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Influence of Some Bacterial and Host Factors on Colonization and Invasiveness of *Escherichia coli* K1 in Neonatal Rats

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Of 209 healthy infants examined, 44 (21.1%) carried *Escherichia coli* K1 in their feces. Of these 44 isolates, 36 (81.8%) were attributed to 10 different known clonal groups of *E. coli* K1 and 4 isolates represented unknown types. The influence of mannose-resistant (MR) adhesins, aerobactin production, and resistance to serum on colonization and invasiveness of *E. coli K1* in orally infected inbred LEW baby rats was investigated. Strains expressing MR adhesins had significantly higher colonization and invasion rates than non-MR strains did. Mixed-infection experiments of LEW rats revealed interactions between different types of *E. coli* K1 strains affecting colonization and invasion rates. P-fimbriated strains appeared to have a selective advantage for colonization. The bacteremic potentials of different *E. coli* K1 strains could not be associated with their resistance to sera from LEW rats free of members of the family *Enterobacteriaceae*. No differences in virulence between fecal *E. coli* K1 isolates and clinical isolates from diseased humans were found. An influence of the major histocompatibility complex on host susceptibility to invasive *E. coli* K1 was indicated by comparing the parental LEW rat strain with different congenic LEW strains (*RT1*).

Escherichia coli carrying the K1 capsule is a commonly occurring bacteria in the feces of healthy humans and is an important pathogen that causes bacteremia and meningitis in newborn infants (23, 34). In addition, some types of *E. coli* K1 strains were found to be of particular virulence in pyelonephritis in humans of all age groups (23, 32, 47). The somatic antigens of *E. coli* K1 are associated with their virulence. The K1 capsule and lipopolysaccharide (LPS) are major determinants for the survival and growth of these strains in human serum (30, 42, 53). It was suggested that a lack of antibodies directed against capsular and LPS surface antigens of *E. coli* and deficiencies of other serum components are responsible for the poor resistance of newborn infants to invasive *E. coli* K1 (7, 28, 29, 43).

Much of the data on the virulence of *E. coli* K1 strains is from experimental infections of newborn animals. The polysialic K1 capsule was found to inhibit antibody-independent serum bactericidal activity and opsonophagocytosis (8, 27, 44). Furthermore, it was found that antibody-independent activation of serum complement depends on the LPS type, which is responsible for the serum sensitivity and low virulence of O1:K1 strains in newborn rats (42, 44). Protection of newborn animals against invasive *E. coli* K1 was effected by the presence of *E. coli* LPS-specific serum antibodies (24, 43). Moreover, animals in experiments could also be protected by immunization with heterologous LPS and its derivatives and by stimulation of nonspecific immune mechanisms (39, 40, 46, 54, 55).

Besides LPS and capsule, fimbrial adhesins were investigated as virulence factors. Colonization of the gastrointestinal tract and translocation of bacteria to the mesenteric lymph nodes were found to be independent of the invasive capacity of *E. coli* strains (22, 45, 50). However, epidemiological and experimental data indicate that *E. coli* K1 expressing mannose-resistant (MR) adhesins might have an increased bacteremic potential (25, 49).

Epidemiological studies have shown that only some of the numerous *E. coli* O serogroups are associated with the K1 antigen and that many *E. coli* K1 strains could be attributed to a small number of clones (2, 3). However, *E. coli* K1 clones were not always uniform in regard to the virulence of strains and geographic differences in clonal predominance and disease were observed (1, 3, 26, 37, 51).

Compiled data from different studies have shown that *E. coli* K1 occurs at a frequency of 23% in the feces of healthy humans (23). In contrast, the incidence of *E. coli* meningitis in newborn infants is low (34, 38). The low incidence might be explained by the fact that certain host and bacterial factors interfere with the pathogenicity of *E. coli* K1 in newborns (34, 38, 42, 43). In this study, we have investigated the influence of some bacterial and host factors on the virulence of *E. coli* K1 strains using newborn rats of the inbred strain LEW and a set of congenic LEW strains differing at the major histocompatibility complex locus (*RT1*).

