INFECTION AND IMMUNITY, Nov. 1985, p. 495–499
0019-9567/85/110495-05$02.00/0

Vol. 50, No. 2

Role of Lipopolysaccharide and Complement in Susceptibility of *Haemophilus ducreyi* to Human Serum

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Received 5 July 1985/Accepted 17 July 1985

The role of lipopolysaccharide (LPS) in the susceptibility of *Haemophilus ducreyi* to human serum and the mechanism of complement activation by serum-susceptible (Ser^s) strains were investigated. Serum treated with 2 mM Mg^{2+} and 20 mM ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N'*,*N'*-tetraacetic acid was nonbactericidal, but inulin-treated serum remained bactericidal. Absorption of serum with heat-killed whole cells of an Ser^s strain removed its bactericidal activity against the absorbing strain and also against other Ser^s strains. LPS obtained from Ser^s strains inhibited the bactericidal activity of serum against all Ser^s strains, whereas LPS from serum-resistant (Ser^r) strains and an Ser^r isogenic strain did not. However, high concentrations of LPS from the Ser^r strain inhibited the bactericidal activity of serum, an indication that part of the structural site involved in serum susceptibility is retained in the LPS of this strain. The LPS of Ser^s strains exhibited higher anticomplement activity than the LPS of Ser^r strains. These findings suggest that the classical pathway of complement activation is involved in the serum killing of *H. ducreyi* and that LPS composition may contribute to their susceptibility to complement-mediated serum bactericidal activity.

Complement-mediated lethal action of human serum and phagocytosis and killing by human polymorphonuclear leukocytes are important components of the host defense against infection with gram-negative bacteria (3, 4, 24). Resistance to the bactericidal activity of serum and phagocytosis by human polymorphonuclear leukocytes is a property of many bacterial strains causing severe infection involving tissue penetration and damage (23, 28, 34, 40). Several studies have implicated bacterial envelope components such as outer membrane lipopolysaccharide (LPS) (10, 20–22, 32), capsular polysaccharide (9, 17, 36), and outer membrane proteins (12, 14, 18, 39) in resistance of bacterial strains to the bactericidal activity of serum.

Complement activation by gram-negative bacteria can occur via the classical or the alternative pathway. The classical pathway can be activated by the interaction of antibody with bacterial surface antigens or directly by the lipid A moiety of LPS (11, 19). The alternative pathway may be activated by bacterial surface polysaccharides independent of antibody (19).

A previous study (23) of the pathogenesis of *Haemophilus ducreyi* infection indicated that virulent strains positive in the rabbit intradermal test were resistant to complement-mediated bactericidal action of human serum, whereas the avirulent strains were susceptible. In the present study, we have investigated the mechanism of complement activation by *H. ducreyi* strains and the role of LPS composition in the susceptibility of these strains to the bactericidal activity of serum. Results suggest that the classical complement pathway is involved in the serum killing of *H. ducreyi* strains, and that the LPS composition of these strains plays a critical role in their susceptibility to human serum.

MATERIALS AND METHODS

Bacterial strains and growth conditions. The *H. ducreyi* strains used in this study include reference strains A77, A75, and A76, all obtained from the Pasteur Institute in Paris; strain 409 is an isolate obtained from Kenya, and strain 35000 is a Winnipeg isolate. Serum resistant isogenic strain A77* was obtained by passage of strain A77 in the presence of increasing concentrations of serum, until a 70% survival rate of organisms was reached. Serum-susceptible *Haemophilus influenzae* NCTC 8143 was included in the study.

Strains were kept as frozen skimmed milk stock cultures at −70°C. When needed, they were cultured on hemoglobin agar composed of gonococcus medium base with CVA enrichment (GIBCO Diagnostics, Madison, Wis.) and incubated at 35°C under 5% CO_2 and high humidity.

Virulence testing. The virulence of stock strains was tested as previously described (13). The test involved intradermal injection of 0.2 ml of a sheep brain heart infusion broth suspension containing 10^9 CFU of *H. ducreyi* per ml into 2- to 3-kg, 1-year-old female rabbits. Induration and necrosis were determined daily from day 1 until day 11 postinoculation. The criteria for virulence were as follows: by day 4, induration exceeded 0.5 cm, and the lesion progressed to an eschar by day 11. According to this test, strains 409, 35000, and A77* were virulent, and strains A77, A75, and A76 were avirulent.

