INFECTION AND IMMUNITY, July 1979, p. 293-298
0019-9567/79/07-0293/06$02.00/0

Vol. 25, No. 1

Capsular K1 Polysaccharide of *Escherichia coli*: Relationship to Virulence in Newborn Rats and Resistance to Phagocytosis

ROBERT BORTOLUSSI,† PATRICIA FERRIERI,* BENGT BJÖRKSTÉN,‡ AND PAUL G. QUIE

Department of Pediatrics, University of Minnesota, Minneapolis, Minnesota 55455

Received for publication 18 April 1979

The virulence of *Escherichia coli* strains for newborn rats was related to opsonic requirements of the strains, sensitivity to the bactericidal activity of serum, and K1 capsular polysaccharide content. K1 *E. coli* strains were more virulent than non-K1 strains after intraperitoneal injection in newborn rats ($P < 0.05$) and were more resistant to phagocytosis than non-K1 strains when the classical complement pathway was blocked with Mg-ethyleneglycoltetraacetic acid ($P < 0.0005$). Sensitivity to the bactericidal activity of serum was similar among K1 and non-K1 *E. coli* strains. Two groups of K1 *E. coli* strains were defined on the basis of opsonic requirements. Group I strains were efficiently opsonized by the alternative complement pathway, while group II strains required the classical complement pathway for opsonization. Group I strains had less detectable K1 polysaccharide in the washed whole cell fraction than group II strains (10.3 versus 18.9 μg of K1 polysaccharide per 10^{10} colony-forming units) and were less virulent than group II strains (mortality, 44 versus 77%, $P < 0.05$). The K1 capsular polysaccharide appears to play an important role in determining virulence in newborn rats and opsonic requirements of these strains, but does not contribute to the sensitivity of strains to the bactericidal activity of serum.

Meningitis in newborn infants differs, because of its high incidence and mortality rate, from meningitis in older children (8). *Escherichia coli* is the commonest organism isolated from the cerebrospinal fluid (CSF) of newborn infants and accounts for approximately 50% of meningeal infections (4). Although the *Escherichia* genus is antigenically complex with over 250 somatic and capsular antigens, one capsular polysaccharide antigen, K1, has been associated with 84% of strains isolated from the CSF of neonates with meningitis (20).

Development of infection depends on the interactions between the host and the microorganism, and differences in virulence of bacterial strains may influence this relationship. In 1974 McCracken et al. (14) determined the median lethal dose of *E. coli* strains injected together with hog gastric mucin intraperitoneally (i.p.) into adult mice. Strains with K1 antigen isolated from infants with meningitis who subsequently died were significantly more virulent than K1 *E. coli* strains isolated from the CSF of infants who survived.

Others have suggested that virulence may be influenced by bacteria-associated factors. Resistance to serum bactericidal activity may contribute to virulence since *E. coli* strains isolated from the urine of patients with pyelonephritis more often are serum resistant than strains with the same serotype isolated from children with asymptomatic bacteriuria (1, 16). Howard and Glynn demonstrated that the content of K antigen among *E. coli* strains was related to resistance to bactericidal activity of serum, phagocytosis in vivo, and virulence for mice on intracerebral injection (9). Medearis et al. (15) reported that strains that lack a complete somatic antigen are less virulent in adult mice when injected i.p. together with hog gastric mucin.

The purpose of the present studies was to examine the relationship between the virulence of *E. coli* strains in newborn rats and the behavior of these strains in a phagocytic assay, their sensitivity to the bactericidal activity of serum, and the capsular content of K1 antigen.

MATERIALS AND METHODS

Animals. Outbred, pathogen-free albino Sprague-Dawley pregnant rats were obtained from Bio-Lab Corp. (White Bear Lake, Minn.). Rat pups were housed with their mothers under standardized conditions as described previously (3).

Bacteria. K1 and non-K1 *E. coli* strains randomly

† Present address: I. W. Killam Hospital for Children, Halifax, Nova Scotia, Canada.

