chlamydiae bind HS-like molecules (10). The results we obtained with DS and PP for *C. trachomatis* infection are similar to results obtained by Zaretzky and colleagues (51) but differ with respect to the concentration required to inhibit 50% of infection (NAC and SM have not been tested previously). The variability in concentrations most likely reflects differences in the assays and cell types.

The notion that sulfated polysaccharides inhibit microbial infection is not new. Previous studies have shown that sulfated compounds inhibit the in vitro replication of enveloped viruses such as HSV, cytomegalovirus, and vesicular stomatitis virus (4). Interest in these compounds as potential antiviral agents surfaced with the in vitro observations that DS inhibited HIV replication (3, 4, 6, 7, 21). These observations led to clinical trials with both orally and intravenously administered DS. However, the results were disappointing, and DS was found to be ineffective in the treatment of HIV infection (1, 6, 15, 26).

Other potential therapeutic applications of sulfated carbohydrates as inhibitors of microbial attachment were not initially fully appreciated. Most patients with mucocutaneous HSV, for example, are unaware of their initial exposure. A drug that blocks viral attachment would, presumably, be ineffective against an established primary infection or reactivation of latent virus. However, at the time of sexual transmission, an agent that blocks attachment to cell surfaces might be highly effective and prevent the establishment of infection. In a recent study, Neyts and De Clercq examined the effect of DS and PAVAS (copolymer of acrylic acid with vinyl alcohol sulfate) on vaginitis and death of mice infected intravaginally with HSV-2 (28). In their study, the compounds were mixed with virus immediately prior to intravaginal inoculation of the animals. Results were encouraging. None of the animals infected with HSV-2 in the presence of PAVAS died, and a significantly smaller number of mice died when infected with HSV-2 in the presence of DS compared to controls.

Studies using animal models of vaginal HSV infection and the polysulfated compounds identified in these studies are currently in progress. The compounds will need to be formulated for intravaginal use as a cream, foam, or gel. Potential advantages include the observation that these compounds exhibit little or no cellular cytotoxicity and are not likely to be spermicidal. As such, they offer distinct advantages over N9, which is both spermicidal and cytotoxic to cells. The effect of polysulfated compounds on vaginal microflora and the local immune system will also need to be assessed.

This work was supported by Public Health Service grant AI37940.

We are grateful to E. Wagner for providing the viral strain HSV-1(17)(dUTPase/LAT). We are also grateful to L. Dee and E. Winkfield for technical support.

REFERENCES

- Abrams, D. I., S. Kuno, R. Wong, K. Jeffords, M. Nash, J. B. Molaghan, R. Gorter, and R. Ueno. 1989. Oral dextran sulfate (UA001) in the treatment of the acquired immunodeficiency syndrome and AIDS-related complex. *Ann. Intern. Med.* **110**:183-188.
- Alexander, N. J. 1996. Barriers to sexually transmitted diseases. *Sci. Am. Sci. Med.* **March/April**:32-40.
- Baba, M., D. Schols, E. De Clercq, R. Pauwels, M. Nagy, J. Gyorgyi-Edelenyi, M. Low, and G. Sandor. 1990. Novel sulfated polymers as highly potent and selective inhibitors of human immunodeficiency virus replication and giant cell formation. *Antimicrob. Agents Chemother.* **34**:134-138.
- Baba, M., R. Pauwels, J. Balzarini, J. Arnout, J. Desmyter, and E. De Clercq. 1988. Mechanism of inhibitory effect of dextran sulfate and heparin on replication of human immunodeficiency virus in vitro. *Proc. Natl. Acad. Sci. USA* **85**:6132-6136.
- Baba, M., R. Snoeck, R. Pauwels, and E. De Clercq. 1988. Sulfated polysaccharides are potent and selective inhibitors of various enveloped viruses, including herpes simplex virus, cytomegalovirus, vesicular stomatitis virus, and human immunodeficiency virus. *Antimicrob. Agents Chemother.* **32**:1742-1745.
- Bagasr, O., and H. W. Lischner. 1988. Activity of dextran sulfate and other polyanionic polysaccharides against human immunodeficiency virus. *J. Infect. Dis.* **158**:1084-1087.
- Batinic, D., and F. Robey. 1992. The V3 region of the envelope glycoprotein of human immunodeficiency virus type 1 binds sulfated polysaccharides and CD4-derived synthetic peptides. *J. Biol. Chem.* **267**:6664-6671.
- Bird, B. R., and F. T. Forrester. 1981. Laboratory diagnosis of *Chlamydia*