MATERIALS AND METHODS

Infants. Fecal samples from 209 infants, 124 boys (59.3%) and 85 girls (40.7%), living in different parts of Germany were examined for the presence of *E. coli* and other members of the family *Enterobacteriaceae*. The infants were between 0 and 24 months old and had no apparent signs of disease. Twenty-nine (13.9%) of the infants investigated were less than 1 month old.

Rat strains. The bacteremic potentials of *E. coli* K1 strains were investigated with the inbred rat strain LEW/Han for infection. Congenic LEW strains (*RT1*) LEW.1A, LEW.1AV1, LEW.1F, and LEW.1W (21) were used to study the influence of MHC (*RT1*) on susceptibility to invasive *E. coli* K1. All rats used in the experiments were hysterectomy derived and associated with an apathogenic,

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mostly anaerobic microflora that included the following genera: *Bacillus*, *Bacteroides*, *Bifidobacterium*, *Clostridium*, *Eubacterium*, *Fusobacterium*, *Lactobacillus*, *Peptococcus*, *Propionibacterium*, *Staphylococcus*, and *Veillonella*. The rats were free of any member of the family *Enterobacteriaceae*. Breeding was done in Trexler isolators with negative pressure. The isolators were run under strictly aseptic conditions to avoid any microbial contamination. Experiments have been performed in accordance with the national animal welfare legislation.

Bacteria. Fecal samples from infants were collected and propagated on Endo agar, L agar, and blood-agar plates for detection of members of the family *Enterobacteriaceae*. *E. coli* was identified by standard methods (13). K1-specific bacteriophages (provided by R. J. Gross, Public Health Laboratory Service, Division of Enteric Pathogens, London, United Kingdom) were used for the detection of *E. coli* K1 strains as previously described (17). Complete serotyping of *E. coli* was performed by I. Ørskov and F. Ørskov (Statens Seruminstitut, International Escherichia and Klebsiella Centre, Copenhagen, Denmark) by standard methods (35). Reference strains for the assignment to clones and for the determination of outer membrane protein (OMP) and LPS patterns of *E. coli* K1 (1, 2) were obtained from M. Achtman (Max-Planck-Institut für Molekulare Genetik, Berlin, Germany). *E. coli* K1 isolates that had serotypes and OMP patterns identical with those of the reference strains were assigned to the clonal groups of *E. coli* K1 as previously described (3, 26). Clinical isolates of *E. coli* K1 strains were from blood samples of human adults with bacteremia from the Rudolf-Virchow Hospital in Berlin. One of these strains was CB107 (O45:K1/9), which was used for the infectivity study with congenic LEW rats (*RT1*).

Determination of OMP and LPS patterns. Outer membranes of *E. coli* were isolated by detergent solubilization as previously described (2). The major OMPs were detected by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) by gel method 2 of Achtman et al. (2). OMP patterns of K1 isolates were compared with those of the reference strains used for detection of individual membrane patterns on SDS-polyacrylamide gels. LPS patterns of O2:K1 isolates were analyzed on gradient SDS-PAGE with sarcosyl-insoluble membrane preparations of strains and were visualized by silver nitrate staining (1, 26).

Hemagglutination tests and detection of fimbrial adhesins. Hemagglutination in the presence of 1% D-mannose (MRHA) was performed with bacteria grown on CFA agar (12) and with human, bovine, and sheep erythrocytes (12, 37). P fimbriae were detected by PF Test (Orion Diagnostika, Espoo, Finland) by agglutination of α-D-Gal-(1-4)-β-Gal-disaccharide-coated latex particles following the instructions of the supplier. Prs-specific adhesins were determined by agglutination of sheep erythrocytes (31). The presence of S fimbriae was measured by hemagglutination tests with untreated and neuramidase-treated human erythrocytes as previously described (20, 37).

Detection of aerobactin and hemolysins. Aerobactin production was detected by a bioassay described by Carbonetti and Williams (9) using *E. coli* K-12 indicator strain LG1522, which was kindly provided by P. H. Williams, University of Leicester, Leicester, United Kingdom. Hemolysin production was observed on blood-agar plates containing 5% washed sheep erythrocytes (5).

