INFECTION AND IMMUNITY, Mar. 1980, p. 876-881
0019-9567/80/03-0876/06$02.00/0

Vol. 27, No. 3

Antibacterial Activity of Bladder Surface Mucin Duplicated in the Rabbit Bladder by Exogenous Glycosaminoglycan (Sodium Pentosanpolysulfate)

C. LOWELL PARSONS,* JEFFREY J. POLLEN, HABIBULLAH ANWAR, CHARLES STAUFFER, AND JOSEPH D. SCHMIDT

Urology Section, San Diego Veterans Administration Hospital, San Diego, California 92161, and the Division of Urology, Department of Surgery, University of California Medical Center, San Diego, California 92103

Previous data from our laboratory suggest that the transitional epithelium of the urinary bladder secretes and binds to its surface a glycosaminoglycan. The presence of this substance at the bladder surface markedly reduces the ability of microorganisms to adhere to the mucosa. Furthermore, this glycosaminoglycan can be removed (with acid) and replaced by intravesical instillation of a synthetic sulfonated glycosaminoglycan (heparin), whose presence is as effective as that of the natural glycosaminoglycan in reducing adherence. We conducted the current study with a different sulfonated glycosaminoglycan to determine whether the antiadherence activity is generalized to heparin congeners and whether the antiadherence effect of heparin is independent of its known anticoagulant activity. In this study we examined the sulfonated glycosaminoglycan, sodium pentosanpolysulfate, which lacks significant anticoagulant activity, and found it to have a mechanism of antiadherence analogous to that of heparin and almost equally as active on a weight basis.

The interaction of microorganisms with mucosal surfaces as a prelude to infection has been widely investigated. Research efforts have centered on the concept that the ability of a bacterium to adhere to a mucous membrane is proportional to its virulence in the genitourinary tract, the gastrointestinal tract, and the oral cavity (5, 6, 19, 22-24). If this is the case, it is possible that the host has immunodefenses or "antiadherence factors" directed against this bacterial virulence factor. The operation of such a factor at the surface of the transitional epithelium in the urinary tract could help explain the resistance of the bladder to infection.

We became interested in this concept and developed an in vivo model to quantitatively measure bacterial adherence to the urinary bladder (15). Data obtained using this model suggest that the transitional cells lining the bladder synthesize a glycosaminoglycan (GAG) which appears to prevent bacterial adherence to the mucosal cells (14). We call this substance antiadherence factor. Our experiments show that the layer of GAGs lining the bladder can be removed by acid treatment, with a corresponding rise in bacterial adherence, but that when an exogenous GAG, heparin, is added to bladders rendered mucin deficient, bacterial adherence drops to control levels (10). Additional studies suggested that heparin coats the transitional cells at the bladder surface and acts as a barrier between the bacterium and the transitional cell (17).

One important consideration with regard to this latter finding was whether the anticoagulant effect of heparin was responsible for the antiadherence activity we detected. The current study was conducted to test the antiadherence properties of an exogenous sulfonated GAG possessing far less anticoagulant activity than heparin and to determine whether sulfonated GAGs other than heparin have the capability of blocking bacterial adherence in vivo.

MATERIALS AND METHODS

Preparation of bacteria. *Escherichia coli* type O4 was labeled with ^{14}C as described previously (18). After labeling, the bacteria were concentrated 10-fold and suspended in 0.1 M sodium phosphate buffer (pH 5.5). This buffered solution was employed throughout the experiments.

Basic model of in vivo adherence assay. Step 1. Urethral catheter. Male New Zealand White rabbits weighing 2 to 3 kg were used. All animals were anesthetized with pentobarbital, 18 mg/kg of body weight. Each rabbit was secured and given 100 ml of physiological saline solution intravenously over a 30-min period. A pediatric feeding tube no. 8 French (C. R. Bard, Murray Hill, N.J.) was inserted into the urethra and secured with a 4-O silk purse-string suture tied around the penis. The abdomen was opened to

expose the bladder, permitting visual confirmation that the bladder was totally empty before each instillation. Between treatments the bladder was returned to the abdomen, and the overlying fascia were secured.

Step 2. Preparation of bladder. (i) Control bladders: these were flushed with four 15-ml volumes of physiological saline solution. (ii) Acid-treated bladders: the mucin layer was removed by acid as previously described (16), except that 0.6 N HCl rather than 0.3 N HCl was used. Throughout the experiments, all bladders were emptied of urine before any instillations.