Methods of LPS isolation. The LPS of *H. ducreyi* strains was isolated from 18 g (wet weight) of stationary-phase cultures by the phenol-water procedure of Westphal and Jann (41). All LPS preparations were lyophilized and stored at −20°C.

Serum bactericidal assay. Serum bactericidal assays were performed as previously described (23) with pooled serum from five healthy young adults with no history of chancroid. A 16-h culture of *H. ducreyi* was suspended in 1% proteose peptone (Difco Laboratories, Detroit, Mich.) in physiological saline (PPS), pH 7.2, to a concentration of 5×10^8 CFU/ml. At time zero, 0.1 ml of the bacterial suspension (5×10^7 CFU) was added to a tube containing 0.4 ml of PPS and 0.5 ml of undiluted serum. Control tubes contained heat-inactivated serum. Tubes were incubated at 35°C and rotated on a rotator at 10 rpm. Samples were taken at various time intervals and serially diluted in PPS, and 0.3 ml of the appropriate 10-fold dilutions was plated onto hemoglobin agar in six 0.05-ml drops for the determination of viable

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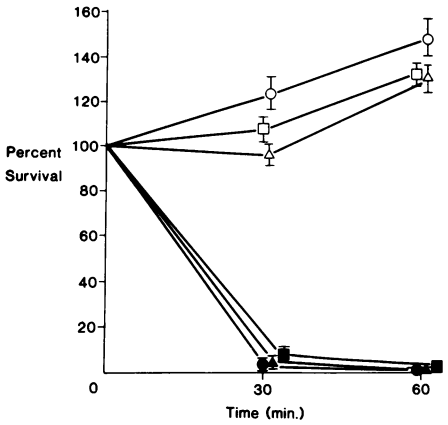

FIG. 1. Kinetics of killing of *H. ducreyi* A77 by 50% human serum. Symbols: serum control (●), serum heated at 56°C for 30 min (○), serum plus 20 mM EDTA (□), serum plus 20 mM EGTA and 2 mM $MgCl_2$ (△), serum plus inulin (2 mg/ml) (▲), and serum heated at 50°C for 20 min (■).

counts. Strains with ≥50% survival in 50% serum after 2 h of incubation were considered resistant. Intermediate resistance was 10 to 49% survival, and strains with <10% survival were considered susceptible.

Treatment of serum. Classical pathway activity in serum was selectively inhibited by chelation with 20 mM EGTA [ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N'*,*N'*-tetraacetic acid] plus 2 mM $MgCl_2$ as previously described (6). Serum was treated with inulin (2 mg/ml) by the method of Gotze and Muller-Eberhard (11) or heated at 50°C for 20 min to inactivate factor B, which is required for the alternative pathway activity in serum (5). Both pathways were inhibited by treatment of serum with 20 mM EDTA. EGTA, EDTA, and inulin, at the above concentrations, had no effect on the survival of *H. ducreyi* in PPS during 2 h of incubation.

Absorption of serum with heat-treated whole cells of *H. ducreyi* or *H. influenzae* was performed as follows: Serum (1 ml) was mixed with a heavy suspension of heat-killed whole cells in PPS (1 ml), tumbled slowly end over end on a rotator at 4°C for 5 h, and then centrifuged to remove the bacteria. The supernatant was either used immediately in serum bactericidal assays or stored at −70°C in small portions.

Inhibition of serum bactericidal activity by LPS. *H. ducreyi* LPS was suspended in PPS (pH 7.2) to a final concentration of 4 mg/ml and sonicated at 4°C until the solution cleared. LPS solution in the concentration range of 0.05 to 1 mg/ml was added to 0.5 ml of serum in a tube. The volume was adjusted to 0.9 ml with PPS, and the solution was incubated at 37°C with shaking for 30 min. Then 0.1 ml of bacterial suspension (5×10^7 CFU) in PPS was added to the tube and incubated at 37°C for an additional 60 min before dilution plating.

