INFECTION AND IMMUNITY, Apr. 1977, p. 75-80

Vol. 16, No. 1
Printed in U.S.A.

Pathogenesis of Neonatal *Escherichia coli* Meningitis: Induction of Bacteremia and Meningitis in Infant Rats Fed *E. coli* K1

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Received for publication 17 November 1976

Escherichia coli K1 strains, isolated from human newborns with meningitis, were fed to pathogen-free Sprague-Dawley infant rats by an oral gastric tube. Feeding of 10^3 to 10^{11} organisms colonized the intestine of approximately 70% of the animals. At 5 days postfeeding of 3- to 5-day-old rats, bacteremia was detected in 60%, and meningitis occurred in 15% of bacteremic animals. Colonization and bacteremia were age-related. Rats 15 days old had only 19% colonization and 10% bacteremia, and those 30 days old were almost completely resistant to colonization and bacteremia. The intranasal route was less effective in inducing colonization and bacteremia. Intralitter transmission from *E. coli* K1-fed rats occurred, with 52% of water-fed controls becoming colonized and 15% becoming bacteremic. Colonization of mothers from their fed infants occurred, but none of five tested developed bacteremia. Other *E. coli* capsular polysaccharide types were studied. A K92 strain isolated from a newborn with meningitis induced a 77% colonization rate, and 8% of these developed bacteremia without detectable meningitis. An *E. coli* K100 strain showed a 32% colonization rate, and 2% developed bacteremia. The age relation, relatively high virulence of K1 compared with other capsular types, spontaneous appearance of colonization, bacteremia, and meningitis, and intralitter transmission of colonization and disease in newborn rats closely parallel *E. coli* epidemiology in human neonates.

The risk for gram-negative bacterial meningitis is highest for neonates. The most frequent gram-negative species causing neonatal bacterial meningitis is *Escherichia coli* (5, 7, 8, 10). Although there are now 154 somatic (O), 34 flagellar (H), and 100 capsular (K) antigens reported for the *Escherichia* species, strains with the K1 capsular polysaccharide account for about 80% of these cases of meningitis (14, 16, 18). The pathogenic role of K1 is also demonstrated by the observations that it is the most common capsular type in neonatal septicemia without meningitis and in childhood pyelonephritis (11, 13).

K1 strains are found frequently in high concentrations in stools of healthy individuals of all ages, including newborns and parturient females (18). Analysis of *E. coli* serotypes in cerebrospinal fluid (CSF) from newborns indicates that most strains can also be simultaneously detected in the infant and maternal stool. Maternal-infant and attendant-infant transmission also occurs without development of disease (18, 19). Previous studies have shown that suckling, but not adult, rats are susceptible to *Haemophilus influenzae* type b meningitis after intranasal challenge (15). In the studies reported here, infant rats fed *E. coli* K1 strains developed a high rate of intestinal colonization, bacteremia, and meningitis. The characteristics of this experimental model are described.

MATERIALS AND METHODS

Bacteria and media. *E. coli* strains C94 (O7:K1:H-), EC3 (O1:K1:H-), Easter (O75:K100:H5), N67 (O13:K92:H4), N70 (O23:K22:H15), and LH (O75:K1:H3), and *H. influenzae* type b strain Eagan (HIB) were lyophilized and stored at 4°C; a new ampoule was rehydrated for each experiment (1, 17, 18, 20). Strains C94, N67, and N70 were isolated from neonatal meningitis (17). Purity and identity of K1 cultures were established by observing halo formation on Davis minimal medium (DMM) agar containing meningococcus group B antiserum (2, 17). (We thank Jane Pitt for suggesting the use of this medium.) This medium supports the growth of *E. coli*, but not *Proteus* species and gram-positive organisms. *E. coli* K100 and K92 were identified with HIB (20) and meningococcus group C antiserum agar, respectively (19).

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Challenge organisms were prepared by inoculating single colonies into tryptic soy broth (Difco) containing 0.1% yeast extract (Difco), shaking the cultures for 2 h at 37°C, and determining the concentration from a standard curve relating optical density at 530 nm to colony-forming units per milliliter. A sample was diluted in phosphate-buffered saline (PBS) to the desired concentration, or the entire culture was centrifuged at 16,000 × *g* for 20 min and resuspended in PBS. The challenge dose was assayed by plate counts in triplicate.