- trachomatis* infections, p. 33–71. U.S. Department of Health and Human Services, Atlanta, Ga.
9. Bourinbaïar, A. S., and S. Lee-Huang. 1994. Comparative *in vitro* study of contraceptive agents with anti-HIV activity: gramicidin, nonoxynol-9, and gossypol. *Contraception* **49**:131–137.
 10. Chen, J. C., J. P. Zhang, and R. S. Stephens. 1996. Structural requirements of heparin binding to *Chlamydia trachomatis*. *J. Biol. Chem.* **271**:11134–11140.
 11. Chen, J. C., 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.
 12. Chen, T., R. J. Belland, J. Wilson, and J. Swanson. 1995. Adherence of pilus-*Op*⁺ gonococci to epithelial cells *in vitro* involves heparan sulfate. *J. Exp. Med.* **182**:511–517.
 13. Cooper, M. D., J. Rapp, C. Jeffrey-Wiseman, R. C. Barnes, and D. S. Stephens. 1990. *Chlamydia trachomatis* infection of human fallopian tube organ cultures. *J. Gen. Microbiol.* **136**:1109–1115.
 14. Feldblum, P. J., and S. S. Weir. 1994. The protective effect of nonoxynol-9 against HIV infection. *Am. J. Public Health* **8**:1032–1034.
 15. Flexner, C., P. A. Barditch-Crovo, D. M. Kornhauser, H. Farzadegan, L. J. Nerhood, R. E. Chaisson, K. M. Bell, K. J. Lorentsen, C. W. Hendrix, B. G. Petty, and P. S. Lietman. 1991. Pharmacokinetics, toxicity, and activity of intravenous dextran sulfate in human immunodeficiency virus infection. *Antimicrob. Agents Chemother.* **35**:2544–2550.
 16. Herold, B. C., S. I. Gerber, B. J. Belval, A. M. Siston, and N. Shulman. 1996. Differences in the susceptibility of herpes simplex virus types 1 and 2 to modified heparin compounds suggest serotype differences in viral entry. *J. Virol.* **70**:3461–3469.
 17. Herold, B. C., S. I. Gerber, T. Polonsky, B. J. Belval, P. N. Shaklee, and K. Holme. 1995. Identification of structural features of heparin required for inhibition of herpes simplex virus type 1 binding. *Virology* **206**:1103–1116.
 18. Herold, B. C., R. Visalli, N. Susmarksi, C. Brandt, and P. G. Spear. 1994. Glycoprotein C-independent binding of herpes simplex virus to cells requires cell surface HS and virion gB. *J. Gen. Virol.* **75**:1211–1222.
 19. Herold, B. C., D. WuDunn, N. Soltys, and P. G. Spear. 1991. Glycoprotein C of herpes simplex virus type 1 plays a principal role in the adsorption of virus to cells and in infectivity. *J. Virol.* **65**:1090–1098.
 20. Holland, T. C., R. M. Sandri-Goldin, L. E. Holland, S. D. Marlin, M. Levine, and J. C. Glorioso. 1983. Physical mapping of the mutation in an antigenic variant of herpes simplex virus type 1 by use of an immunoreactive plaque assay. *J. Virol.* **46**:649–652.
 21. Ito, M., M. Baba, A. Sato, R. Pauwels, E. De Clercq, and S. Shigeta. 1987. Inhibitory effect of dextran sulfate and heparin on the replication of human immunodeficiency virus (HIV) *in vitro*. *Antiviral Res.* **7**:361–367.
 22. Jennings, R., and A. Clegg. 1993. The inhibitory effect of spermicidal agents on replication of HSV-2 and HIV-1 *in vitro*. *J. Antimicrob. Chemother.* **32**:71–82.