Step 3. Introduction of bacteria. The bladders of control rabbits received 0.5 ml of buffered solution, followed immediately by 0.4 ml of bacteria suspended in buffer, and the catheter was clamped. The acid-treated bladders of test control rabbits (mucin layer absent) also received 0.5 ml of buffered solution, followed by 0.4 ml of bacteria (Step 1), and the catheter was clamped.

Sodium pentosanpolysulfate and bacteria experiments. Sodium pentosanpolysulfate (SP54; Benechemie, Munich, Germany) was added to buffered saline in concentrations of either 40, 20, 10, 5, 2.5, 1, or 0.5 mg/ml. To each acid-treated bladder, under direct vision, was added 0.5 ml of this suspension, followed immediately with 0.4 ml of labeled bacteria suspended in buffer, and the catheter was clamped.

Pretreatment of bladder with sodium pentosanpolysulfate. Before the addition of bacteria [Step 3], acid-treated bladders were treated with 1.0 ml of buffered solution containing 20 mg of sodium pentosanpolysulfate per ml for 30 min. This solution was removed by aspiration, and the bladder was washed twice with 15.0-ml volumes of buffered solution. The labeled bacteria (0.4 ml) were then added to the empty bladder, and the catheter was clamped.

Step 4. Interaction of bacteria with bladder mucosa. After the bacteria were introduced into the bladder, the catheter was clamped for 15 min while saline was given intravenously at a rate of 200 ml/h. At the end of 15 min, 10 ml of buffer was introduced into the bladder to dilute the bacteria and terminate the reaction. The penile catheter was left to straight drainage, and, after the rabbit had made 50 to 70 ml of urine, the rabbit was sacrificed, and the bladder was removed. The mucosa was dissected free from the muscle layer, and both mucosal and muscle tissue were assayed for ^{14}C activity.

Step 5. Recording of radioactivity measurements. Bladder tissue was placed in an incubator at 65°C until desiccated, and a dry weight was determined. The mucosal tissue or muscle tissue was dissolved and bleached in a combination of 1.0 ml of 70% perchloric acid and 0.4 ml of 30% hydrogen peroxide. After 30 min, 2 or 3 drops of 15% ascorbic acid was added to remove the remaining hydrogen peroxide. The mucosal samples, muscle samples, and bacteria were suspended in Aquasol (New England Nuclear Corp., Boston, Mass.). Radioactive counts were recorded by a Searle liquid scintillation counter. Each sample was counted for 10 min.

Statistical analysis of data. All data were subjected to variance analysis using Student's *t* test. Differences in mean bacterial adherence values are reported as significant when $P < 0.05$ and not significant when $P > 0.05$.

Bacteria. The bacterial solution injected into the rabbits ranged between 1.0×10^9 and 2.0×10^9 colony-forming units per ml with a ratio of bacteria to counts per minute of between 300 and 500.

RESULTS

Sodium pentosanpolysulfate and bacteria. The data are expressed in two ways: bacteria per milligram (dry weight) of mucosa, and the ratio of bacteria per milligram of mucosa for all groups for each given day of experimentation. This latter ratio is important because we have long since noted in our model that bacterial ability to adhere varies greatly from day to day, perhaps reflecting drifts in pili production, but that the ratio in adherence between experimental and control rabbits on any day is consistent. This ratio is presented in Table 1. In general, inter-experimental variability in adherence values was low. Over 95% of the time, the results obtained on a given day showed higher values for bacterial adherence to acid-treated bladders than to control bladders or to bladders receiving sodium pentosanpolysulfate.

As can be seen in Table 1, the sodium pentosanpolysulfate exerts its maximum effect at blocking adherence at concentrations of 5 to 10 mg/ml. For this reason, the bladders and bacteria were pretreated with sodium pentosan-

TABLE 1. *Effect of sodium pentosanpolysulfate on bacterial adherence in bladders rendered mucin deficient*

Mucosal group	10^3 mean bacteria per mg of mucosa ± SD[a]	*N*	Ratio, acid-treated bladders/controls[b]
Control[c]	1.9 ± 1.7	25	1.0
Acid[d]	56 ± 69	20	49 ± 71
Sodium pentosanpolysulfate[d] (concn, mg/ml):			
40	2.4 ± 3.8	16	3.3 ± 6.0
20	5.0 ± 10	16	5.4 ± 7.2
10	2.5 ± 3.9	16	3.8 ± 4.9
5	7.6 ± 6.0	15	4.1 ± 3.9
2.5	9.8 ± 13	14	25.5 ± 43
1.0	25 ± 39	15	22.4 ± 41
0.5	22 ± 23	15	50 ± 93

[a] SD, Standard deviation.