Animals. Pathogen-free Sprague-Dawley rats with mothers were obtained from Charles River Breeding Laboratories (Wilmington, Mass.) or Taconic Farms (Germantown, N.Y.). Each litter of 8 to 12 pups remained with its mother. The pups were fed 0.5 ml of bacterial suspension through an oral gastric tube (0.024-inch [0.061-cm] outer diameter polyethylene tubing; Clay Adams, Parsippany, N.J.) directly into the stomach. Preliminary experiments showed no escape of methylene blue. Older pups were fed under light ether anesthesia. Intranasal challenge was performed as described previously (15).

Cultural procedures. For stool cultures the anal area was sampled with a sterile cotton swab moistened with sterile water, and the sample was plated directly onto DMM antiserum agar. Blood was obtained from infant rats by puncturing a tail vein with a microlance (Becton-Dickinson & Co., Rutherford, N.J.) and drawing the blood into a micropipette (Corning Glass Works, Corning, N.Y.). The skin was first disinfected with 2% iodine followed by 70% isopropyl alcohol. Samples of 10 to 20 μl of blood were inoculated onto DMM antiserum plates. Adult rats were bled by cardiac puncture. CSF was obtained by cisterna magna puncture (Moxon, in press). Meningitis was defined by positive culture.

Statistics. The data were analyzed by applying a normal distribution approximating a binomial distribution (4) and by chi-square.

RESULTS

Virulence of *E. coli* capsular types. Table 1 summarizes the results of feeding the *E. coli* strains to infant rats. All encapsulated strains established intestinal colonization in 32 to 77% of the animals as measured by continued stool excretion of the fed organism. High rates of bacteremia occurred only in K1-fed animals, ranging from 21% for EC3 to 66% for the LH strain. In contrast, bacteremia was detected in only 8% of K92-fed, 2% of K100-fed, and 5% of strain N70-fed animals. Approximately 15% of the K1-fed bacteremic animals developed meningitis. Colonization, bacteremia, and meningitis were all significantly higher in K1-fed animals than in animals fed other strains ($P < 0.01$). The overall mortality of animals fed the three K1 strains varied. Although EC3-fed animals had a lower rate of bacteremia than animals fed the other two K1 strains, 21% of animals fed strain EC3 died as compared with 8% and 0% for the other two.

Meningitis. CSF of 65 bacteremic rats fed *E. coli* K1 and of 2 water-fed littermates was obtained for culture and Gram stain. Eleven had positive CSF cultures. CSF colony counts ranged from 10^2 to 10^3/ml, and one CSF smear had numerous gram-negative rods. Seven of these specimens were clear but contained microscopic amounts of blood, and we could not exclude the possibility that the positive CSF culture represented contamination by small amounts of infected peripheral blood. Four samples contained no blood and had colony counts of 2×10^2 to 4×10^2/ml. When the number of K1 organisms exceeded 5×10^2/ml in blood cultures, meningitis developed more frequently (in 3 of 11, compared to 8 of 44 with $<5 \times 10^2$/ml).

In an attempt to increase the number of animals developing meningitis, six litters were isolated from their mothers 4 h prior to and for 36 h after feeding of strain C94 (Table 2). These animals were kept warm and given glucose-water by tube to prevent dehydration. Bacteremia occurred in 81%, and in 25 of the 42 rats with bacteremia the number of organisms was

TABLE 1. *Colonization, bacteremia, and meningitis after feeding of E. coli to 3- to 5-day-old rats*[a]

Strain	Colonization	Bacteremia[b]	Meningitis[c]	Mortality[d]
C94 (O7:K1:H-)	69 (118/170)	64 (107/167)	13 (6/45)	8 (13/170)
EC3 (O1:K1:H-)	48 (9/19)	21 (5/24)	ND	21 (5/24)
LH (O75:K1:H3)	74 (26/35)	66 (23/35)	20 (4/20)	0 (0.35)
Easter (O75:K100:H5)	32 (20/63)	2 (1/63)	ND	0 (0/63)
N67 (O13:K92:H4)	77 (20/26)	8 (2/26)	0 (0/2)	4 (1/26)
N70 (O23:K22:H15)	ND	5 (1/20)	ND	0 (0/20)