‡ Present address: Department of Pediatrics, University of Umea, Umea, Sweden.

selected from isolates of blood and CSF (23 K1 and 11 non-K1 isolates) or stools (23 K1 and 6 non-K1 isolates) of newborn infants were studied in a phagocytic assay and in an assay of sensitivity to the bactericidal activity of serum. All K1 *E. coli* strains were identified by the agarose halo technique described by Sarff et al. (21). Strains were obtained from C. Krishnan (Department of Bacteriology, Hospital for Sick Children, Toronto, Canada), J. B. Robbins (Division of Bacterial Products, Bureau of Biologics, Food and Drug Administration, Bethesda, Md.), D. Blazevic (Diagnostic Bacteriology Department, University of Minnesota, Minneapolis, Minn.), and W. L. Albritton (Department of Pediatrics, Health Sciences Center, Winnipeg, Manitoba, Canada). Strains were stored at −20°C in 20% glycerol in small vials until needed. Serotyping was performed by Fritz Ørskov, Seruminstitut, Copenhagen, and by H. Lior of the Laboratory of the Center for Disease Control, Ottawa, Canada. The serotypes of 12/46 K1 *E. coli* strains have been reported previously (3). Eleven of the 17 non-K1 strains were serotyped, and 2 strains possessed K antigen, 1 a K7 and the other a K36A.

Logarithmic-phase organisms as described earlier (3) were used for virulence studies. Bacteria were diluted in 0.85% NaCl to give a concentration of 5×10^4 colony-forming units (CFU) in a volume of 0.1 ml and injected i.p.

Opsonic studies were done with bacteria that had been incubated overnight at 37°C, washed three times in phosphate-buffered saline (PBS) at pH 7.4, and adjusted to 5×10^7 CFU per ml of PBS by a spectrophotometric method.

Opsonic assay and serum sensitivity. Human leukocytes were collected and separated as described previously (17). Serum pooled from three normal donors (PHS) and frozen in 0.5-ml portions at −70°C was thawed immediately before use. A phagocytic mixture consisted of 0.5 ml of polymorphonuclear leukocyte (PMN) suspension (5×10^6 PMN per ml of mixture), 0.4 ml of serum (final concentration of 20%), and 0.1 ml of bacterial suspension (5×10^6 CFU per ml of mixture). The assay was done as described previously (17). Samples were obtained at 0 and 60 min and diluted in distilled water, and the viable bacterial count was estimated by pour-plate technique. Bacterial killing was expressed as the percentage of the original inoculum. Opsonic studies were repeated at least twice for each of the 63 strains. Reduction in viable bacterial count of less than 20% was considered resistance to phagocytosis. The standard error of the assay was ±1.2% for the 46 K1 strains and was ±4% for the 17 non-K1 strains.

Opsonization via the alternative complement pathway was evaluated in serum containing 0.1 M ethyleneglycoltetraacetic acid (Sigma Chemical Co., St. Louis, Mo.) with 0.1 M $MgCl_2$ (MgEGTA) (7). Controls with 20% MgEGTA-chelated serum but without PMN were included. Strains with less than a 20% decrease in viable bacterial count were considered resistant to phagocytosis. In addition, four K1 and one non-K1 *E. coli* strains were examined in serum (provided by Y. Kim) from a patient with no detectable C2 complement (11).

Sensitivity of *E. coli* strains to the bactericidal activity of serum was done in 40% PHS with omission of PMN in the phagocytic mixture. The same serum pool was used for all the studies. Strains which showed less than a 20% decrease in viable bacterial count were considered resistant to the bactericidal activity of serum. The standard error of the assay was ±1.4%. In a few assays of bactericidal activity, the serum was heat inactivated and the bacterial killing was abolished. This supports the concept that complement mediates the bactericidal activity of serum.

Virulence studies. Mortality in 5-day-old rats after i.p. injection of 5×10^4 CFU of *E. coli* was used as an indicator of virulence. Strains used in the virulence studies were those studied in the phagocytic system except for two *E. coli* isolates. Because non-K1 *E. coli* strains that were both serum resistant and resistant to phagocytosis in chelated serum were not found among the isolates of newborn infants, two strains having these characteristics were obtained from children with urinary tract infection. Five animals from multiple litters were injected with each strain and observed every 12 h and every 24 h thereafter. Control animals from the same litters were injected with saline or a sterile broth filtrate of the test strain. The median lethal dose (LD_{50}) of 14 of these *E. coli* strains was determined at 72 h after i.p. injection in 5-day-old animals by the method of Reed and Muench (18). Between 20 and 30 animals were used for each LD_{50} determination.