[b] Ratio of acid-treated bladders to controls (±standard deviation) on each separate day of experimentation. This ratio is slightly higher than that obtained comparing overall mean ratio of adherence, since day-to-day variation of bacterial adherence has more effect on the overall means (see text).

[c] Bladder mucin present; no acid treatment.

[d] Bladder mucin absent; pretreated with acid.

polysulfate at a concentration of 20 mg/ml, four times that needed to ensure an effect.

After acid treatment of the bladder mucosa, there was a rise in bacterial adherence to experimental rabbit bladders of an average of 50-fold, reflected in the ratio of bacteria bound per milligram of mucosa, between experimental and control rabbits. Bacterial adherence after acid treatment in experimental rabbits was significantly greater ($P < 0.001$) than that in control rabbits but was not significantly greater than adherence in the acid-treated bladders that received sodium pentosanpolysulfate in concentrations of 40, 20, 10, and 5 mg/ml ($P > 0.05$). Bacterial adherence in bladders at concentrations of sodium pentosanpolysulfate of 2.5, 1.0, and 0.5 mg/ml was significantly greater than adherence in control rabbits ($P < 0.001$), but was not significantly different from that in the acid-treated controls (Table 1). No bacteria were detected in the bladder muscle.

Pretreatment of bacteria with sodium pentosanpolysulfate. As shown in Table 2, pretreatment of bacteria with 20 mg of sodium pentosanpolysulfate per ml did not interfere with bacterial adherence in acid-treated bladders. A 54-fold increase in bacterial adherence to experimental bladders was seen when the ratio of bacteria adherent per milligram of mucosa of experimental bladders was compared to the ratio for control bladders on each day of experimentation. No statistically significant difference was found between adherence in the acid-treated bladder and that in the group of bladders that received bacteria pretreated with sodium pentosanpolysulfate.

Pretreatment of bladders with sodium pentosanpolysulfate. The data presented in Table 2 indicate that pretreatment of the bladder with sodium pentosanpolysulfate partially blocked bacterial adherence to the experimental bladder. Adherence values demonstrated only a 9-fold rise, compared to the 55-fold rise in adherence seen in the acid-treated bladders without sodium pentosanpolysulfate. Statistically, these values were not significantly different from those for the control group of bladders.

TABLE 2. *Effect of sodium pentosanpolysulfate on bacterial adherence when either the bacteria or the bladder is pretreated with this compound*

Mucosal group	Mean bacteria per mg of mucosa ± SD[a]	N
Control[b]	2.6 ± 3.3	16
Acid[c]	47.6 ± 78	20
Bacteria pretreated with sodium pentosanpolysulfate[c]	57.4 ± 92	15
Bladder pretreated with sodium pentosanpolysulfate[c]	9.4 ± 11.7[d]	16

[a] SD, Standard deviation.
[b] Bladder mucin present; no acid treatment.
[c] Bladder mucin absent; pretreated with acid.
[d] This difference does not attain statistical significance when compared to controls ($P > 0.1$).

DISCUSSION

It has long been known that the urinary bladder in the normal state is remarkably resistant to infection, although little has been described concerning any intrinsic antibacterial defense mechanism. A vesical mucosal bactericidal activity has been suggested (3, 4, 13), but its existence has not been corroborated by other investigators (11, 12). It seems unlikely that chance alone accounts for the bladder's ability to maintain a sterile lumen in the face of direct contact with environmental organisms; rather, antibacterial defense mechanisms might actively maintain this equilibrium. In this regard, we became interested in the concept of bacterial virulence depending on the ability of bacteria to adhere to a mucous surface.