[a] Rats 3 to 5 days old were fed 10^8 to 10^{10} organisms by oral gastric tube. Blood and stool were cultured on antiserum agar on days 2 and 5 postfeeding. CSF cultures were taken 1 day after demonstration of positive blood culture. Results show the percentage of rats, with the number/total tested given in parentheses. Colonization, bacteremia, or meningitis in K1-fed vs. non-K-1-fed: $P < 0.01$. ND = Not done.

[b] Includes heart blood cultures done postmortem.

[c] Positive cultures; only bacteremic animals were examined for the presence of meningitis.

[d] Death occurring more than 12 h postfeeding, excludes all rats sacrificed for CSF studies.

$>5 \times 10^2$/ml. Forty-seven percent of bacteremic rats had meningitis, as defined by positive CSF culture. Contamination of CSF by minute quantities of blood was unlikely since no red cells were seen on microscopic examination of CSF. In addition, two rats had grossly purulent CSF and positive Gram stains (no cultures obtained) and two rats had CSF pleocytosis (>70 cells/mm^3) but were culture negative. Gram-stained smears of CSF revealed gram-negative rods in 6 of 17 samples.

Inoculum size related to bacteremia. Rats were fed from 10^3 to 10^{11} strain C94 cells at 5 days of age. There were no differences in colonization rates or bacteremia (Table 3). In animals fed 10^3 cells, 8 of 15 positive rats first exhibited bacteremia on day 8 or 10. In a long-term study of animals fed 10^8 cells, 91% developed bacteremia by day 6 and no new positive blood cultures were detected thereafter. Water-fed littermates developed bacteremia as late as day 10. The delayed appearance of bacteremia in rats fed low doses suggests that organisms may be obliged to multiply in the gastrointestinal tract until a concentration necessary for bloodstream invasion is reached.

Effect of route of inoculation. Table 4 shows the effect of challenging the infant rats with HIB and *E. coli* K1 by the intranasal or intestinal route. The highest bacteremic rates were observed for the nasally administered HIB (56%) and for the fed *E. coli* (56%). The differences in rates for each route were significant ($P = 0.01$) for both organisms. Some bacteremia occurred in the other groups, but swallowing and subsequent intestinal multiplication of the nasally administered K1 strain may explain the 15% bacteremia observed in this group.

Effect of age upon induction of colonization, bacteremia, and meningitis. No significant difference in the rates of bacteremia and meningitis resulted from feeding C94 at 3 or 5 days of age (Table 5). Colonization was readily achieved after feeding at all ages. However, difficulties in feeding and stool samples may explain the apparent age-related differences between 3- and 5-day-old animals. Colonization and bacteremia were both significantly lower in animals fed at 15 days or older ($P = 0.01$). Bacteremia was observed in 10% of the animals fed at 15 days of age, but there were no positive

TABLE 2. *Colonization, bacteremia, and meningitis, in colostrum-deprived infant rats*[a]

Determination	Percent	No./total
Colonization	65	34/52
Bacteremia[b]	81	42/52
Meningitis[c]	47	15/32
Mortality[d]	21	11/52

[a] Four-day-old rats were fed strain C94 by oral gastric tube. Stool and blood were cultured on antiserum agar on days 2 and 3. CSF was cultured after demonstration of a positive blood culture.

[b] Includes heart blood cultures done postmortem.

[c] Positive culture and/or gram-negative rods in CSF; only bacteremic rats were examined for meningitis.

[d] Death occurring more than 12 h postfeeding, excluding rats sacrificed for CSF studies.