Quantitation of K1 polysaccharide. A 10^{-4} dilution of an overnight broth culture was inoculated into 10 ml of brain heart infusion broth (Difco Laboratories, Detroit, Mich.) and incubated at 35°C with agitation for 18 h. Viable bacteria count was estimated by pour-plate technique in triplicate. The remaining sample was centrifuged at $6,000 \times g$ for 15 min at 4°C. The bacterial sediment was washed twice in 0.85% NaCl and resuspended to 1 ml in saline. A portion of the supernatant and sediment was stored at −20°C. Before quantitation studies, bacterial sediment was thawed and refrozen six times to disrupt the cells. K1 polysaccharide was quantitated by using a rocket immunoelectrophoresis technique as described by Weeke (22). Equine group B meningococcus antiserum, which cross-reacts with *E. coli* K1 polysaccharide, was incorporated into agarose plates. Horse serum and purified K1 polysaccharide (which was used as a standard for quantitation) were kindly provided by John B. Robbins.

Statistical methods. Data are expressed in the text and figures as mean ± standard error. Analysis of the difference of means used the unpaired *t* test. Incidence data were analyzed by the chi-square test. Probability of 0.05 or less was accepted as significant.

RESULTS

Opsonic assay and serum sensitivity. The opsonic requirements and sensitivity to the bactericidal activity of pooled human serum were determined in 46 K1 and 17 non-K1 *E. coli* strains. The median percent bacterial killing in the phagocytic system using 20% PHS was 98.2% for non-K1 *E. coli* and 89.5% for K1 *E. coli*

strains ($P < 0.05$). When serum chelated with MgEGTA was used in the phagocytic system, the median percent killing for K1 *E. coli* strains was 17% compared to 94% for non-K1 *E. coli* strains ($P < 0.0005$) (Fig. 1). Most K1 strains resisted phagocytic killing by human leukocytes in serum chelated with MgEGTA, while most non-K1 strains were killed under these conditions. In both K1 and non-K1 *E. coli* groups, however, considerable variability from strain to strain was present. The non-K1 *E. coli* separated into two distinct groups (one with a high percentage and one with a low percentage of killing), whereas the K1 *E. coli* strains included some that showed intermediate levels of killing.

To confirm results obtained using chelated serum, the phagocytic studies were repeated with five *E. coli* strains using 20% serum deficient in the second component of complement (Fig. 2). Three of the strains that were opsonized and killed in 20% serum chelated with MgEGTA were well opsonized and killed in the presence of C2-deficient serum. Two of the K1 *E. coli* strains were not opsonized in chelated serum or in C2-deficient serum.

Resistance to the bactericidal activity of PHS was similar in K1 *E. coli* strains (32% of strains) and non-K1 *E. coli* strains (41% of strains).

Virulence studies. The relationship of opsonic requirement and capsular antigen to virulence was determined by studying mortality of 5-day-old rats after i.p. injection with 5×10^4 CFU of *E. coli*. Mortality at 24 to 72 h after injection in animals receiving the 20 K1 *E. coli* strains differed significantly from mortality of animals injected with 13 non-K1 *E. coli* strains ($P < 0.01$) (Fig. 3). Approximately 80% of the K1 isolates from blood and CSF of newborns and 50% of the K1 strains isolated from the stools of infants without evidence of infection were resistant to opsonization by the alternative comple-

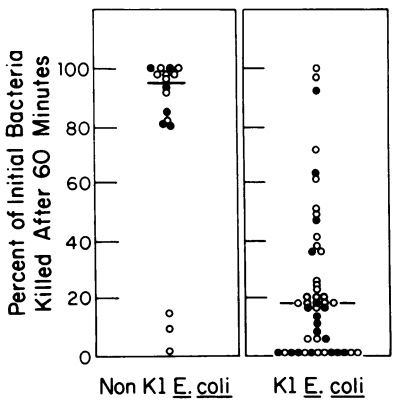

FIG. 1. *Percentage of bacterial killing of 46 K1 E. coli and 17 non-K1 E. coli strains in the presence of human leukocytes and 20% pooled human serum chelated with MgEGTA: serum-sensitive (○) and serum-resistant (●) E. coli strains. Median indicated by horizontal line for K1 and non-K1 E. coli strains ($P < 0.005$).*