 23. Kreiss, J., E. Ngugi, K. K. Holmes, J. Nidinya-Achola, P. Waiyaki, P. L. Roberts, I. Ruminjo, R. Sajabi, J. Kimata, R. T. R. Fleming, A. Anzala, D. Holtan, and F. Plummer. 1992. Efficacy of nonoxynol-9 contraceptive sponge use in preventing heterosexual acquisition of HIV in Nairobi prostitutes. *JAMA* **268**:477–482.
 24. Kuo, C., S. Wang, and J. T. Grayston. 1972. Differentiation of TRIC and LGV organisms based on enhancement of infectivity by DEAE-dextran in cell culture. *J. Infect. Dis.* **125**:313–317.
 25. Lopalco, L., F. Ciccomascolo, P. Lazna, G. Zoppetti, I. Caramazza, F. Leoni, A. Beretta, and A. G. Siccardi. 1994. Anti-HIV type 1 properties of chemically modified heparins with diminished anticoagulant activity. *AIDS Res. Hum. Retroviruses* **10**:787–793.
 26. Lorentsen, K. J., C. S. Hendrix, J. M. Collins, D. M. Kornhauser, B. G. Petty, R. W. Klecker, C. Flexner, R. H. Eckel, and P. S. Lietman. 1989. Dextran sulfate is poorly absorbed after oral administration. *Ann. Intern. Med.* **111**:561–566.
 27. Mitsuya, H., D. J. Looney, S. Kuno, R. Ueno, F. Wong-Staal, and S. Broder. 1988. Dextran sulfate suppression of viruses in the HIV family: inhibition of virion binding to CD4⁺ cells. *Science* **226**:172–174.
 28. Neyts, J., and E. De Clercq. 1995. Effect of polyanionic compounds on intracutaneous and intravaginal herpesvirus infection in mice: impact on the search for vaginal microbicides with anti-HIV activity. *J. Acquired Immune Defic. Syndr.* **10**:8–12.
 29. Ohshiro, Y., T. Murakami, K. Matsuda, K. Nishioka, K. Yoshida, and N. Yamamoto. 1996. Role of cell surface glycosaminoglycans of human T cells in human immunodeficiency virus type-1 (HIV-1) infection. *Microbiol. Immunol.* **40**:827–835.
 30. Patel, M., M. Yanagishita, G. Roderiquez, D. C. Bou-Habib, T. Oravec, V. C. Hascall, and M. A. Norcross. 1993. Cell-surface heparan sulfate proteoglycan mediates HIV-1 infection of T-cell lines. *AIDS Res. Hum. Retroviruses* **9**:167–174.
 31. Patton, D. L., C. C. Kuo, S. P. Wang, and S. A. Halbert. 1987. Distal tubal obstruction induced by repeated *Chlamydia trachomatis* salpingeal infections in pig-tailed macaques. *J. Infect. Dis.* **155**:1292–1299.
 32. Patton, D. L., S. A. Halbert, C. C. Kuo, S. P. Wang, and K. K. Holmes. 1983. Host response to primary *Chlamydia trachomatis* infection of the fallopian tube in pig-tailed monkeys. *Fertil. Steril.* **40**:829–840.
 33. Phillips, J. J. 1973. Dye exclusion tests for cell viability, p. 406–408. *In P. F. Kruse and M. K. Patterson (ed.), Tissue culture: methods and application.* Academic Press, New York, N.Y.
 34. Piot, P., and M. Islam. 1994. Sexually transmitted diseases in the 1990s: global epidemiology and challenges for control. *Sex. Transm. Dis.* **21**:S7–S13.
 35. Roderiquez, G., T. Oravec, M. Yanagishita, D. C. Bou-Habib, H. Mostowski, and M. A. Norcross. 1995. Mediation of human immunodeficiency virus type 1 binding by interaction of cell surface heparan sulfate proteoglycans with the V3 region of envelope gp120-gp41. *J. Virol.* **69**:2233–2239.
 36. Roller, R. J., and B. C. Herold. 1997. Characterization of a BHK(TK⁻) cell clone resistant to postattachment entry by herpes simplex virus types 1 and 2. *J. Virol.* **71**:5805–5813.
