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K-1 Antigen of *Escherichia coli*: Epidemiology and Serum Sensitivity of Pathogenic Strains

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K-1 *Escherichia coli* are far more frequent in neonatal sepsis (36% of *E. coli* sepsis) and meningitis (80% of *E. coli* meningitis) than would be expected by the frequency of K-1 *E. coli* colonization in neonates (11 to 25%). There is no apparent parallel in cases of sepsis in adults. To study further this apparent age-related difference in virulence, *E. coli* K-1 clinical isolates were tested for their sensitivity to sera. Strains isolated from cases of neonatal meningitis were more sensitive to serum bactericidal activity than those from cases of neonatal or adult sepsis or adult meningitis ($P < 0.01$). Serum sensitivity did not appear to be determined by K or O antigens. Four isolates sensitive to serum bactericidal activity obtained from neonatal cerebrospinal fluid were killed by adult serum chelated with 0.05 M Mg^{2+} ethyleneglycol-bis(β-aminoethyl ether)-*N,N*-tetraacetic acid (EGTA), suggesting that the alternative pathway was activated. Although untreated neonatal sera killed these strains as well as adult sera did, EGTA-treated neonatal sera were less effective than EGTA-treated adult sera. This suggests that the alternative pathway function was not activated in neonatal sera. The bactericidal defect of neonatal EGTA-treated serum was partially corrected by addition of either A or B hyperimmune equine meningococcal antiserum.

Recent studies have pointed out that about 80% of strains of *Escherichia coli* causing neonatal meningitis and 36% of those causing neonatal sepsis contain K-1 capsular antigen (20, 21). These figures are considerably higher than those reported in adults. The present study was undertaken to determine whether the clinical isolates of *E. coli* from infants and adults differed from each other in any way that might help to define mechanisms of virulence of K-1 *E. coli*.

MATERIALS AND METHODS

Bacterial isolates studied. Most *E. coli* K-1 strains isolated from infants with bacteremia and meningitis were obtained from John Robbins and George McCracken. The strains had been identified as K-1 by antibody-agar technique (22) and in some cases also by full crossed immunoelectrophoresis performed by Fritz and Ida Orskov (17). Five additional infant K-1 pathogenic isolates and all of the infant stool isolates were identified by me immediately after isolation by using a modification of the antibody-agar technique described below. Unselected *E. coli* bloodstream and cerebrospinal fluid isolates from nonpediatric patients at the Presbyterian Hospital in New York City, Los Angeles County General Hospital, and Peter Bent Brigham Hospital in Boston were kindly provided by Paul Ellner, Lowell Young, and Thomas O'Brien, respectively. Organisms with K-1 capsule were identified by the antibody-agar technique described below. Variant clones of K-1 strain were selected for deficiency in capsular antigen on antibody agar.

Bacteriological identification. The organisms were identified as *E. coli* by the methods of Edwards and Ewing (7). K-1 strains were identified on the modified medium of Davis and Mignoli (5) containing 10% equine B-meningococcal antiserum (taking advantage of the cross-reactivity between meningococcus B and *E. coli* K-1 capsules [13]) and 1.5% agar (Bacto; Difco Laboratories) Halos were read after 24 h of incubation at 37°C and 48 and 72 h at 4°C.

Media used. The antibody agar medium used by Robbins et al. (20) containing tryptic soy broth (Difco) 1.5% agarose, and 10% equine B-meningococcal antiserum was initially compared with modified minimal medium of Davis and Mingioli (5), 1.5% agar (Bacto), and the same concentration of antiserum. Overnight cultures of isolates in tryptic soy broth (Difco) were diluted 1:1 with glycerol (Fisher certified grade), and samples were stored at −40°C.

Stability of strains. Twelve strains of *E. coli* K-1 whose susceptibility to serum bactericidal activity had been previously tested were passed 16 times on Trypticase soy agar by picking five colonies for each passage. The final strains were then retested for susceptibility to serum bactericidal activity.

Susceptibility to serum bactericidal action. Sera studied were stored at −70°C within 2 h of collection. The adult sera were obtained from healthy volunteers who were not on antimicrobial therapy and had no recent recognized bacterial infections. Cord

sera were checked for maternal blood contamination by immunoglobulin M (IgM) determinations, and only those cord sera that had levels of IgM below 10 mg/dl were used. Horse sera were prepared by ultracentrifugation at 110,000 × *g* for 3 h to remove anticomplementary activity, and their effect on serum bactericidal activity was tested within 24 h of preparation. Meningococcal antisera A and B and K-1 polysaccharide (from strain 235) were provided by John Robbins. A total of 2×10^7 to 5×10^7 log-phase organisms were tested in 0.5 ml of Hanks medium containing Mg^{2+} and Ca^{2+} (Baltimore Biological Laboratory) and 20 or 80% serum. In some studies sera were pooled; in others several sera were used to test each strain. Duplicate samples of 0.1 ml were taken at zero time and after 60 min of incubation. Colonies counted were on Trypticase soy agar after overnight incubation at 37°C of serial 10-fold dilutions of the specimens in Trypticase soy broth. Serum sensitivity was scored as shown in Table 1. Repeated assays were reproducible within one score point.