Adherence is postulated to play a role in bacterial virulence at many mucous surfaces including the gastrointestinal tract, the genitourinary tract, and the oral cavity (5, 6, 19, 22–27). The main theme of data obtained in these systems is that microbial ability to infect a surface is directly proportional to its ability to adhere to the mucosal cells. If adherence is important to bacterial virulence, it is possible that the body produces antiadherence factors as a counter measure. In the urinary bladder, an antiadherence factor preventing bacteria from adhering to the bladder wall would explain both the need for and the efficiency of the urine washout factor (4). Human immunoglobulin A and glycoproteins have been studied as possible antiadherence factors acting in an antibody-like fashion, inactivating bacterial adherence mechanisms such as pili or the glycocalyx (11, 12). Such a mechanism would be less effective in the urinary bladder than at other mucous surfaces because it would require specific antibody production, which in turn requires prior exposure to antigens. Such a model does not adequately serve to explain the bladder's resistance to infection in the presence of a variety of environmental microorganisms.

To explore the possibility that an active antiadherence factor exists in the urinary tract, we developed and reported an in vivo method of quantitating bacterial adherence to the vesical mucosa (15). We discovered that pretreatment

of the bladder with acid causes a 50-fold rise in bacterial adherence to the transitional cells. The increase in adherence was found to be pH dependent and independent of the bacterial species employed (16, 18). Histochemical studies revealed that bacterial adherence is increased in the absence of bladder surface GAG (which is removed by acid treatment) and that the ability of the bladder surface to resist bacterial adherence is correlated with the natural regeneration of the GAG after its removal with acid (14). Additional histochemical studies corroborated these findings (21).

It is important to note that the manner by which we remove this surface layer of mucin (namely, with 0.6 N HCl) is harsh treatment; several important points are to be made in this regard. No gross effects were noted on the bladders. Microscopically, the histological studies showed that the mucosa was basically intact; the only consistent finding was the loss of the surface mucin layer (14, 15). In fact, the time of acid treatment and the concentration of acid were reduced in a stepwise fashion to the minimum level, which would produce a rise in bacterial adherence. It is at this level that the loss of the mucin layer occurred (14). This layer was remarkably resistant to attempts at removal. Attempts to hydrolyze it with trypsin, hyaluronidase, neuraminidase, and acetylcysteine produced a modest but significant rise in bacterial adherence, but only acid treatment resulted in a histologically demonstrable removal of the mucin layer (21a).

Based on these data, we formulated the thesis that the surface GAG (or its contents) in the urinary bladder is capable of acting as an antiadherence factor. Subsequently, we found that we could remove the endogenous GAG with a corresponding rise in bacterial adherence. When the sulfonated GAG, heparin, was placed intravesically into these mucin-deficient bladders, the bacterial adherence returned to control levels (10). It appeared that heparin blocked bacterial adherence by coating the bladder epithelium (17), since adherence was unaffected by pretreatment of the bacteria with heparin, but was prevented by pretreatment of the bladder. Additional studies employing [^{3}H]heparin made it possible to demonstrate that the heparin did, indeed, adhere to the surface of the transitional cells (17).

The results of the current study corroborate our thesis that bladder surface GAG is an antiadherence factor active against bacteria. We found that sodium pentosanpolysulfate blocked bacterial adherence to the transitional cells when it was added to the mucin-deficient (acid-treated) bladders before the introduction of bacteria into the bladders. The antiadherence activity persisted even after the sodium pentosanpolysulfate solution was irrigated from the bladders. Pretreatment of bacteria with sodium pentosanpolysulfate, however, had no effect on adherence. These data are important in several regards. First, our thesis that sulfonated GAGs act as antiadherence factors in the urinary tract is supported by our finding that we can remove the natural mucin, replace it with either of two synthetic GAGs instilled intraluminally, and achieve an antiadherence effect. Second, whereas the antiadherence activity of sodium pentosanpolysulfate is almost equal to that of heparin on a weight basis (17), sodium pentosanpolysulfate has little anticoagulant activity (G. Wakisaki and K. Anei, 11th Congress of the Japanese Society for Cardiovascular Research, 1961); this suggests that these two effects are independent of one another. Since sodium pentosanpolysulfate is commercially available in an oral form which is excreted into the urine, this could be an important new type of antibacterial agent, acting in a novel fashion, since the data presented here show that the intracavitary presence of sodium pentosanpolysulfate is all that is needed to ensure an antibacterial effect.