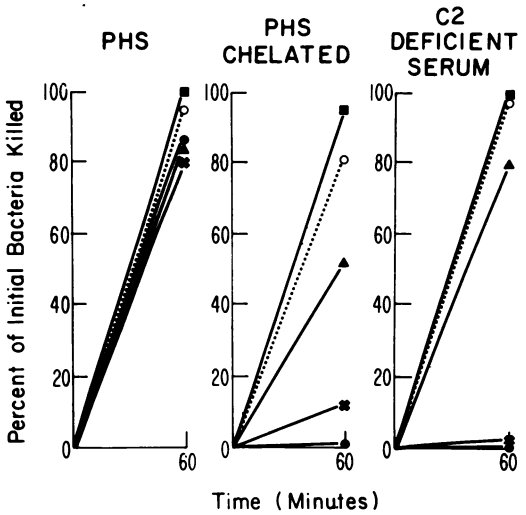

FIG. 2. *Percentage of bacterial killing after 60 min in presence of human leukocytes and PHS, PHS chelated with MgEGTA, and serum from a patient with absence of C2. Four K1 E. coli strains are indicated by solid symbols (●, ■, ▲, ×) and one non-K1 E. coli strain is indicated by open circles (○). Two K1 strains, ● and ×, were not opsonized in chelated serum or C2-deficient serum.*

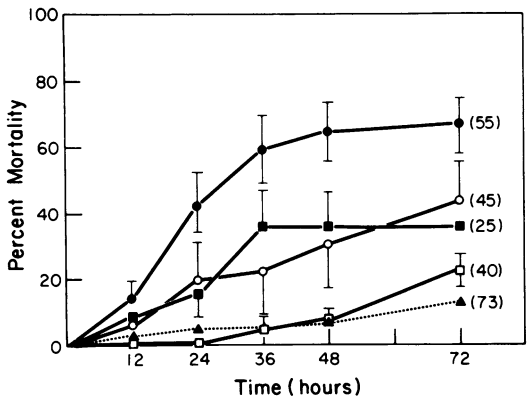

FIG. 3. *Percent mortality (mean ± standard error of the mean of mortality in animals) in suckling rats after i.p. injection of 5×10^4 CFU of E. coli: animals injected with K1 E. coli (○, ●); animals injected with non-K1 E. coli (□, ■); animals injected with saline (▲). Closed symbols (●, ■) indicate strains resistant to opsonization in 20% chelated serum, open symbols (○, □) indicate strains sensitive to opsonization in 20% chelated serum. Numbers in parentheses indicate total number of animals injected.*

ment pathway (ACP). At 72 h after challenge, K1 *E. coli* strains that avoided opsonization in chelated serum (group II strains) were associated with a higher mortality rate (77%) than either K1 strains (group I) that were opsonized in chelated serum (44%, $P < 0.05$) or non-K1 *E. coli* strains (28%, $P < 0.001$). However, animals injected with group I K1 strains had a mortality rate at 72 h after injection that was not significantly different from that of animals injected with non-K1 strains ($0.1 > P > 0.05$). Both K1 and non-K1 strains that resisted opsonization in chelated serum were more virulent than strains that were opsonized in chelated serum (cumulative mortality of 57 versus 34% at 72 h, $P < 0.005$).

The LD_{50} was determined in eight serum-sensitive and six serum-resistant K1 *E. coli* strains selected because of their various opsonic requirements (Fig. 4). Strains that resisted both the bactericidal activity of serum and opsonization via the ACP were most virulent. The median LD_{50} for eight group I K1 *E. coli* strains was higher than the LD_{50} for six group II strains ($10^{4.95}$ CFU versus $10^{3.15}$ CFU, $P < 0.02$). The most virulent strains were resistant to both the bactericidal activity of serum and to opsonization by the ACP (LD_{50} of $10^{0.8}$ to $10^{3.15}$).

Quantitation of K1 polysaccharide: relationship to opsonization. When the amount of K1 polysaccharide in the broth supernatant or whole cell fraction was measured, a considerable variability from strain to strain was noted (Table 1). K1 antigen was measurable in high concentration in the supernatant fluid in most strains. The concentration of K1 polysaccharide found in the bacterial pellet was always less than that in the supernatant fluid. Four strains (no. 6, 9, 10, and 11) with less than 12 μg of K1 polysaccharide per 10^{10} CFU in the washed cell fraction were phagocytized well in chelated serum (82 ± 14% killing). The remaining strains had more than 12 μg of K1 polysaccharide per 10^{10} CFU and usually were more resistant to phagocytosis in chelated serum (27 ± 10.6% killing, $P < 0.01$). However, three of these strains (no. 5, 7, and 8) were opsonized well despite having higher amounts of cell-associated K1 polysaccharide. The mean concentration of K1 polysaccharide in whole washed cell fractions for group I strains was 10.3 ± 2.6 μg per 10^{10} CFU and for group II strains 18.9 ± 2.6 μg per 10^{10} CFU ($P < 0.05$).