Quantitative measurement of bacterial growth in the presence of rat serum. Bactericidal activity of rat serum was determined by the method of Pluschke et al. (44). Pooled serum samples were obtained from LEW rats more than 3 weeks old with different microbial statuses. Two types of sera were used: LEW:As serum derived from rats associated with an anaerobic flora (see above) and LEW:Gf serum taken from germ-free LEW rats. Overnight cultures of bacteria were diluted 40-fold in L broth (44) and grown at 37°C under aeration to a titer of 4×10^8 CFU/ml. Bacteria were then diluted in prewarmed L broth containing 90% serum to a final titer of 4×10^5 CFU/ml and incubated for 3 h at 37°C. Viable-cell counts were determined before and after incubation with serum. Bactericidal activity was calculated as the percentage of viable cells from the initial inoculum found after incubation with serum for 3 h at 37°C.

Monoinfection of rats. A modified version of the method developed by Pluschke et al. (45) was used. Bacterial cultures in 100-ml Erlenmeyer flasks containing 50-ml portions of tryptone soy broth (Oxoid, Wesel, Germany) were grown from single colonies with aeration for 16 h at 37°C. For infection, bacteria were harvested by centrifugation, suspended in sterile phosphate-buffered saline (PBS), and adjusted photometrically to an optical density at 546 nm of 0.2. Rat pups between 5 to 7 days old were orally infected with 20-μl portions (approximately 4×10^6 CFU per animal) of the bacterial suspension. The pups were then returned to their mother in a sterile macrolon cage type III equipped with a filter hood. At 3 to 4 days after infection, the pups were anesthetized with ether. Bacteremia in rats was detected by plating 0.1 ml of heart blood sample on MacConkey agar (Oxoid). To measure colonization, 3-cm sections of rat intestine were dissected upstream from the rectum and homogenized in 1-ml portions of sterile PBS in a glass homogenizer (Braun, Melsungen, Germany). The number of bacteria (CFU) was determined by plating appropriate dilutions on MacConkey agar and then incubating for 24 h at 37°C. The colonization rate is indicated as the number of CFU per 3-cm section of intestinal tissue and contents.

Successive, mixed infections of rats. Successive, mixed infections of rats were done by inoculation of pups with a low-bacteremic K1 strain followed 2 days later by administration of a highly bacteremic K1 isolate. The four strains used in these infections were independently isolated from humans and could be distinguished by their lactose fermentation properties. Two different combinations of strains were applied as follows: (i) C1, O1:K1/5 Lac^+ together with O45:K1/9 Lac^-; and (ii) C2, O16:K1/12 Lac^- together with O45:K1/9 Lac^+. The pups (3 days old) were infected orally with the first strain and 2 days later, the second strain was administered. The inoculum concentrations for each animal was 4×10^6 CFU. The pups were sacrificed 3 days after the second strain had been administered, and the pups were treated as described above. Bacterial colonies were distinguished by their phenotypes for lactose utilization on MacConkey agar.

RESULTS

Frequency and characteristics of *E. coli* K1 strains isolated from healthy infants. Fecal samples of 209 healthy infants were examined for the presence of *E. coli* carrying the K1 capsule by testing their sensitivities of isolates to five K1-specific bacteriophages. K1-positive *E. coli* was detected in 44 (21.1%) of the 209 infants. The serotypes and OMP profiles of the K1 isolates were examined (1, 2). The LPS patterns of O2:K1 isolates were additionally analyzed (1). The results are summarized in Table 1. Of the 44 isolates, 36 (81.8%) could be assigned to 1 of 10 different clonal groups of

TABLE 1. Clonal groups, serotypes, and virulence markers of *E. coli* K1 isolates from infants

Serotype or OMP type	No. of isolates									
	Positive for aerobactin	Exhibiting MRHA	With MR adhesins				Exhibiting growth in LEW:As serum[a]			Total
			P	Prs	S	?[b]	S	P	R	
O1:K1:H$^-$/5	0	1	0	1	0	1	6	0	0	6
O1:K1:H7/9	6	6	3	3	0	3	6	0	0	6
O2:K1:H4/9[c]	3	3	1	0	0	2	1	2	0	3
O2:K1:H7/9[c]	0	3	1	0	0	2	3	0	0	3
O2:K1:H6/29[d]	0	0	0	0	0	0	1	0	0	1
O2:K1:H$^-$/*[e]	0	0	0	0	0	0	0	0	1	1
O7:K1:H$^-$/3	6	6	3	2	2	0	0	3	3	6
O12:K1:H$^-$/7	0	1	0	0	1	0	1	0	0	1
O16:K1:H$^-$/12	0	1	1	0	0	0	0	0	1	1
O18ac:K1:H7/9	4	1	0	0	0	1	0	2	4	6
O18ac:K1:H7/6	0	1	0	1	0	0	0	0	1	1
O45:K1:H7/9	0	1	1	0	0	0	1	0	0	1
O46:K1:H31/*[f]	0	0	0	0	0	0	0	1	0	1
O83:K1:H33/32	1	0	0	0	0	0	0	0	2	2
O117:K1:H$^-$/9	0	0	0	0	0	0	0	0	1	1
Rough LPS:K1:H/[g]	1	1	1	0	0	0	4	0	0	4