Measurement of the anticomplement activity of *H. ducreyi* LPS. The anticomplement activity of LPS was measured as previously described (33), with slight modifications. Serum (0.1 ml) was mixed with LPS (0.005 to 0.1 mg) suspended in PPS or with PPS alone to a final volume of 0.2 ml and incubated with shaking at 37°C for 30 min. Antibody-sensitized sheep erythrocytes in 0.2 ml of PPS were added to a fourfold dilution of treated human serum and incubated for an additional 30 min in a 37°C water bath. Ice-cold saline (3 ml) was added to the mixture, the cells were pelleted by centrifugation, and the absorbance of the supernatant was measured at 412 nm. The positive control was sensitized erythrocytes plus serum without added LPS, and the negative control was LPS plus erythrocytes without added serum.

RESULTS

Mechanism of complement activation by *H. ducreyi* strains. The mechanism of complement activation by serum-susceptible (Ser^S) strains was examined. Serum treated with Mg^{2+}-EGTA, which selectively inhibits the classical complement pathway, and serum treated with EDTA, which inhibits both the classical and the alternative pathway, were nonbactericidal to Ser^S *H. ducreyi* A77 (Fig. 1). The strain also survived well in serum heated at 56°C for 30 min to inactivate complement. However, it was rapidly killed in untreated serum and in serum pretreated with inulin or serum heated at 50°C for 20 min (which depleted it of alternative pathway activity). Similar results were obtained with the other Ser^S strains, A76 and A75 (data not shown). This suggests that the serum killing of *H. ducreyi* strains is mediated by the classical pathway of complement activation.

Absorption of serum with heat-killed *H. ducreyi* A77 removed bactericidal activity against Ser^S strains A77, A75, and A76 (Fig. 2; data shown for strain A77). Similar results were obtained with strain A75 as the absorbing isolate. Absorption of serum with the Ser^r strain 409 or the Ser^r isogenic strain A77* resulted in partial reduction in the bactericidal activity against strain A77, suggesting that these strains differ in the heat-stable cell components involved in serum bactericidal reactions. Absorption of serum with Ser^S *H. influenzae* NCTC 8143 also inhibited serum killing of *H. ducreyi* (data not shown). Absorbed sera had ≥70% complement activity in relation to unabsorbed samples.

Inhibition of serum bactericidal activity by *H. ducreyi* LPS. Strain A77 LPS at various concentrations completely inhibited the bactericidal activity of serum against Ser^S strain A77. The percent survival of strain A77 cells in serum after 60 min of incubation at 35°C was 0, 57, 112, 110, and 108% at LPS concentrations of 0, 0.05, 0.1, 0.2, and 1.0 mg/ml, respectively.

Table 1 shows the percent survival of *H. ducreyi* strains in normal serum treated with 0.1 mg of LPS per ml from Ser^r and Ser^S strains. Ser^S strains A76, A75, and A77 did not survive in control serum during the 60-min incubation time, whereas these strains survived well in serum preincubated with LPS obtained from any of the three Ser^S strains. LPS

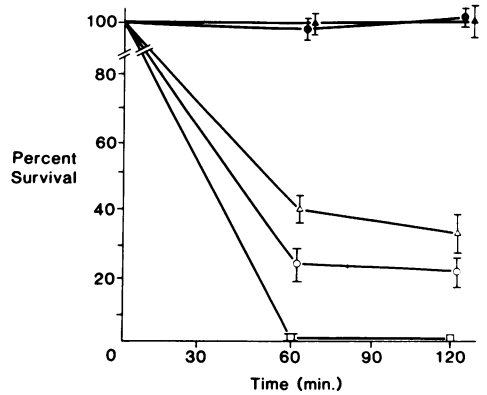

FIG. 2. Bactericidal activity of 50% human serum on *H. ducreyi* A77 after absorption with heat-killed whole cells of strains A75 (●), A77 (▲), A77* (△), 409 (○), and PPS (□).

from Ser^r strains 409 and 35000 and Ser^r isogenic strain A77* did not inhibit serum bactericidal activity against the Ser^s strains (<5% survival, 60 min). The viability of Ser^r strains improved in serum treated with homologous and heterologous LPS (data shown for Ser^r strain A77*). High concentrations of LPS (1 mg/ml) of Ser^r strains 35000 and 409 did not inhibit the bactericidal activity of human serum against Ser^s strains A77, A75, and A76. However, LPS from Ser^r isogenic strain A77* at this high concentration inhibited the bactericidal activity of serum (data not shown).