A possible mechanism for the antiadherence effect of sulfonated GAGs is provided by data obtained in the field of synthetic membrane technology (7–9; T.W. Jeffries, D.R. Omstead, R.R. Cardenas, and H.P. Gregor, Biotechnol. Bioeng., in press), where it is well established that sulfonated GAGs act as "nonfouling" polymers. The sulfonate groups (SO_3^-) of the GAGs are extremely hydrophilic and will bind several molecules of water in preference even to heavy metal cations (such as calcium or barium) and hydrogen (even at low pH's). What the sulfonate group does, in effect, is place water between the environment and the membrane to which the polymer is bound. This prevents fouling of the surface of the membrane, since there is a reduction in the net electrical charge to which compounds may adhere. Such sulfonated polymers are also called "wetting agents," since they specifically bind water molecules and "wet" the surface.

We feel that sodium pentosanpolysulfate, heparin, and mucin may operate in an analogous fashion in the urinary tract, preventing bacterial adherence by providing a tightly bound molecular layer of water between the bacterium and the transitional cell. In fact, by acting in such a fashion, the GAG could conceivably function as a nonspecific antiadherence factor whose activity could explain other phenomena in the uri-

nary tract. For example, the presence of a molecular layer of water bound to the surface of the transitional cells could be responsible for the fact that calcium, often supersaturated in urine, does not encrust the epithelium as it will foreign bodies inserted into the urinary tract.

An antiadherence factor acting in this fashion would preclude the specificity and hence the limited activity required of an antigen-antibody response. Such activity has been suggested for IgA and glycoprotein (11, 12). The fact that such specificity would not be required seems logical, since an efficient antiadherence factor would ideally act in a generalized manner to be effective against a host of environmental organisms. This explains our earlier finding that various bacterial species behave similarly in our model (16) and that adherence of live and dead bacteria and colloid particles is controlled by the presence of mucin (20).

Little is known about the function of the sulfonated GAGs that are present at the surface of the eye, in the gastrointestinal tract, and at other mucosal surfaces in the body. Perhaps there, too, they act as antiadherence factors and play a role in host immunodefense. It is interesting to note that heparin is secreted by and adherent to the endothelial lining of blood vessels (1, 2); the presence of an antiadherence factor in the vascular tree could be important in preventing blood elements from adhering to vessel walls. Our findings, complemented by data from the field of synthetic membrane technology, suggest a simple and artificially reproducible model of antiadherence activity for sulfonated GAGs at the bladder surface. Furthermore, the data raise the possibility that exogenous GAGs have potential for clinical use in the genitourinary tract.

ACKNOWLEDGMENT

This work was supported by the Medical Research Service of the Veterans Administration.