TABLE 4. *Comparison of bacteremia in infant rats inoculated intranasally or fed with E. coli K1 or H. influenzae type b*[a]

Organism	Route of administration	
	Intranasal	Fed
E. coli K1	15 (3/20)	56 (10/18)
H. influenzae b	56 (5/9)	11 (1/9)

[a] Five-day-old rats were challenged with 10^5 *E. coli* K1 strain C94 cells or 10^7 *H. influenzae* b strain Eagan cells. Blood cultures were taken 2 and 5 days postfeeding. Results show the percentage of rats, with the number/total given in parentheses. Intranasal vs. oral: $P = 0.01$ for both organisms.

TABLE 3. *Dosage effect of E. coli K1 fed to infant rats upon induction of colonization, bacteremia, and meningitis*[a]

Determination	No. of organisms fed			
	10^3	10^5–10^7	$>10^7$–10^9	$>10^9$–10^{11}
Colonization	100 (32/32)	75 (15/20)	76 (47/62)	78 (36/46)
Bacteremia[b]	47 (15/32)	50 (10/20)	76 (47/62)	63 (29/46)
Meningitis[c]	ND	ND	ND	22 (4/18)
Mortality[d]	0 (0/32)	15 (3/20)	11 (7/62)	0 (0/46)

[a] Five-day-old rats were fed strain C94 by oral gastric tube. Stool and blood were cultured on antiserum agar on days 2 and 5 postfeeding. CSF was cultured after demonstration of positive blood cultures. Results show the percentage of rats, with the number/total tested given in parentheses. ND = Not done.

[b] Includes heart blood cultures done postmortem.

[c] Positive cultures; only bacteremic animals were examined for the presence of meningitis.

[d] Death occurring more than 12 h postfeeding; excludes all rats sacrificed for CSF studies.

TABLE 5. *Age-related disease susceptibility of infant rats fed 10^8 to 10^{10} E. coli K1 cells*[a]

Determination	Age when fed			
	3 days	5 days	15 days	30 days
Colonization	44 (19/43)	78 (99/127)	19 (17/90)	32 (6/18)
Bacteremia[b]	53 (21/40)	68 (86/127)	10 (9/90)	0 (0/19)
Meningitis[c]	6 (1/17)	18 (5/28)	ND	ND
Mortality[d]	7 (3/43)	8 (10/237)	6 (5/90)	0 (0/19)

[a] Rats were fed *E. coli* strain C94. Stool and blood cultures were plated on antiserum agar 2 and 5 days after feeding. CSF cultures were taken 1 day after demonstration of positive blood cultures. Results show the percentage of rats, with the number/total given in parentheses. Colonization or bacteremia in rats aged 3 to 5 days vs. 15 to 30 days: $P = 0.01$. ND = Not done.

[b] Includes heart blood cultures done postmortem.

[c] Positive cultures; only bacteremic animals were examined for the presence of meningitis.

[d] Death occurring more than 12 h postfeeding; excludes all rats sacrificed for CSF studies.

blood cultures among rats fed at 30 days of age, although colonization occurred in 32%.

Intralitter transmission. Two animals in each of 15 litters were fed sterile water at the same time their littermates received strain C94 organisms, and all were caged together with their mother. Table 6 shows that 78% of the K1-fed rats had positive stool cultures and 68% developed bacteremia. Within several days, 52% of the controls were colonized, and 15% subsequently demonstrated bacteremia. The rates of colonization were significantly different at $P = 0.05$, and the rates of bacteremia, at $P = 0.01$. Although 75% of the mothers became colonized, none of five studied developed bacteremia.

Duration of colonization related to bacteremia. Figure 1 shows the duration of colonization and the development of bacteremia in 5-day-old rats fed 10^8 strain C94 organisms and observed for 8 weeks. At 30 days of age, the infants were caged separately in groups of five siblings. The highest incidence of colonization (98%) occurred 6 days after feeding, and 20% of the fed animals were still colonized at 8 weeks. In contrast, bacteremia was observed most frequently 3 days after feeding and was not observed in any of the animals 13 days after feeding. Stool cultures were intermittently positive in most animals, possibly resulting from failure to detect small numbers of organisms and/or from a loss and reinfection from colonized littermates.