Anticomplement activity of *H. ducreyi* LPS. The anticomplement activity of *H. ducreyi* LPS was measured to determine whether its inhibitory effect on serum bactericidal activity was due to its ability to activate and deplete complement. Figure 3 shows the inhibitory effect of the LPS of Ser^r and Ser^s strains on complement-mediated hemolysis of sensitized sheep erythrocytes. The anticomplement activity of the LPS of Ser^s strains A76, A75, and A77 was dose dependent. There was complete inhibition of complement-mediated hemolysis of erythrocytes when serum was preincubated with LPS (1 mg/ml) from Ser^s strains. The LPS of Ser^r strains, on the other hand, had low anticomplement activity even at this concentration. However, LPS of Ser^r isogenic strain A77*, unlike other Ser^r strains, exhibited high anticomplement activity when high concentrations of LPS (0.5 to 1 mg/ml) were used to treat the serum. The LPS of strain A77* at these concentrations inhibited serum bactericidal activity (data not shown). Thus, there is a correlation between anticomplement activity of LPS and its inhibitory effect on serum bactericidal activity.

DISCUSSION

The bactericidal effects of immune or nonimmune serum are mediated by activated components of the classical or alternative complement pathway (11, 25, 38). Activation of either can lead to membrane damage culminating in cell death (38). Our study of the mechanism of complement activation by *H. ducreyi* strains indicates that the classical pathway is involved in serum killing of Ser^s strains. Selective inhibition of the classical complement pathway activation by treatment of serum with Mg^{2+}-EGTA abolished serum bactericidal activity. Serum treated with inulin, which depletes it of the alternative complement pathway, was bactericidal. Thus, the alternative pathway of complement activation is probably not involved in the serum killing of Ser^s *H. ducreyi* strains. Other gram-negative bacteria such as *H. influenzae* (26), *Salmonella* spp. (15), and *Escherichia coli* (39) are known to activate both complement pathways, but *Neisseria*

TABLE 1. Inhibition of serum bactericidal activity against *H. ducreyi* strains by homologous and heterologous LPS[a]

Strain	% Survival[b] of strains after 60 min of incubation in:						
	Control serum	Serum plus LPS from strains:					
		Ser^s			Ser^r		
		A76	A75	A77	A77*[c]	409	35000
A76	0	136	140	132	0.1	0.1	0.3
A75	0	115	108	116	1.7	0.7	0.5
A77	0	128	107	132	3.7	1.1	0.5
A77*	70	112	108	104	122	132	108

[a] The concentration of LPS was 0.1 mg/ml.
[b] Means of three experiments.
[c] A Ser^r isogenic strain obtained from Ser^s strain A77.

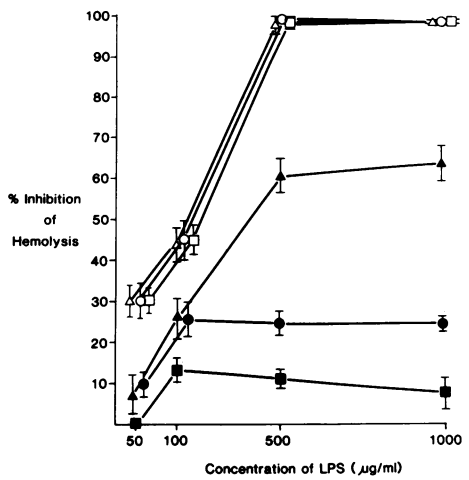

FIG. 3. Inhibition of the complement-mediated hemolysis of sensitised sheep erythrocytes after 30 min of incubation of human serum with purified LPS from *H. ducreyi* strains 409 (■), 35000 (●), A77* (▲), A77 (□), A75 (△), and A76 (○).

gonorrhoeae (33) and *Pseudomonas aeruginosa* (30) activate mainly the classical pathway.

