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Escherichia coli K5 Capsule Expression Enhances Colonization of the Large Intestine in the Gnotobiotic Rat

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The role of capsule expression in the capacity of *Escherichia coli* to colonize in the large intestinal environment was studied in a gnotobiotic rat model. The rats were given perorally a mixture of two mutant strains differing in K5 expression. After 2 weeks, the rats were sacrificed, and subsequently intestinal contents, intestinal mucosae, and mesenteric lymph nodes were homogenized and bacterial numbers were quantified. Two *E. coli* mutant pairs were used, the first pair (972-998) lacking the O-specific side chain and the second pair (973-997) carrying the O75 lipopolysaccharide. The $K5^+$ mutants established themselves at a higher level than the $K5^-$ mutants (10^9 versus 10^6 CFU/g [$P < 0.001$] for the first pair and 10^9 versus 10^8 CFU/g [$P < 0.01$] for the second pair, respectively). The results were confirmed by serology showing a $K5^+$ phenotype for practically all isolates. The bacterial population associated with the mucosa was similar to that in the luminal contents with respect to the proportions of the respective mutants, and translocation occurred in numbers proportional to the intestinal population densities of the respective mutants. All mutants were able to express type 1 as well as P fimbriae. After colonization, the expression of P fimbriae remained high whereas only a minority of the isolates expressed type 1 fimbriae. The results suggest that capsule expression and P fimbriae enhance intestinal colonization by *E. coli* and that these virulence factors, by increasing bacterial densities in the intestine, secondarily increase translocation.

Encapsulated bacteria are often associated with invasive or otherwise serious diseases (28, 34). Capsules increase bacterial virulence by inhibiting the opsonizing activities of complement, thereby decreasing the elimination by phagocytes, or escape the immune response by mimicking host molecules (3, 6, 9). The *Escherichia coli* K1 and K5 capsules are frequently found among isolates causing extraintestinal infections, K1 being more commonly associated with neonatal septicemia and K5 being more frequently associated with sepsis and urinary tract infection (9, 19, 34, 35).

E. coli strains causing extraintestinal disease derive from the normal intestinal microbiota (29, 35). This bacterial population consists of both resident strains, which persist for months and years, and transient strains, which are unable to become permanently established in the large intestine (36–38). We have recently shown in a gnotobiotic rat model that P fimbriae, which are a virulence factor for urinary tract infection, facilitate intestinal colonization by *E. coli* (17). This is in accordance with epidemiological studies which show a clear association between P fimbriae and intestinal persistence (2, 44, 49) and may suggest that the role of certain virulence factors is to enable long-term persistence in the colon and that virulence may be coincidental (11, 42, 49).

E. coli and other intestinal commensal bacteria may cause disease by spreading from the intestinal environment and colonizing the periurethral area (22) or by directly translocating over the intestinal mucosa (4). Although translocation may be a normal physiological process necessary for regulating immunity to gut bacteria (46), the bacteria may also reach the bloodstream and cause sepsis and meningitis (23). This process is more likely to occur in immunocompromised patients or in the very young than in persons with intact defense mechanisms (35, 43). Bacterial factors governing the capacity to translocate have not been defined.

In the above-mentioned gnotobiotic rat model, we found that apart from P fimbriae, other bacterial traits linked to uropathogenicity were important for intestinal colonization by *E. coli*. Thus, mutant strains derived from a fecal isolate of serotype O19,22:K1:H^- colonized at much lower levels than mutants derived from a pyelonephritogenic isolate of serotype O75:K5:H^- (17). In the present study, the importance of the K5 capsule for intestinal colonization of gnotobiotic rats and for translocation to the mesenteric lymph nodes was investigated by using isogenic mutant strains derived from *E. coli* GR-12 differing in K5 expression.

MATERIALS AND METHODS

Rats. Male and female rats of the AGUS strain were reared in isolators under germfree conditions (16). Prior to the experiment, the germfree status of the rats and isolators was checked as reported before (17). The rats were 8 to 10 months old when used for colonization experiments.

Bacteria. All the *E. coli* strains used in the study were derived from the wild-type pyelonephritogenic isolate GR-12 of serotype O75:K5:H^- (41). This strain expresses both type 1 and P fimbriae and has the capacity for phase variation; i.e., it can switch its fimbrial expression on or off depending on the environmental conditions. The production of mutants differing from this strain in O and K expression has been previously described (40). Briefly, mutations were induced by nitrous acid treatment, with two mutants being obtained, 748 and 734. The 748 mutant is a nonreverting histidine auxotroph identified after ampicillin–D-cycloserine enrichment. A spontaneous nalidixic acid-resistant derivative was selected and mated with an *E. coli* K-12 Hfr donor; coinheritance of the *rfb* locus was tested using anti-O75 serum. Two mutants were selected, one of which (972) expressed the $O75^-$ $K5^+$ phenotype. The other mutant, of the $O75^+$ $K5^+$ phenotype, was plated on agar containing rifampin, and a spontaneous rifampin-resistant mutant, named 973, was selected. K5-deficient mutants (998 and 997) were obtained by selecting spontaneously resistant colonies in the presence of K5-specific bacteriophages. The K5-deficient strain 998 ($O75^-$ $K5^-$) originated from a derivative of 972 which was also selected for rifampin resistance. Strain 997 ($O75^+$ $K5^-$) was a derivative of strain 734 (the other mutant obtained after nitrous acid treatment). It was lactose negative and was selected for spontaneous rifampin resistance (15, 40).