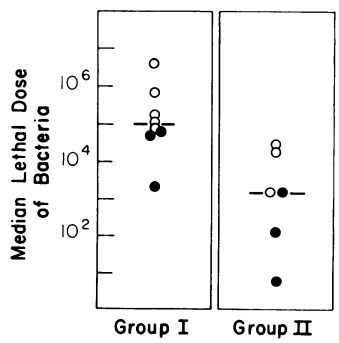

FIG. 4. *Relationship of LD_{50} of K1 E. coli strains to opsonic resistance in presence of MgEGTA-chelated serum and human leukocytes. Serum-sensitive* (○) *and serum-resistant* (●) *E. coli strains. K1 E. coli strains were considered sensitive to opsonization if more than 20% of the bacteria were killed after 60 min (group I). Group II K1 E. coli strains were resistant to opsonization (less than 20% bacterial killing in 60 min). Median shown by horizontal bar.*

K1 polysaccharide does not appear to influence a strain's susceptibility to the bactericidal activity of serum since two of four strains with limited K1 polysaccharide (no. 10 and 11) and four of the seven strains with higher concentrations of K1 antigen in the whole cell fraction were serum sensitive.

DISCUSSION

In 1974, Robbins et al. (20) reported the high frequency of *E. coli* strains having the K1 capsular polysaccharide among isolates from the CSF of newborn infants with meningitis. It was of interest that the capsular polysaccharide of K1 *E. coli* was antigenically identical to that of group B meningococcus (10) and that both organisms cause meningitis in humans. These similarities suggest that the capsule may play a role in the pathogenicity of these two organisms.

We compared 46 K1 *E. coli* strains with 17 non-K1 *E. coli* strains. Those with the K1 capsular polysaccharide were significantly more resistant to uptake and killing than non-K1 strains in a phagocytic system with serum chelated with MgEGTA. Since 10 mM MgEGTA selectively blocks the classical complement pathway while allowing activation of the ACP (5–7), the lack of uptake and killing in serum with MgEGTA suggests that most of the K1 *E. coli* strains we studied require the classical complement pathway for efficient opsonization. However, three K1 strains (no. 9, 10, and 11 in Table 1) were opsonized well in chelated or unchelated serum. Furthermore, three non-K1 strains resisted opsonization in chelated serum, suggesting that K1 polysaccharide may not be the only factor affecting resistance to opsonization.

Four K1 *E. coli* strains that resisted phagocytosis and killing in chelated serum were also resistant in C2-deficient serum. This strongly suggests that most K1 *E. coli* strains require the classical complement pathway for efficient opsonization.

TABLE 1. *Concentration of K1 capsular polysaccharide per 10^{10} CFU in supernatant and washed whole cell fraction of K1 E. coli strains grown in brain heart infusion broth*[a]

Strain	Serotype	% Phagocytosis[b]	Serum sensitivity[c]	Log LD_{50}	K1 content	
					Supernatant (μg/ml per 10^{10} CFU)	Whole cell fraction (μg/ 10^{10} CFU)
1	O7:K1	0	S	3.14	164	13.5
2	NA[d]	0	S	4.45	210	17.1
3	O18ac:K1:H7	13	R	0.8	208	19.0
4	O7:K1	17	R	3.15	232	25.8
5	NA	27	S	5.05	164	16.4
6	O2:K1:H6	40	R	4.80	209	9.8
7	O7:K1:NM	62	R	3.39	346	15.7
8	SpAg:K1:H7	69	S	5.86	428	16.5
9	NA	92	R	4.69	187	11.4
10	O7:K1:NM	97	S	5.25	254	<1
11	SpAg:K1:H7	99	S	6.60	8.5	1

[a] Relationship of phagocytosis in MgEGTA-chelated serum, sensitivity to bactericidal activity of serum, and LD_{50} of strains injected into 5-day-old rats.

[b] Percent reduction in CFU of *E. coli* strain after 60 min in presence of human PMN and 20% pooled human serum chelated with 10 mM MgEGTA.

[c] Sensitivity to the bactericidal activity of 40% pooled human serum after 60 min. Resistant strains (R) had less than a 20%, and sensitive strains (S) had more than a 20% reduction in CFU.