[a] S, fully serum sensitive (<1% surviving bacteria); P, partially serum resistant (2 to 80% surviving bacteria); R, fully serum resistant (>100% viable bacteria).
[b] MR hemagglutinins different from P, Prs, and S fimbriae.
[c] LPS type O2A (1).
[d] LPS type O2E (1).
[e] Unknown OMP (*) and LPS types (1, 2).
[f] Unknown OMP type (*).
[g] Strains with rough LPS exhibited heterogeneity in the H antigens and OMP types.

E. coli K1 strains (3, 37). Four isolates with O2:K1:H$^-$, O12:K1:H$^-$, O46:K1:H31, and O117:K1:H$^-$ serotypes could not be assigned to any of the known clonal groups. Four other isolates with rough LPS also could not assigned to a group.

Virulence factors of fecal *E. coli* K1 strains. The *E. coli* K1 isolates were examined for the presence of MR adhesins, resistance to bactericidal activity of serum, and production of hemolysin and aerobactin (Table 1). Of the 44 isolates, 25 (54.8%) were positive for MR hemagglutinins (MRHA$^+$). P fimbriae were detected in 11 strains in five clonal groups. The Prs fimbrial phenotype was present in seven strains belonging to four groups, and S fimbriae were found in three strains. Nine strains caused MRHA of human or bovine erythrocytes but were different from the P, Prs, or S fimbrial phenotypes. Aerobactin was produced by 21 (47.7%) of the 44 isolates. The production of aerobactin was highly associated with O1:K1/9 and O7:K1/3 strains. Only one strain (rough LPS:K1:H$^-$) was positive for α-hemolysin, and all other strains were hemolysin negative.

The 44 *E. coli* K1 strains from healthy infants were investigated for survival and growth in pooled samples of LEW:As and LEW:Gf rat serum. All fecal K1 strains except three rough LPS isolates grew well in 90% LEW:Gf serum (data not shown). In contrast, only 13 (29.5%) of the K1 isolates from healthy infants grew in 90% LEW:As serum. Resistance to LEW:As serum was mainly associated with the O7:K1/3, O18:K1/9, and O83:K1/32 groups.

Within a clonal group of strains, no differences in the serum response were observed between fecal K1 strains from healthy infants, reference K1 strains, and those strains of clinical origin.

Intestinal colonization of rats. The influence of MR adhesins on colonization of a rat pup's distal intestine was investigated with 30 *E. coli* K1 isolates of different origin belonging to 15 different clonal types (Table 2). Highly significant differences ($P < 0.001$ by Student's t test) for colonization of rat intestine were found between MR adhesin-negative strains and all groups of MR adhesin-positive strains, except those expressing S fimbriae (Table 2). By comparing groups of strains expressing MR adhesins, P-fimbriated strains were significantly more efficient in colonization ($P < 0.01$) than S-fimbriated strains or those expressing undetermined MR hemagglutinins ($P < 0.05$). A possible

TABLE 2. MR adhesins and colonization of LEW rat intestine by *E. coli* K1

MR adhesin type	No. of isolates/ no. of clonal types	Mean colonization rate (10^8 CFU/3-cm ITC[a]) ± SD (n)
None	9/8[c]	2.2 ± 1.6 (74)
P	5/4[d]	4.4 ± 3.0 (53)
Prs[b]	4/3[e]	3.7 ± 2.0 (28)
S	3/3[f]	2.3 ± 1.6 (27)
?	9/3[g]	3.3 ± 2.4 (97)