TABLE 6. *Transmission of E. coli K1 within litters*[a]

Determination	K1-fed	Controls (water-fed)	Mothers
Colonized	78 (99/127)	52 (14/27)	75 (18/24)
Bacteremic	68 (86/127)	15 (4/27)	0 (0/5)

[a] Five-day-old rats were fed 10^8 to 10^{10} C94 organisms, and stool and blood cultures were obtained at 2 and 5 days postfeeding. Within each litter, one or two rats were fed sterile water and were cultured at the same time intervals as the C94-fed rats. Nursing mother rats were also cultured at 2 and 5 days after feeding the infant rats. Results show the percentage of rats, with the number/total given in parentheses. Colonization in K1-fed vs. controls: $P = 0.05$. Bacteremia in K1-fed vs. controls: $P = 0.01$.

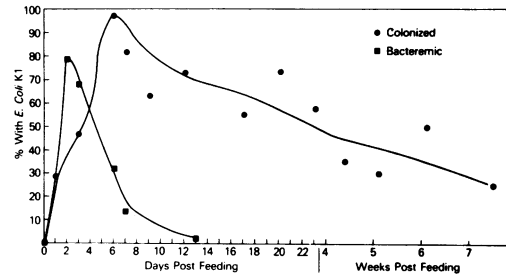

FIG. 1. *Persistence of colonization and bacteremia in 5-day-old infant rats fed 3.4 × 10^8 E. coli K1. Infant rats were fed strain C94 by oral gastric tube. Stool was cultured on antiserum agar at 1, 3, 6, 7, 9, 12, 17, 20, and 23 days and 4, 5, 6, and 7 weeks postfeeding. Blood was cultured at 1, 3, 6, and 7 days and 2 weeks postfeeding.*

DISCUSSION

Our results demonstrate that the K1-induced disease in infant rats has many characteristics similar to *E. coli* systemic disease in humans. These are (i) the invasiveness of K1 as contrasted to other *E. coli* capsular polysaccharide types, (ii) the superiority of gastrointestinal over nasopharyngeal challenge for disease induction, (iii) the remarkable susceptibility of newborns to bacteremia and meningitis as compared with older animals, (iv) the ease of animal to animal transmission, and (v) the high rate of colonization without disease in maturing and adult animals. Epidemiological studies have shown that K1 colonization is common in individuals of all ages. Thus, 45% of pregnant females studied had positive stool cultures for *E. coli* K1 based on a single culture, and ap-

proximately two-thirds of babies born to women K1-positive at the time of delivery were colonized with *E. coli* K1. Yet, systemic *E. coli* K1 disease is a comparatively rare event, occurring in approximately 1 in 200 colonized newborns (18). Studies have documented that the identical serotype of *E. coli* is usually isolated from neonatal CSF, blood, and stool and maternal stool, suggesting that contamination with maternal flora occurs either at the time of birth or possibly in utero (19). *E. coli* C94 (O7:K1:H-) is a representative serotype of strains from neonatal *E. coli* K1 meningitis (18). Feeding of strain C94 consistently resulted in colonization and bacteremia. Meningitis occurred in 13% of bacteremic animals. EC3 (O1:K1:H-), another K1 strain isolated from a neonate with meningitis, tended to give poorer halos on antiserum agar plates and was not as virulent as C94 in the infant rat when bacteremia was used as the criterion. However, mortality in animals fed EC3 was greater than that caused by C94. The third K1 organism, LH (O75:K1:H3), was isolated from the urine of an adult female with pyelonephritis. (We are grateful to Lars Hansen for providing this strain.) This strain was as virulent for infant rats as strain C94.

Three encapsulated but non-K1 *E. coli* strains were also used as challenge organisms. Strain Easter is a nonpathogenic *E. coli* (O75:K100:H4) isolated from normal stool and possessing a polysaccharide capsule cross-reactive with the capsule of HIB (20). Strain Easter was always less effective in colonizing animals than was C94. Bacteremia was noted in only 1 of 63 fed animals, indicating that this encapsulated *E. coli* is less likely to invade the bloodstream (9). The other organisms were isolated from the CSF of neonates with meningitis. One of these, strain N67 (O13:K92:H4), has a polysaccharide capsule cross-reactive with meningococcus group C (19). Colonization was readily achieved with N67, but bacteremia with either N67 or N70 (O23:K22:H15) was infrequently observed (<10%) and no meningitis was detected. Colonization with N70 could not be determined because of lack of a specific marker in mixed stool cultures. N70 blood cultures were identified by culture and stain characteristics.