Mechanism of bactericidal activity. Bactericidal assays were performed on selected strains with sera treated in the following ways: (i) chelated with 0.05 Mg^{2+}-ethyleneglycol-bis(β-aminoethyl ether)-*N*,*N*-tetraacetic acid (EGTA) to ablate classical pathway activity (15); (ii) heated for 30 min at 50°C to ablate alternative pathway activity (12); (iii) chelated and heated as above.

RESULTS

Comparison of antibody-agar media for detection of K-1-positive *E. coli*. Twenty-four rectal swabs from healthy pregnant or nonpregnant volunteers were tested on two media described above. Seven individuals had organisms with K-1 antigen detectable with either media. Twenty-five known K-1-positive strains were stamped with a replicator on both media. Halos appeared promptly on both media and were of similar size.

Prevalence of K-1 antigen in strains identified as K-1 *E. coli* recovered from blood cultures of adults. Forty-one *E. coli* K-1 strains were detected among 230 *E. coli* bacteremia isolates from adults from three institutions (Table 2). The prevalence varied from a low of 11% of Presbyterian Hospital to 24% at Los Angeles County Hospital.

Susceptibility to serum bactericidal activity. Isolates from infants with meningitis were significantly more susceptible to serum bactericidal activity than isolates from infants or adults whose infection was limited to sepsis (Table 3). To determine whether this was a phenotypic change due to growth in the cerebrospinal fluid, three matched pairs of cerebrospinal fluid and blood isolates from infants were tested. In each pair the blood stream and cerebrospinal fluid isolates had the same score. Adult septicemia isolates did not differ in susceptibility from infant septicemia isolates. *E. coli* meningitis in adults is rare. For this reason, only two K-1 isolates from cases of meningitis in adults were tested. They were both more resistant than any isolate from an infant with meningitis (Fig. 1).

Twelve fresh isolates of *E. coli* K-1 were tested for susceptibility before and after 16 serial passages on Trypticase soy agar. In no case was there a change in serum sensitivity greater than one score point. Thus, differences in susceptibility are not likely to be due to the number of laboratory passages of the strains in question. The infant strains and adult strains which were fresh isolates from New York and were not subcultured more than twice after initial isolation were comparable in susceptibility to the other

TABLE 1. *Scoring of serum sensitivity*

Score	% of 0°C bacterial counts in:	
	20% Serum	80% Serum
1	≥200	≥200
2	≥200	51-199
3	≥200	≤50
4	51-199	51-199
5	51-199	≤50
6	≤50	≤50

TABLE 2. *Incidence of K-1 serotype in adult E. coli isolates from cases of bacteremia in adults*

Source	No. of *E. coli* tested	No. K-1	% K-1
Peter Bent Brigham Hospital	72	13	18
Los Angeles County Hospital	78	19	24
Presbyterian Hospital	80	9	11
Total	230	41	18

TABLE 3. *Serum sensitivity scores of clinical isolates by source*

Source	Serum sensitivity score
Adult sepsis and meningitis[a] ($n = 29$)	3.07
Adult sepsis[b] ($n = 27$)	3.19
Adult meningitis ($n = 2$)	1.50
Infant sepsis and meningitis[a] ($n = 34$)	3.41
Infant sepsis[b, c] ($n = 23$)	2.87
Infant meningitis[c] ($n = 11$)	4.73

[a] Adult strains versus infant strains, $P > 0.05$.
[b] Adult sepsis strains versus infant sepsis strains, $P > 0.05$.
[c] Infant sepsis versus infant meningitis, $P < 0.01$. The above statistical analyses were performed by using the Wilcoxon rank sum test (24).