Serum absorption experiments with heat-killed *H. ducreyi* cells suggest that heat-stable components of the bacterial cell surface are involved in the serum killing. Absorption of serum with whole cells of an Ser^s strain removed the bactericidal effect of serum against the absorbing strain and other Ser^s strains, whereas absorption of serum with Ser^r strains resulted in partial removal of the bactericidal effect. These results suggest that Ser^r and Ser^s strains differ in heat-stable cellular components involved in serum susceptibility. There is a possibility that antibody present in human serum is involved in the killing of these strains, since the sera used in the assays had ≥70% complement activity after absorption with whole cells at 4°C. This must, however, be nonspecific or natural antibody directed against the heat-stable components of the organism, since the serum was obtained from persons with no history of chancroid and since absorption with Ser^s *H. influenzae* also abrogated killing of *H. ducreyi*. Natural antibodies directed against the surface antigens of a number of gram-negative bacteria have been demonstrated in nonimmune serum (7, 31, 32, 35). Their production is believed to be initiated by normal flora in the human intestinal tract (16).

Sensitivity of a number of gram-negative bacteria to the bactericidal activity of normal or immune sera has been attributed to their LPS composition (20, 27, 29, 30, 33, 37). We found that LPS from Ser^s strains inhibited serum bactericidal activity against Ser^s strains, whereas LPS from Ser^r strains was not inhibitory even at high concentrations. These data suggest that the LPS composition of *H. ducreyi* strains may be a determinant factor of their serum susceptibility. Purified LPS from Ser^s strains in *E. coli* (2) and *N. gonorrhoeae* (10, 33) has also been shown to inhibit serum bactericidal activity in vitro as a result of anticomplement activity of the LPS (33) or by binding to the bacterial cells, such that they are protected from complement-mediated bactericidal reaction (1, 2). The ability of *H. ducreyi* LPS to inhibit serum bactericidal activity against Ser^s strains correlated with the anticomplement activity of the LPS. Thus, the inhibitory effect of the LPS of Ser^s *H. ducreyi* strains on the bactericidal activity of serum may be due to their ability to activate and deplete serum complement. The inhibitory

effect of the LPS of Ser^r isogenic strain A77* at high concentrations (0.5 to 1 mg/ml) on serum bactericidal activity may indicate that part of the LPS structural site involved in serum sensitivity is retained in the LPS of this isogenic strain.

Anticomplementary activity of the LPS of *Salmonella* spp. and *E. coli* smooth and rough mutant strains has been attributed to the lipid A moiety of LPS (8). However, the polysaccharide portion of the LPS molecule has a modifying influence on its potential anticomplement activity, which decreases with an increase in the length of the polysaccharide moiety (19). Differences in the anticomplement activity of the LPS of *H. ducreyi* strains may be due to variations in the chemical structure of the LPS of these strains.

In conclusion, the data presented in this report suggest that the classical pathway of complement activation is involved in serum killing of *H. ducreyi* strains and that the LPS composition of these strains may play an important role in the serum bactericidal reaction.

ACKNOWLEDGMENTS

This work was supported by a grant from the Medical Research Council of Canada (MA-6368). J.A.O. is the recipient of a Manitoba Health Research Council studentship.