[a] ITC, intestinal tissue and contents (see Materials and Methods).
[b] Two of these strains also expressed P-type fimbriae.
[c] O1:K1/5 (one isolate), O2:K1/29 (one isolate), O2:K1/* (one isolate), O83:K1/32 (two isolates), O46:K1/* (one isolate), O117:K1/9 (one isolate), O75:K1/11 (one isolate), and rough LPS:K1/9 (one isolate).
[d] O1:K1/9 (one isolate), O2:K1/9 (two isolates), O45:K1/9 (one isolate), and O16:K1/12 (one isolate).
[e] O1:K1/9 (one isolate), O18:K1/6 (two isolates), and O7:K1/3 (one isolate).
[f] O18:K1/9 (one isolate), O7:K1/3 (one isolate), and O12:K1/7 (one isolate).
[g] O2:K1/9 (seven isolates), O45:K1/9 (one isolate), and O18:K1/9 (one isolate).

TABLE 3. Aerobactin, MR adhesins, and invasiveness

No. of isolates/no. of clones	Expression of:		No. of pups with the following no. of bacteria/0.1 ml of blood:				% of rat pups	
	Aerobactin	MR adhesins	0	1–10	11–100	>100	With bacteremia	Without bacteremia
8/8[a]	–	–	94	5	2	0	6.9	93.1
12/6[b]	–	+	153	15	18	5	19.9	80.1
1/1[c]	+	–	10	1	0	0	9.1	90.9
9/3[d]	+	+	90	10	9	1	18.2	81.8

[a] O1:K1/5 (one isolate), O2:K1/29 (one isolate), O2:K1/* (one isolate), O83:K1/32 (one isolate), O46:K1/* (one isolate), O117:K1/9 (one isolate), O75:K1/11 (one isolate), and rough LPS:K1/9 (one isolate).
[b] O2:K1/9 (four isolates), O12:K1/7 (one isolate), O16:K1/12 (one isolate), and O18:K1/9 (three isolates), O18:K1/6 (one isolate), and O45:K1/9 (two isolates).
[c] O83:K1/32 (one isolate).
[d] O1:K1/9 (one isolate), O2:K1/9 (six isolates), and O7:K1/3 (two isolates).

effect of aerobactin production alone on colonization could not be measured, since there was only one isolate which was positive for aerobactin and negative for MR adhesins. The strains expressing aerobactin and MR adhesins or only MR adhesins were significantly better colonizing the rats ($P < 0.001$ and 0.01, respectively, by Student's t test) than the strains which were negative for both virulence markers (data not shown). No significant differences in colonization rates were found between strains which were negative for aerobactin and positive for MR adhesins and those which were positive for both (data not shown).

Invasiveness of K1 strains for LEW rats. Invasiveness was measured by the rate and degree of bacteremia in LEW baby rats. The groups of strains which were investigated for colonization were further investigated for their invasiveness in rats (Table 3). Strains with no MR adhesins and aerobactin were significantly less invasive than those which were only MR adhesin positive ($P < 0.01$ by Chi-square test) or those which were positive for both aerobactin and MR adhesins ($P < 0.05$).

The influence of serum resistance on invasiveness in neonatal rats was tested with 32 different *E. coli* K1 isolates. On the basis of survival and growth of bacteria in 90% LEW:As serum, three groups of isolates were established (Table 4). No significant differences in the rate and degree of bacteremia in newborn LEW rats was found (chi-square test).

The association of clonal types with invasiveness was not measured, since only a few isolates for each group or serotype were investigated. Within a given group of isolates, no differences in invasiveness were found between the fecal isolates from healthy infants and isolates from feces, blood, or cerebrospinal fluid samples from diseased human patients. Chi-square analysis on results (bacteremic versus nonbacteremic pups) obtained with five clusters of isolates (O1, O2, O7, O18, and O45) showed significant differences in the ability to induce bacteremia between O1 and O45 strains ($P < 0.01$). Weak significant differences were found between the O1 and O7 groups and the O2 and O45 groups ($P < 0.05$). All other correlations were not statistically significant.

Infectivity study with congenic LEW rats. The possible influence of the MHC on the bacteremic effect of the virulent strain CB107 (O45:K1/9) was investigated with four different congenic rat strains with LEW background but with different MHC loci (*RT1*). These results are shown in Table 5.