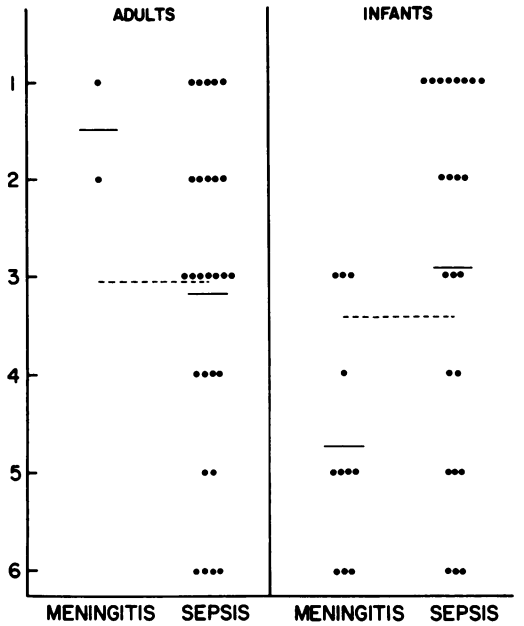

FIG. 1. *Serum sensitivity of virulent strains of E. coli K-1. (For scoring system, see text). Adult sepsis strains versus infant sepsis strains $P > 0.05$ by Wilcoxon rank sum test (24). Infant sepsis strains versus infant meningitis strains $P < 0.01$ (24). -----, overall mean; ——, mean for meningitis, mean for sepsis.*

TABLE 4. *Serum sensitivity of individual strains to individual and pool adult sera*

Strain	Serum sensitivity score of:			
	JP	MR	MF	Serum pool
C 77 (O7)	3	3	3	3
C 88 (O7)	6	1	6	6
C 93 (sa H34)	5	6	5	5
C 98 (O123 H−)	5	5	5	5
C 99 (O18a6:H7)	3	3	3	3
C 105 (sa H6)	5	4	6	5
C 106 (O7 H−)	3	3	4	3
C 117 (sa H6)	6	6	6	6
C 119 (O7 H−)	6	6	6	6
N 105 (O1 H−)	3	2	2	2
2371	3	3	3	3
2524	3	1	1	2
2820	1	1	3	2

TABLE 5. *Activity of one adult and four cord sera against four E. coli K-1 strains*

Strain	JP (adult)	Bu	Do	Gib	La
88	6	4	5	1	4
93	6	4	6	1	5
117	6	6	6	4	6
119	6	6	6	4	6

strains studied: the six neonatal bacteremia strains had a mean score of 2.8, the one neonatal meningitis strain had a score of 5, the five adult sepsis strains had a mean score of 3, and the one adult meningitis strain a score of 1.

Variation among individual sera. Eleven strains were tested in sera from three healthy adult donors and in an adult serum pool (n = 10) (Table 4). Although individual sera differed little from each other in their effect on the various strains, individual strains, even those which bore the same O and K antigens, differed widely in serum sensitivity. For example C 106 and C 119, both K-1 O7, had a mean score of 3.25 ± 0.5 and 6, respectively. The activity of four untreated cord sera against four very susceptible strains from cases of neonatal meningitis is shown in Table 5. Only one serum (Gib) was significantly less active than the simultaneously tested adult serum.

Effect of heating and/or chelation of sera. To separate the activity of the alternative and classical pathways of complement activation in bactericidal activity, the effect of heating and/or chelating adult and infant sera was investigated. All 13 susceptible strains tested were killed by 0.05 M ethylenediaminetetraacetic acid alone, whereas 5/13 were killed by 0.05 M Mg^{2+}-EGTA. Because of this difficulty, only four strains were available to evaluate influence of the alternative pathway of complement activation on serum bactericidal effect. Susceptible K-1 isolates were killed by untreated and EGTA-treated adult sera (Table 5). Strain 117 was killed by either EGTA or 50°C heated serum but not by sera which had received both treatments (Table 6).

Untreated cord sera, but not EGTA-treated cord sera, killed all four strains of *E. coli* studied (Table 6). Both meningococcal antisera A and B at 1:100 dilution restored bactericidal activity of newborn EGTA-treated serum. Treatment of these horse antisera with K-1 polysaccharide absorbed onto aluminum hydroxide did not alter their ability to restore bactericidal activity (11).

DISCUSSION

The prinicpal finding of this study is that *E. coli* K-1 isolates from infants with meningitis are considerably more serum sensitive than are isolates from sepsis alone in infants and sepsis or meningitis in adults.

It is unlikely that in this study differences in the handling of the strains from isolation to testing could account for the differences ob-

TABLE 6. *Role of alternative pathway of complement activation in serum bactericidal activity of adult and cord sera*

Strain	Adult sera ($n = 3$)		Cord sera ($n = 4$)			
				EGTA-treated[a]		
	Untreated	EGTA-treated[a]	Untreated	Without anti-meningococcus serum	Anti-meningococcus B serum	Anti-meningococcus A serum
C 117 (sa H6)	6	6	6	1.3	5	4.7
C 88 (O7)	4.3	4.6	4.3	1	4.3	4
C 93 (sa H34)	5.3	5	5	1.7	6	5
C 119 (O7 H−)	6	6	6	1.7	5.7	6
C 117; serum heated at 50°C for 30 min.	6	1				

[a] Treated with .05 mM Mg^{2+}-EGTA.

served because (i) growth rates of serum-sensitive and -resistant strains were comparable, (ii) serial passage of freshly isolated resistant strains did not result in alteration of serum resistance, and (iii) a small sample of fresh isolates tested promptly after initial isolation had a distribution of serum sensitivity similar to that of the strains previously collected and stored.