LITERATURE CITED

1. **Allen, R. J., and G. K. Scott.** 1980. The effect of purified lipopolysaccharide on the bactericidal reaction of human serum component. J. Gen. Microbiol. **117**:65–72.
2. **Allen, R. J., and G. K. Scott.** 1981. Comparison of the effects of different lipopolysaccharides on the serum bactericidal reactions of two strains of *Escherichia coli*. Infect. Immun. **31**:831–832.
3. **Brooks, G. F., I. Ingwer, and B. H. Petersen.** 1978. Importance of serum bactericidal and opsonic activity and complement in *Neisseria gonorrhoeae* infections, p. 199–203. *In* G. F. Brooks, E. C. Gotschlich, K. K. Holmes, W. D. Sawyer, and F. E. Young (ed.), Immunobiology of *Neisseria gonorrhoeae*. American Society for Microbiology, Washington, D.C.
4. **Corrall, C. J., J. A. Winkelstein, and E. R. Moxon.** 1982. Participation of complement in host defense against encapsulated *Haemophilus influenzae* types a, c, and d. Infect. Immun. **35**:759–763.
5. **Eidinger, D., E. Bello, and A. Mates.** 1977. The heterocytotoxicity of human serum. I. Activation of the alternative complement pathway by heterologous target cells. Cell. Immunol. **29**:274–186.
6. **Fine, D. P., S. R. Marney, Jr., D. G. Colley, J. S. Sergent, and R. M. DesPrez.** 1972. C3 shunt activation in human serum chelated with EGTA. J. Immunol. **109**:807–809.
7. **Gaines, S., and M. Landy.** 1955. Prevalence of antibody to *Pseudomonas* in normal human serum. J. Bacteriol. **69**:628–633.
8. **Galanos, C., and O. Luderitz.** 1976. The role of the physical state of lipopolysaccharide in the interaction with complement. High molecular weights as prerequisite for the expression of anticomplementary activity. Eur. J. Biochem. **65**:403–408.
9. **Glynn, A. A., and C. J. Howard.** 1970. The sensitivity to complement of strains of *Escherichia coli* related to their K antigens. Immunology **18**:331–346.
10. **Glynn, A. A., and M. E. Ward.** 1970. Nature and heterogeneity of the antigens of *Neisseria gonorrhoeae* involved in the serum bactericidal reactions. Infect. Immun. **2**:162–168.
11. **Gotze, O., and H. J. Muller-Eberhard.** 1971. The C3-activator system: an alternate pathway of complement activation. J. Exp. Med. **134**(Suppl.):90–108.
12. **Guymon, L. F., T. J. Lee, D. Walstad, A. Schmoyer, and P. F. Sparling.** 1978. Altered outer membrane components in serum-sensitive and serum-resistant strains of *Neisseria gonorrhoeae*, p. 139-141. *In* G. F. Brooks, E. C. Gotschlich, K. K. Holmes, W. D. Sawyer, and F. E. Young (ed.), Immunobiology of *Neisseria gonorrhoeae*. American Society for Microbiology, Washington, D.C.
13. **Hammond, W. H., C. J. Lian, J. C. Wilt, and A. R. Ronald.** 1978. Antimicrobial susceptibility of *Haemophilus ducreyi*. Antimicrob. Agents Chemother. **1**:283–288.
14. **Hildebrant, J. F., L. W. Mayer, S. P. Wang, and T. M. Buchanan.** 1978. *Neisseria gonorrhoeae* acquire a new principal outer membrane protein when transformed to resistance to serum bactericidal activity. Infect. Immun. **20**:267–273.
15. **Joiner, K. A., C. H. Hammer, E. J. Brown, R. J. Cole, and M. M. Frank.** 1982. Studies on the mechanism of bacterial resistance to complement-mediated killing. I. Terminal complement components are deposited and released from Salmonella minnesota S218 without causing bacterial death. J. Exp. Med. **155**:797–808.
16. **Kunin, C. M.** 1962. Antibody distribution against non-enteropathogenic *E. coli*. Arch. Intern. Med. **110**:676–686.
17. **McCabe, W. R., B. Kaijser, S. Olling, M. Vwaydah, and L. A. Hanson.** 1978. *Escherichia coli* in bacteremia: K and O antigens and serum sensitivity of strains from adults and neonates. J. Infect. Dis. **138**:33–41.
18. **Moll, A., P. A. Manning, and K. N. Timmis.** 1980. Plasmid-determined resistance to serum bactericidal activity: a major outer membrane protein, the traT gene product, is responsible for plasmid-specified serum resistance in *Escherichia coli*. Infect. Immun. **28**:359–367.
19. **Morrison, D. C., and L. F. Kline.** 1977. Activation of the classical and properdin-pathways of complement by bacterial lipopolysaccharides (LPS). J. Immunol. **118**:362–368.
20. **Munn, C. B., E. E. Ishiguro, W. W. Kay, and T. J. Trust.** 1982. Role of surface components in serum resistance of virulent *Aeromonas salmonicida*. Infect. Immun. **36**:1069–1075.
21. **Mushel, L. H., and L. J. Larsen.** 1970. The sensitivity of smooth and rough gram-negative bacteria to the immune bactericidal reaction. Proc. Soc. Exp. Biol. Med. **133**:345–348.
22. **Nelson, B. W., and R. J. Roantree.** 1967. Analyses of lipopolysaccharides extracted from penicillin-resistant, serum-sensitive Salmonella mutants. J. Gen. Microbiol. **48**:179–188.
23. **Odumeru, J. A., G. M. Wiseman, and A. R. Ronald.** 1984. Virulence factors of *Haemophilus ducreyi*. Infect. Immun. **43**:607–611.
24. **Olling, S.** 1977. Sensitivity of gram-negative bacilli to the serum bactericidal activity: a marker of the host parasite relationship in acute and persisting infections. Scand. J. Infect. Dis. Suppl. **10**:1–40.
25. **Pangburn, M. K.** 1983. Activation of complement via the alternative pathway. Fed. Proc. **42**:139–143.
26. **Quinn, P. H., F. J. Crosson, Jr., J. A. Winkelstein, and E. R. Moxon.** 1977. Activation of the alternative complement pathway by *Haemophilus influenzae* type b. Infect. Immun. **16**:400–402.
27. **Rice, P. A., and D. L. Kasper.** 1977. Characterization of gonococcal antigens responsible for induction of bactericidal antibody in disseminated infection: the role of gonococcal endotoxins. J. Clin. Invest. **60**:1149–1158.
28. **Roantree, R. J., and L. A. Rantz.** 1960. A study of the relationship of the normal bactericidal activity of human serum to bacterial infection. J. Clin. Invest. **39**:72–81.
29. **Rowley, D.** 1968. Sensitivity of rough gram-negative bacteria to the bactericidal action of serum. J. Bacteriol. **95**:1647–1650.
30. **Schiller, N. L., M. J. Alazard, and R. S. Borowski.** 1984. Serum sensitivity of a *Pseudomonas aeruginosa* mucoid strain. Infect. Immun. **45**:748–755.
31. **Schoolnik, G. K., T. M. Buchanan, and K. K. Holmes.** 1976. Gonococci causing disseminated infection are resistant to the bactericidal action of normal human serum. J. Clin. Invest. **58**:1163–1173.
32. **Schwab, G. E., and P. R. Reeves.** 1966. Comparison of the bactericidal activity of different vertebrate sera. J. Bacteriol. **91**:106–112.
33. **Shafer, W. M., K. Joiner, L. F. Guymon, M. S. Cohen, and P. F. Sparling.** 1984. Serum sensitivity of *Neisseria gonorrhoeae*: the role of lipopolysaccharide. J. Infect. Dis. **149**:175–183.
34. **Simberkoff, M. S., I. Ricupero, and J. J. Rahal.** 1976. Host