[d] NA, Not available.

When *E. coli* strains were injected i.p. into suckling rats, K1 strains were associated with a higher mortality rate than non-K1 strains ($P < 0.05$). K1 and non-K1 *E. coli* strains that avoided opsonization by the ACP were more virulent than strains that were opsonized. The association of K1 antigen to virulence in newborn rats was not, however, related solely to the higher frequency of opsono-resistance, since non-K1 *E. coli* strains that were not opsonized by the ACP were less virulent than similar K1 strains. A relationship was also found between serum sensitivity and virulence in K1 strains. The most virulent strains were those which were both resistant to opsonization by the ACP and resistant to the bactericidal activity of serum. The ability of K1 *E. coli* strains to resist opsonization by the ACP and to resist the bactericidal activity of serum are important virulence factors in newborn rats. These findings also suggest that antibody specific for the K1 polysaccharide may be required for efficient phagocytosis.

Rottini in 1974 found that *E. coli* strains with a K antigen were more virulent in mice and more resistant to phagocytosis than strains lacking the K antigen (19). Other investigators have found virulence to be related to the presence of somatic antigens (15) or to the ability of a strain to resist the bactericidal activity of serum (13, 16). Björkstén and Kaijser (2) found that *E. coli* strains isolated from children with asymptomatic bacteriuria were significantly more often serum sensitive and opsonized via the ACP than strains isolated from children with pyelonephritis. In our study both serum sensitivity and resistance to phagocytosis were assessed. Of the strains tested, those which avoided opsonization by the ACP were more virulent in infant rats (mortality, 57%) than those which were opsonized by the ACP (mortality, 34%). The ability of strains to resist the bactericidal activity of serum may enhance their virulence, and this may be of particular importance in strains that resist opsonization by the ACP.

When K1 polysaccharide antigen was measured in the washed whole cell fraction of broth-grown *E. coli*, strains well opsonized by the ACP (group I strains) often had less than 12 μg of K1 antigen per 10^{10} CFU, while strains that were poorly opsonized by it (group II strains) always had greater than 12 μg/10^{10} CFU. The level of K1 polysaccharide in the supernatant of broth cultures was not related to opsonic requirements. The ability to produce and release K1 antigen was not, by itself, a determinant of resistance to phagocytosis. However, it is possible that K1 polysaccharide contributes to inhibition of phagocytosis when a critical level is present on the surface of bacteria. No relationship was found between the amount of K1 antigen content or production and resistance to the bactericidal activity of serum.

Our studies have shown that the opsonic requirements of K1 and non-K1 *E. coli* strains are related to virulence in newborn rats. K1 *E. coli* strains with small amounts of polysaccharide in their washed whole cell fractions behaved in a phagocytic system as if they were non-K1

strains. Two factors, the ability to resist opsonization by the ACP and serum resistance, were determinants of the virulence of K1 *E. coli* strains when injected into newborn rats. They may be determinants of virulence in newborn humans as well and suggest that specific immunoglobulin against K1 polysaccharide for activation of the classical complement pathway may be a major factor in host defense against these organisms.

ACKNOWLEDGMENTS

This work was supported by a grant-in-aid from the Graduate School, University of Minnesota. R. Bortolussi was a recipient of the Hospital for Sick Children Foundation Fellowship, Toronto. P. Ferrieri was supported in part by Public Health Service grants HL-06314 and AI-13926 from the National Institutes of Health. P. Quie was supported in part by grants AI-08821 and AI-06931. P. Quie is an American Legion Memorial Heart Research Professor. B. Björkstén was supported by grant B-77-19X-04990 from the Swedish Medical Research Council.

We thank Lewis W. Wannamaker for his encouragement and for his critical review of the manuscript.