Cord sera, uncontaminated by maternal serum and therefore containing very little IgM, had adult levels of serum bactericidal activity but had diminished activity when calcium was chelated by, 0.05 M Mg^{2+}-EGTA to inhibit the classical pathway of complement activation. This suggests a defective participation of the alternate pathway of complement activation in neonatal sera as influencing the serum bactericidal effect. Previous studies have shown depression of alternate pathway-mediated opsononization (26) and hemolysis (8) in the neonate. As has been shown by others (28), the susceptibility of individual isolates to serum varied anong strains of identical serotype, suggesting that antibodies directed against the serotype antigens are not the sole determinants of the serum bactericidal reaction. Consistent with this were the findings that activity was not absorbed by alum-K-1 flocculates and that both group A and B antimeningococcal antisera appeared to correct the impaired activity of EGTA-treated neonatal sera.

The study of the role of K-1 antibody in the serum bactericidal reaction is limited by the lack of a sensitive, specific assay. Hyperimmune equine antisera can be evaluated by counterimmunoelectrophoresis. Although preliminary reports suggested that human antibody by enzyme-linked immunoadsorbent assay was virtually ubiquitous (G. H. McCracken, L. B. Sarff, J. B. Robbins, M. Glode, B. Kaiser, and L. Hanson, Pediatr. Res. **10**:401, 1976), work in this laboratory with the same batch of K-1 antigen and evaluating hyperimmune and normal equine antisera suggests that that assay is probably detecting binding to *E. coli* antigens other than the K-1 polysaccharide (J. Pitt and M. Fencick, manuscript in preparation). Moreover, radioactive binding studies for B meningococcal antibody detected antibody in about 10% of healthy young adults (3, 27, 29).

A number of studies in animals and humans correlate the likelihood of meningitis with the intensity of bacteremia (9, 6, 16). The increased susceptibility to serum of infant meningitis K-1 isolates and the greater incidence in neonates than in adults of meningitis as a complication of *E. coli* K-1 sepsis suggest that the infant who develops *E. coli* K-1 meningitis has a defect specifically impairing the clearance of *E. coli* K-1. Taken together, these findings suggest that (i) neonates who develop *E. coli* K-1 meningitis are more susceptible to serum-sensitive organisms than neonates or adults in general; (ii) this susceptibility is not a reflection of *E. coli* K-1 antibody deficiency; and (iii) it may be due to a quantitative deficiency in the alternative pathway of complement deficiency, some nonserotypic specific antibody, or a non-immunological clearance mechanism. Uncovered sialic acid receptors (25) or deficient neuraminidase sialidase activity (4) would be examples of such mechanisms.

This study confirmed the findings of others that K-1 *E. coli* account for a lower percentage of adult than infant bacteremias. Of interest was a moderate variation in percentage between the Presbyterian Hospital and the Los Angeles County Hospital isolates. The difference in patient population and difference in virulence of K-1 strains in the two institutions may be related to the differing incidence (J. Pitt, J. Infect. Dis., in press; R. J. Weinstein, and L. S. Young, Pro-

gram Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 17th, New York, N.Y., Abstr. no. 332, 1977).

Serum bacteriolysis has previously been reported as a normal host defense in a case of recurrent gonococcal bacteremia in an individual with C-8 deficiency (19) and by Vosti (28) in his study of *E. coli* bacteremia isolates. A recent study by Shaw and associates, however, (23) suggested that *H. influenzae* b serum sensitivity is an artifact of in vitro culture and that serum bactericidal activity, which is often detectable in the bacteremic patient, does not protect against bacteremia. This study revealed that the characteristics of serum sensitivity and resistance of *E. coli* K-1 are more stable than those of *H. influenzae*. Consistent with the findings of Shaw et al., anticapsular antibody did not seem to play a critical role in the serum bactericidal effect, but, contrary to their findings, there was a role for the serum bactericidal effect in host defense,

In summary, this study provides evidence that the sensitive pressures found in adults which favor serum resistant strains causing disease are relatively weak in newborns. This study and others have shown defective bactericidal and opsonic activity of the alternative pathway of complement activation in many newborn infants, and these defects may be responsible for the defective clearance. Because there is no evidence that K-1 antigen activates the alternative pathway (2) and the evidence for K-1 antibody deficiency in the neonate is contradictory, the mechanism for K-1 virulence in neonates remains unknown.

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