The results of inoculating animals intranasally versus oro-gastrically suggest that *E. coli* K1 is less likely to invade the nasopharyngeal mucosa and prefers the milieu of the gastrointestinal tract. HIB is rarely recovered from stool cultures and is not suited for the gastrointestinal tract. In humans and infant rats, HIB invades the bloodstream via the nasopharyngeal mucosa (15). The one positive blood culture seen after HIB was fed may represent contamination of the pharynx when the tube was withdrawn.

Colonization and invasive disease were both age-related. Rats 15 or 30 days old did not colonize as readily as 5-day-old rats and had a significantly lower incidence of bacteremia. Failure to colonize may be due to several factors, such as the presence of established gut flora, the increased gastric acidity, or the development of local or systemic immunity in older animals, including passive transfer of colostral antibody. Further, meningitis was not detected in any animal fed at 15 days or older.

Transmission of encapsulated bacteria from *E. coli*-fed infant rats to water-fed littermates occurred with all the organisms tested. This ease of transmission parallels the demonstration of infant to attendant to infant transmission of K1 colonization and disease observed in humans (17–19). The peak incidence of colonization and bacteremia in controls with K1-fed littermates occurred at 6 days postfeeding. The delayed peak of bacteremia may represent transmission of small numbers of organisms which colonize and invade after reaching a critical concentration in the gastrointestinal tract. A similar observation has been mentioned recently with *Salmonella* infection (3). It is of interest that nursing mother rats were readily colonized when caged with their K1-fed pups. This may be due to prolonged exposure to large numbers of K1 organisms harbored by the colonized pups.

Colonization of 5-day-old rats persisted for up to 8 weeks. Bacteremia occurred within 10 days of feeding and reached a peak incidence at 3 days postfeeding. It would appear that bacteremia occurs shortly after colonization in the susceptible animal and leads either to death or to clearing of the bacteremia with persistence of colonization. Long-term studies of colonization are not available in human infants or adults.

The development of meningitis in infant rats occurred spontaneously and was detected in only 15% of bacteremic animals. In several instances, CSF culture was positive with no evidence of pleocytosis. Since animals were sacrificed at the time CSF culture was obtained, it was not possible to determine whether progression of disease would have occurred. Animals with the highest level of bacteremia were more likely to have positive CSF cultures. Colostrum deprivation increased the magnitude of bacteremia and the percentage of bacteremic animals developing meningitis. The nutritional and/or immunological consequences of colostrum deprivation were not investigated in these studies. By manipulating certain variables such as hy-

poxia, cold, and colostrum deprivation, it may be possible to increase the incidence of spontaneous meningitis. Similar environmental stresses are well documented in the human neonatal period.

The K1 antigen is structurally similar or identical to the meningococcal group B capsular polysaccharide (2, 6). Thus, this capsular antigen confers virulence upon two different bacterial species. The isolated and purified meningococcal group B and *E. coli* K1 polysaccharides, in contrast to other bacterial polysaccharides, are nonimmunogenic in adult humans (12, 21); however, purified anticapsular antibody conferred protection against lethal mucine-enhanced infection in mice (13, 17, 19). The establishment of a valid model for neonatal *E. coli* K1 bacterial meningitis could provide the basis for study of the pathogenesis of disease and mechanisms of immunity.

ACKNOWLEDGMENTS

We gratefully acknowledge the continued support of Frits and Ida Ørskov of the WHO Escherichia Reference and Research Center for serotyping of these organisms. Howard Hoffman, National Institute of Allergy and Infectious Diseases, kindly performed the statistical analyses. Technical assistance was provided by Robert J. Kapko and Linda Spruill.

M.P.G. was recipient of postdoctoral fellowship 5F22-HDO-2034-02 from the National Institute of Child Health and Human Development. E.R.M. was supported by grant 1-RO1 NS 12554-01 from the National Institutes of Health and by the Hospital for Consumptives of Maryland (Eudowood), Baltimore.

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