TABLE 4. Association of resistance to serum with invasiveness of different *E. coli* K1 isolates in LEW rats

Growth in LEW: As serum[a]	No. of isolates/no. of clonal types	No. of pups with the following no. of bacteria/0.1 ml of blood:				No. of LEW rat pups (%)	
		0	1–10	11–100	>100	With bacteremia	Without bacteremia
S	18/9[c]	239	22	22	5	49 (17.1)	239 (82.9)
P	5/4[d]	70	8	5	3	16 (18.6)	70 (81.4)
R	9/7[e]	86	7	7	1	15 (14.8)	86 (85.2)

[a] S, fully serum sensitive (<1% surviving bacteria); P, partially serum resistant (2 to 80% surviving bacteria); R, fully serum resistant (>100% viable bacteria).
[c] O1:K1/5 (one isolate), O1:K1/9 (two isolates), O1:K1/* (one isolate), O2:K1/9 (seven isolates), O2:K1/29 (one isolate), O12:K1/7 (one isolate), O45:K1/9 (three isolates), rough LPS:K1/* (one isolate), and O75:K1/11 (one isolate).
[d] O45:K1/9 (one isolate), O46:K1/* (one isolate), O2:K1/9 (two isolates), and O7:K1/3 (one isolate).
[e] O2:K1/* (one isolate), O7:K1/3 (one isolate), O16:K1/12 (one isolate), O18:K1/9 (one isolate), O18:K1/6 (two isolates), O83:K1/32 (two isolates), and O117:K1/9 (one isolate).

Although there were no significant differences in the colonization of animals (Student's t test), significant differences ($P < 0.001$ by Chi-square test) were found for the number of bacteremic pups when the progenitor strain (LEW) and congenic strains (*RT1*) (results of all four rat strains) were compared.

Successive, mixed infections of LEW rats. The effects of successive oral infection with two different *E. coli* K1 strains on colonization of rat intestine and bacteremia were tested with two pairs of strains. In both experiments, low-bacteremic strains were administered to rats first followed 2 days later by the administration of highly bacteremic O45:K1/9 strains. These results are presented in Table 6. With the C1 combination, both strains exhibited reductions in colonization rates similar to those of monoinfections. The resident O1:K1/5 strain did not specifically impair the subsequent colonization of rats by the P-fimbriated O45:K1/9 isolate. Both strains did not have significantly altered (Chi-square test) invasion rates when the results of monoinfections and mixed infections of rats were compared (Table 6). The two strains used in the C2 combination both expressed MR hemagglutinins. With this combination, the P-fimbriated O16:K1/12 strain which was given first had a significant ($P < 0.001$ by Student's t test) advantage in the colonization of rat intestine over the O45:K1/9 strain given later. Compared

TABLE 5. Intestinal colonization and bacteremia caused by *E. coli* CB107 (O45:K1:H7) in congenic LEW rats with different MHC loci

Rat strain (*RT1* haplotype[a])	No. of rat pups investigated	No. of pups with the following no. of bacteria/0.1 ml of blood:				Mean colonization rate (10^8 CFU/3 cm of ITC[b]) ± SD
		0	1–10	11–100	>100	
LEW (*l*)	27	13	5	8	1	2.1 ± 0.9
LEW.1A (*a*)	8	8	0	0	0	2.2 ± 1.1
LEW.1AV1 (*av1*)	11	11	0	0	0	2.3 ± 0.4
LEW.1F (*f*)	17	16	1	0	0	2.9 ± 1.2
LEW.1W (*u*)	23	20	2	0	1	2.3 ± 0.8

[a] Haplotypes from reference 21.
[b] ITC, intestinal tissue and contents (see Materials and Methods).