resistance to *Serratia marcescens* infection: serum bactericidal activity and phagocytosis by normal blood leukocytes. J. Lab. Clin. Med. **87**:206–217.
35. **Skarnes, R. C.** 1978. Humoral bactericidal systems: nonspecific and specific mechanisms. Infect. Immun. **19**:515–522.
36. **Sutton, A., R. Schneerson, S. Kendall-Morris, and J. B. Robbins.** 1982. Differential complement resistance mediates virulence of *Haemophilus influenzae* type b. Infect. Immun. **35**:95–104.
37. **Taylor, P. W.** 1975. Genetical studies of serum resistance in *Escherichia coli*. J. Gen. Microbiol. **89**:57–66.
38. **Taylor, P. W.** 1983. Bactericidal and bacteriolytic activity of serum against gram-negative bacteria. Microbiol. Rev. **47**:46–83.
39. **Taylor, P. W., and R. Parton.** 1977. A protein factor associated with serum resistance in *Escherichia coli*. J. Med. Microbiol. **10**:225–232.
40. **Vosti, K. L., and E. Randall.** 1970. Sensitivity of serological classified strains of *Escherichia coli* of human origin to the serum bactericidal system. Am. J. Med. Sci. **259**:114–119.
41. **Westphal, O., and K. Jann.** 1965. Bacterial lipopolysaccharides: extraction with phenol-water and further application of the procedure. Methods Carbohydr. Chem. **5**:83–91.