LITERATURE CITED

1. **Buonassisi, V.** 1973. Sulfated mucopolysaccharide synthesis and secretion in endothelial cell cultures. Exp. Cell Res. **76**:363–368.
2. **Buonassisi, V., and M. Root.** 1975. Enzymatic degradation of heparin related mucopolysaccharide from the surface of endothelial cell cultures. Biochim. Biophys. Acta **385**:1–10.
3. **Cobbs, C. G., and D. Kaye.** 1967. Antibacterial mechanisms in the urinary bladder. Yale J. Biol. Med. **40**:93–108.
4. **Cox, C. E., and F. Hinman, Jr.** 1961. Experiments with induced bacteriuria, vesical emptying and bacterial growth on the mechanism of bladder defense to infection. J. Urol. **86**:739–748.
5. **Ellen, R. P., and R. J. Gibbons.** 1972. M protein-associated adherence of *Streptococcus pyogenes* to epithelial surfaces: prerequisite for virulence. Infect. Immun. **5**:826–830.
6. **Gibbons, R. J., and J. van Houte.** 1971. Selective bacterial adherence to oral epithelial surfaces and its role as an ecological determinant. Infect. Immun. **3**:567–573.
7. **Gregor, H. P.** 1975. Anticoagulant activity of sulfonate polymers and copolymers, p. 51–56. *In* H. P. Gregor (ed.), Polymer science and technology, vol. 5. Plenum Press, New York.
8. **Gregor, H. P.** 1976. Fixed-charge ultrafiltration membranes, p. 235. *In* E. Selegny (ed.), Charged gels and membranes, part I. D. Reidel, Holland.
9. **Gryte, C. C., and H. P. Gregor.** 1976. Poly-(styrene sulfonic acid)-poly(vinylidene fluoride) interpolymer ion-exchange membranes. I. Preparation and characterization. J. Polymer Sci. (Part A-2: Polymer Physics Edition) **14**:1839–1854.
10. **Hanno, P. M., C. L. Parsons, S. H. Shrom, R. Fritz, and S. G. Mulholland.** 1978. The protective effect of intravesical heparin in experimental bladder infection. J. Surg. Res. **25**:324–329.
11. **Mulholland, G., E. Foster, J. Gillenwater, and A. Paquin.** 1966. Effect of vesical mucosa on bacterial growth. Clin. Res. **14**:341.
12. **Mulholland, S. G., E. A. Foster, A. J. Paquin, Jr., and J. Y. Gillenwater.** 1969. The effect of rabbit vesical mucosa on bacterial growth. Invest. Urol. **6**:593–604.
13. **Norden, C. W., G. M. Green, and E. H. Kass.** 1968. Antibacterial mechanisms of the urinary bladder. J. Clin. Invest. **47**:2689–2700.
14. **Parsons, C. L., C. Greenspan, S. W. Moore, and S. G. Mulholland.** 1977. Role of surface mucin in primary antibacterial defense of bladder. Urology **9**:48–52.
15. **Parsons, C. L., C. Greenspan, and S. G. Mulholland.** 1975. The primary antibacterial defense mechanism of the bladder. Invest. Urol. **13**:72–76.
16. **Parsons, C. L., and S. G. Mulholland.** 1978. Bladder surface mucin: its antibacterial effect against various bacterial species. Am. J. Pathol. **93**:423–432.
17. **Parsons, C. L., S. G. Mulholland, and H. Anwar.** 1979. Antibacterial activity of bladder surface mucin duplicated by exogenous glycosaminoglycan (heparin). Infect. Immun. **24**:552–557.
18. **Parsons, C. L., S. H. Shrom, P. Hanno, and S. G. Mulholland.** 1978. Bladder surface mucin: examination of possible mechanisms for its antibacterial effect. Invest. Urol. **16**:196–200.
19. **Punsalang, A. P., Jr., and W. D. Sawyer.** 1973. Role of pili in the virulence of *Neisseria gonorrhoeae*. Infect. Immun. **8**:255–263.
20. **Shrom, S. H., C. L. Parsons, A. Alavi, and S. G. Mulholland.** 1977. Vesical defense: assessment of adsorption potential using live, dead, and metabolically inhibited *E. coli* and ^{99m}Tc-labeled sulfur colloid particles. Surg. Forum **28**:565.
21. **Shrom, S. H., C. L. Parsons, and S. G. Mulholland.** 1977. Role of urothelial surface mucoprotein in intrinsic bladder defense. Urology **9**:526–533.
21a. **Shrom, S. H., C. L. Parsons, and S. G. Mulholland.** 1978. Vesical defense: further evidence for a charge-related mucosal anti-adherence mechanism. Surg. Forum **29**:632–633.
22. **Sobelavsky, O., B. Prescott, and R. M. Chanock.** 1968. Adsorption of *Mycoplasma pneumoniae* to neuraminic acid receptors of various cells and possible role in virulence. J. Bacteriol. **96**:695–705.
23. **Svanborg-Edén, C., B. Eriksson, and L. Å. Hanson.** 1977. Adhesion of *Escherichia coli* to human uroepithelial cells in vitro. Infect. Immun. **18**:767–774.
24. **Swanson, J.** 1973. Studies on gonococcus infection. IV. Pili: their role in attachment of gonococci to tissue culture cells. J. Exp. Med. **137**:571–589.
25. **Swanson, J., G. King, and B. Zeligs.** 1975. Studies on

gonococcus infection. VIII. ^{125}Iodine labeling of gonococci and studies on their in vitro interactions with eukaryotic cells. Infect. Immun. 11:453–459.
26. **Ward, M. E., and P. J. Watt.** 1972. Adherence of *Neisseria gonorrhoeae* to urethral mucosal cells: an electron-microscopic study of human gonorrhea. J. Infect. Dis. 126:601–605.
27. **Ward, M. E., P. J. Watt, and J. N. Robertson.** 1974. The human fallopian tube: a laboratory model for gonococcal infection. J. Infect. Dis. 129:650–659.