TABLE 6. Intestinal colonization and bacteremia in LEW rats infected with different *E. coli* K1 strains

Strain combination[a]	MR adhesin	Mean colonization rate (CFU/3 cm of ITC[b]) ± SD		No. of bacteremic pups/nonbacteremic pups in:	
		Mixed infection	Monoinfection	Mixed infection	Monoinfection
C1					
O1:K1/5	None	$(3.8 \pm 3.8) \times 10^7$	$(8.0 \pm 4.0) \times 10^7$	1/45	1/16
O45:K1/9	P type	$(1.6 \pm 0.9) \times 10^8$	$(5.4 \pm 3.6) \times 10^8$	4/42	9/30
C2					
O16:K1/12	P type	$(1.6 \pm 0.9) \times 10^8$	$(2.3 \pm 1.0) \times 10^8$	9/32	1/9
O45:K1/9	?[c]	$(3.3 \pm 3.9) \times 10^7$	$(1.4 \pm 1.3) \times 10^8$	1/40	14/13

[a] For both combinations (C1 and C2), the strains are listed in the order of application.
[b] ITC, intestinal tissue and contents (see Materials and Methods).
[c] MR hemagglutinin different from P-, Prs-, and S-type fimbriae.

with the rates of monoinfections, the colonization rate of the O16:K1/12 strain was not significantly altered, whereas that of the O45:K1/9 strain was significantly reduced ($P < 0.001$ by Student's t test). In accordance with this finding, the number of pups with bacteremia caused by the O45:K1/9 isolate was significantly lower ($P < 0.001$ by Chi-square test) in a mixed infection with combination C2 than in a monoinfection with the same strain.

DISCUSSION

Epidemiological studies have shown that many *E. coli* K1 strains isolated from different sources and geographic locations belong to a small number of genetic clones (2, 3, 37, 51). Some of these genetic clones were associated with particular virulence in human urinary tract infection and others were associated with neonatal bacteremia and meningitis (3, 32, 47). Studies with laboratory animals have shown that the clonal type is not always in conformity with the virulence of strains and virulence markers of strains belonging to the same clonal type are often diverse (1, 3, 37, 45, 51; also this study).

Many virulence studies in animals were done with *E. coli* K1 strains isolated from diseased humans and it was suggested that the source of a strain could have an influence on its virulence (2, 45). In this study, strains from the feces of healthy infants were similar to clinical isolates in virulence to animals and other phenotypical traits, supporting previous findings that the source of a strain is not the decisive factor causing virulence (15, 48).

It was originally thought that the number of *E. coli* K1 clones was very limited (2, 11, 42). However, more clonal types and new clones of virulent *E. coli* K1 have recently been described (2, 3, 37). It appears possible that the human fecal flora which is an important reservoir of *E. coli* K1 (23) serves as a source for hitherto unknown clonal types of K1 strains. Our finding that 4 of the 44 *E. coli* K1 isolates from feces did not belong to one of the clonal groups described so far points to that possibility.

Different studies have shown that the immune response to bacterial surface antigens is the main determinant of host resistance to invasive *E. coli* K1. LPS, K1 capsule, and fimbrial antigens proved to be protective when used as vaccines in experimental infections of laboratory animals (19, 24, 46, 55). Activation of serum complement was found to be dependent on the LPS type when O1:K1, O7:K1, and O18:K1 strains were compared (45), and it was suggested that the virulence of K1 strains correlates with the O serotype and resistance to the bactericidal activity of serum complement (42, 43). However, in O2:K1 strains, the O serotype could not be associated with induction of bacteremia in rats (1). Only some of the O2:K1 strains caused bacteremia, and no association between phenotypical traits, clonal type, and virulence was found. Originally, the O2:K1 strains were not analyzed for serum resistance (1). In this work, we were interested in studying these isolates and other *E. coli* K1 isolates for the association of serum resistance with induction of bacteremia in newborn rats. The sera we used were from rats free of members of the family *Enterobacteriaceae* in order to minimize activation of complement activity by the presence of *E. coli* surface-specific antibodies (43, 46). With these sera used for testing bactericidal activity, killing of *E. coli* should be due to only nonspecific activation of complement, as described for O1:K1 strains (39, 40, 42). Our finding that almost all *E. coli* K1 strains grew well in LEW:Gf serum might be explained by the reduced complement hemolytic activity of LEW:Gf compared with LEW:As serum (data not shown). The finding that O7:K1 and O18:K1 strains were resistant to LEW:As serum and O1:K1 strains were sensitive is in accordance with previously published results (42). Interestingly, the ability of *E. coli* K1 strains to induce bacteremia in neonatal rats was not limited to LEW:As serum-resistant strains like O7:K1 and O18:K1 but was also found with O2:K1 and O45:K1 isolates, which are highly sensitive to serum. This observation might be explained by recent findings showing that neonatal sera from humans and rats are inefficient for bacteriolysis because of complement factor deficiencies (27–29, 43). Our finding that LEW:As serum-sensitive *E. coli* K1 strains induce bacteremia in newborn rats fits epidemiological data from human infections. Serum-sensitive strains are not rare among *E. coli* K1 isolated from blood and cerebrospinal fluid samples from diseased humans (33, 41). Among these strains, rough LPS:K1 and O1:K1 types which are relatively avirulent in rats were frequent (11, 25, 26, 34, 45, 48).