 37. Rostand, K. S., and J. D. Esko. 1997. Microbial adherence to and invasion through proteoglycans. *Infect. Immun.* **65**:1–8.
 38. Shieh, M.-T., D. WuDunn, R. I. Montgomery, J. D. Esko, and P. G. Spear. 1992. Cell surface receptors for herpes simplex virus are heparan sulfate proteoglycans. *J. Cell Biol.* **116**:1273–1281.
 39. Singh, J., and E. K. Wagner. 1995. Herpes simplex virus recombination vectors designed to allow insertion of modified promoters into transcriptionally “neutral” segments of the viral genome. *Virus Genes* **10**:127–136.
 40. Spear, P. G. 1993. Entry of alphaherpesviruses into cells. *Semin. Virol.* **4**:167–180.
 41. Stanley, M. A., and E. K. Parkinson. 1979. Growth requirements of human cervical epithelial cells in culture. *Int. J. Cancer* **24**:407–414.
 42. Stephens, R. S. 1994. Molecular mimicry and *Chlamydia trachomatis* infection of eukaryotic cells. *Trends Microbiol.* **2**:99–101.
 43. Stratton, P., and N. J. Alexander. 1993. Prevention of sexually transmitted infections: physical and chemical barrier methods. *Infect. Dis. Clin. N. Am.* **7**:841–859.
 44. Su, H., L. Raymond, D. D. Rockey, E. Fischer, T. Hackstadt, and H. D. Caldwell. 1996. A recombinant *Chlamydia trachomatis* major outer membrane protein binds to heparan sulfate receptors on epithelial cells. *Proc. Natl. Acad. Sci. USA* **93**:11143–11148.
 45. Swain, S. M., B. Parker, A. Wellstein, M. E. Lippman, C. Steakley, and R. DeLap. 1995. Phase I trial of pentosan polysulfate. *Invest. New Drugs* **13**:55–62.
 46. Turyk, M., G. D. Wilbanks, and K. DeGeest. Growth and differentiation of endocervical and ectocervical epithelial cells. *In A. Doyle and J. G. Griffiths (ed.), Cell and tissue culture: laboratory procedures, in press.* Clear Academic Press of Holland, The Netherlands.
 47. Turyk, M. E., T. R. Golub, N. B. Wood, J. L. Hawkins, and G. D. Wilbanks. 1989. Growth and characterization of epithelial cells from normal human uterine ectocervix and endocervix. *In Vitro Cell. Dev. Biol.* **25**:544–556.
 48. van Putten, J. P., and S. M. Paul. 1995. Binding of syndecan-like cell surface proteoglycan receptors is required for *Neisseria gonorrhoeae* entry into human mucosal cells. *EMBO J.* **14**:2144–2154.
 49. Weir, S. S., P. J. Feldblum, L. Zekeng, and R. E. Roddy. 1994. The use of nonoxynol-9 for protection against cervical gonorrhoea. *Am. J. Public Health* **84**:910–914.
 50. WuDunn, D., and P. G. Spear. 1989. Initial interaction of herpes simplex virus with cells is binding to heparan sulfate. *J. Virol.* **63**:52–58.
 51. Zaretzky, F. R., R. Pearce-Pratt, and D. M. Phillips. 1995. Sulfated polyanions block *Chlamydia trachomatis* infection of cervix-derived human epithelia. *Infect. Immun.* **63**:3520–3526.
 52. Zekeng, L., P. J. Feldblum, R. M. Oliver, and L. Kaptue. 1993. Barrier contraceptive use and HIV infection among high-risk women in Cameroon. *AIDS* **7**:725–731.
 53. Zhang, J. P., and R. S. Stephens. 1992. Mechanism of *C. trachomatis* attachment to eukaryotic host cells. *Cell* **69**:861–869.