LITERATURE CITED

1. **Björkstén, B., R. Bortolussi, L. Gothefors, and P. G. Quie.** 1976. Interaction of *E. coli* strains with human serum: lack of relationship to K1 antigen. J. Pediatr. **89**:892–897.
2. **Björkstén, B., and B. Kaijser.** 1978. Interaction of human serum and neutrophils with *Escherichia coli* strains: differences between strains isolated from urine of patients with pyelonephritis or asymptomatic bacteriuria. Infect. Immun. **22**:308–311.
3. **Bortolussi, R., P. Ferrieri, and L. W. Wannamaker.** 1978. Dynamics of *Escherichia coli* infection and meningitis in infant rats. Infect. Immun. **22**:480–485.
4. **Bortolussi, R., C. Krishnan, D. Armstrong, and P. Tovichayathamrong.** 1978. Prognosis for survival in neonatal meningitis: clinical and pathologic review of 52 cases. Can. Med. Assoc. J. **118**:165–168.
5. **Forsgren, A., R. H. McLean, A. F. Michael, and P. G. Quie.** 1975. Studies of the alternate pathway in chelated serum. J. Lab. Clin. Med. **85**:904–912.
6. **Forsgren, A., and P. G. Quie.** 1974. Influence of the alternate complement pathway on opsonization of several bacterial species. Infect. Immun. **10**:402–404.
7. **Forsgren, A., and P. G. Quie.** 1974. Opsonic activity in human serum chelated with ethylene glycoltetra-acetic acid. Immunology **26**:1251–1256.
8. **Goldacre, M. J.** 1976. Acute bacterial meningitis in childhood. Lancet **i**:28–31.
9. **Howard, C. J., and A. A. Glynn.** 1971. The virulence for mice of strains of *Escherichia coli* related to the effects of K antigens on their resistance to phagocytosis and killing by complement. Immunology **29**:767–777.
10. **Kasper, D. L., J. L. Winkelhake, W. D. Zollinger, B. L. Brandt, and M. S. Artenstein.** 1973. Immunochemical similarity between polysaccharide antigens of *Escherichia coli* O7:K1(L):NM and group B *Neisseria meningitidis*. J. Immunol. **110**:262–268.
11. **Kim, Y., P. S. Friend, I. G. Dresner, E. J. Yunis, and A. F. Michael.** 1977. Inherited deficiency of the second component of complement (C2) with membranoproliferative glomerulonephritis. Am. J. Med. **62**:765–771.
12. **McCabe, W. R., P. C. Carling, and S. Bruins.** 1975. The relation of K-antigen to virulence of *Escherichia coli*. J. Infect. Dis. **131**:6–10.
13. **McCabe, W. R., B. Kaijser, S. Olling, M. Uwaydah, 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.
14. **McCracken, G. H., Jr., M. P. Glode, L. D. Sarff, S. G. Mize, M. S. Schiffer, J. B. Robbins, E. C. Gotschlich, I. Ørskov, and F. Ørskov.** 1974. Relation between *Escherichia coli* K1 capsular polysaccharide antigen and clinical outcome in neonatal meningitis. Lancet **ii**:246–250.
15. **Medearis, D. N., Jr., B. M. Camitta, and E. C. Heath.** 1968. Cell wall composition and virulence in *Escherichia coli*. J. Exp. Med. **128**:399–414.
16. **Olling, S., L. A. Hanson, J. Holmgren, U. Jodal, K. Lincoln, and U. Lindberg.** 1973. The bactericidal effect of normal human serum on *E. coli* strains from normals and from patients with urinary tract infections. Infection **1**:24–28.
17. **Quie, P. G., J. G. White, B. Holmes, and R. A. Good.** 1967. In vitro bactericidal capacity of human polymorphonuclear leukocytes: diminished activity in chronic granulomatous disease of childhood. J. Clin. Invest. **46**: 668–679.
18. **Reed, L. J., and H. Muench.** 1938. A simple method of estimating fifty percent endpoints. Am. J. Hyg. **27**:493–497.
19. **Rottini, G., P. Dri, M. R. Soranzo, and P. Patriarca.** 1975. Correlation between phagocytic activity and metabolic response of polymorphonuclear leukocytes toward different strains of *Escherichia coli*. Infect. Immun. **11**:417–423.
20. **Robbins, J. B., G. H. McCracken, Jr., E. C. Gotschlich, F. Orskov, I. Orskov, and L. A. Hanson.** 1974. *Escherichia coli* K1 capsular polysaccharide associated with neonatal meningitis. N. Engl. J. Med. **290**:1216–1220.
21. **Sarff, L. D., G. H. McCracken, Jr., M. S. Schiffer, M. P. Glode, J. B. Robbins, I. Ørskov, and F. Ørskov.** 1975. Epidemiology of *Escherichia coli* K1 in healthy and diseased newborns. Lancet **i**:1099–1104.
22. **Weeke, B.** 1973. Rocket immunoelectrophoresis. Scand. J. Immunol. **2**(Suppl. 1):37–46.