Epidemiological data and virulence studies in animals indicate that host factors play a major role in neonatal infections with *E. coli* K1. The host's immune status and predisposing factors are of major importance in the host's susceptibility to infection (7, 15, 28, 29, 34, 38). To standardize genetically dependent host factors, this study was conducted with the inbred rat strain LEW and not with commonly used outbred strains like Wistar or Sprague-Dawley. Although the breeding of the LEW rat is complicated, since approximately 30% of all matings are sterile (16), this inbred strain is helpful to study the influence of certain genes on

disease processes (21). Many congenic strains, like MHC (*RT1*), non-MHC, and pathophysiological mutants are present in rats with LEW host background (21). In this study, we have investigated the influence of MHC (*RT1*) on the susceptibility of LEW rats to the virulent O45:K1:H7 strain CB107. These results indicate that the *RT1* haplotype *l* might render the LEW rat more susceptible to bacteremia with *E. coli* K1 than the other haplotypes tested. Since only a few gnotobiotic litters were available for investigation, more experiments are needed.

Colonization of the host intestine is the first step in neonatal infections by invasive *E. coli* K1. Invasive and noninvasive *E. coli* strains colonized the alimentary tracts of newborn experimental animals equally well (22, 50). The invasive potential of *E. coli* K1 was found to be independent of the presence of pili, which serve as colonization factors (6, 18, 49). However, invasion rates could be associated with the intestinal concentration of bacteria and might thus depend on bacterial colonization capacity (10, 14, 52). In this study, it was shown that the expression of some MR adhesins significantly contributes to colonization and invasiveness of *E. coli* K1. Among strains expressing MR adhesins, P-fimbriated types were most efficient for colonization. A possible specific advantage of strains expressing P fimbriae for colonization affecting invasion rates was also detectable in mixed infections of animals. Because our virulence studies were performed with nonisogenic *E. coli* K1 wild-type strains, our studies need to be confirmed by further colonization and invasiveness tests using isogenic fimbriated and nonfimbriated mutant strains.

It was reported previously that the presence of genes specific for aerobactin and for P, Prs, and S fimbriae are more common than the expression of their phenotypes in *E. coli* (37). In this study, we had examined only the expression of phenotypes corresponding to these virulence markers, not the presence of the genes. Therefore, it is possible that more strains than indicated carry DNA sequences specific for P, Prs, or S fimbriae or for aerobactin.

It has been shown that colonization of the gut and translocation of bacteria to the mesenteric lymph nodes vary widely, depending on the resident bacterial flora of the host (4, 45, 52). In order to prevent unspecific effects resulting from interference between *E. coli* K1 and the resident *E. coli* flora of the laboratory animals, we conducted our study with gnotobiotic rats carrying a defined gut flora free of members of the family *Enterobacteriaceae*. Our experience in monitoring the health of laboratory animals has shown that conventional and also specific-pathogen-free rats commonly harbor members of the family *Enterobacteriaceae*, mostly *E. coli*, in their intestine. In a similar way, interactions between bacteria might also play a role in neonatal infections with *E. coli* K1. In feces from healthy infants, *E. coli* K1 is frequently accompanied by other types of *E. coli* or members of the family *Enterobacteriaceae* (data not shown) which might limit their growth by bacterial interference (36; also this study). In contrast, monoinfections of newborn infants with *E. coli* K1 might result in an increased risk for invasion caused by bacterial overgrowth.

Epidemiological data and virulence studies have shown that *E. coli* K1 strains are generally able to invade their host. However, these strains have different pathogenic potentials. In addition to host factors, interference between bacteria might be of major influence on the risk of infection.

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