The mutants were cultivated as described in Table 1. Before colonization of

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TABLE 1. Some characteristics of the strains used in this study

Strain	Serotype	Fimbriae	Hemagglutination pattern[a]	Growth medium[b]	Reference
972	$O75^-$ $K5^+$	Type 1 and P	MRHA, MSHA	TSA	40
998	$O75^-$ $K5^-$	Type 1 and P	MRHA, MSHA	TSA-rifampin (200 μg/ml)	40
973	$O75^+$ $K5^+$	Type 1 and P	MRHA, MSHA	TSA-nalidixic acid (50 μg/ml)	40
997	$O75^+$ $K5^-$	Type 1 and P	MRHA, MSHA	TSA	40

[a] MRHA, mannose-resistant hemagglutination; MSHA, mannose-susceptible hemagglutination.
[b] Before colonization, the bacteria were passaged three times in static Luria broth with 0.1% $CaCl_2$ in order to favor the expression of type 1 fimbriae.

the rats, however, all strains were cultivated three times in static Luria broth with 0.1% $CaCl_2$ to maximize the expression of type 1 fimbriae (12).

Bacterial colonization. Two colonization experiments were carried out. One experiment was performed with a pair of mutant strains lacking the O75-specific side chain, 972 ($O75^-$ $K5^+$) and 998 ($O75^-$ $K5^-$). The other experiment was performed with a pair of mutant strains having a complete lipopolysaccharide: 973 ($O75^+$ $K5^+$) and 997 ($O75^+$ $K5^-$). Bacteria from an overnight Luria broth culture were harvested and adjusted to a concentration of approximately 10^8/ml. The strains were mixed in equivalent amounts and poured into 10-ml glass ampoules which were sterilized on the outside with chromosulfuric acid and transferred into the rats' isolators. One milliliter of the bacterial suspension was given perorally with a syringe to each rat, and some drops were also spread onto the fur. The animals were kept within the isolators for the whole course of the experiment. To determine the actual concentration of each strain in the mixture given, viable counts were performed on the proper antibiotic-containing agar plates. Selected colonies from unsubstituted tryptic soy agar (TSA) plates were also tested for O75 and K5 expression as well as for their hemagglutination patterns (see below).

Determination of bacterial numbers in the intestinal lumen and in association with the gut wall. After 11 to 15 days of colonization, the rats were anesthetized with sodium pentobarbital (60 mg/kg of body weight; Apoteksbolaget, Umeå, Sweden) and exsanguinated by heart puncture. A sample from the large intestinal contents was obtained with a calibrated loop. Serial dilutions were performed in sterile saline and distributed on agar plates appropriate for each bacterial strain (Table 1). After overnight culture, the number of colonies was counted and expressed as CFU per gram of gut contents.

To assess the composition of the bacterial population closely associated with the mucosal surface, pieces of large intestine were excised, rinsed, and homogenized. The homogenate was diluted and cultivated on agar plates as described above. The ratio of the bacterial strains in this fraction was calculated and compared with the ratio of the same strains in the luminal contents.

To determine bacterial translocation, the whole mesenteric lymph node chain of each rat was aseptically removed and homogenized. The homogenate of the individual rat's lymph nodes was plated on agar after serial dilution in phosphate-buffered saline (PBS). The results were expressed as the number of CFU recovered from the mesenteric lymph nodes for each rat.

K5 typing. K5 was identified by using a K5-specific bacteriophage (15), which only infects $K5^+$ isolates of *E. coli*. The analyses were performed as previously described (19). Each of the colonies selected was spread onto an agar plate containing 1% nutrient agar and 1% glucose. A drop of a phage suspension consisting of 4×10^7 PFU/ml was added, and the plates were incubated at 37°C overnight. A clear zone where the drop was deposited was indicative of the presence of the $K5^+$ phenotype in the bacteria.

O75 typing. The same colonies selected for K5 typing were also tested for O75 expression (25, 26). Each colony was suspended in nutrient broth and cultivated at 37°C overnight. The next day, the tubes were autoclaved at 120°C for 30 min, and 0.05 ml of each bacterial suspension was mixed with an anti-*E. coli* O75 rabbit antiserum (18) in 96-well plates (flat-bottomed Microwell; Nunc, Roskilde, Denmark) and incubated for 18 h at 50°C. Bacterial agglutination was read in the microscope at a magnification of ×10 to ×16 and indicated the presence of the O75 antigen.

Hemagglutination pattern. The hemagglutination pattern was determined for colonies obtained from the original mixture given to the rats, as well as for those obtained from the rats after colonization. The following erythrocyte suspensions (3% [vol/vol]) were made: human erythrocytes in PBS, human erythrocytes in PBS with 2.5% methyl-α-D-mannoside, guinea pig erythrocytes in PBS, and guinea pig erythrocytes in PBS with 2.5% methyl-α-D-mannoside. A drop of each of these suspensions was mixed on a microscope slide with a loopful of bacteria from each colony selected. Hemagglutination was read by the naked eye after gentle tilting of the slide for 1 to 3 min. Agglutination of guinea pig erythrocytes in the absence, but not in the presence, of methyl-α-D-mannoside was defined as mannose-sensitive hemagglutination (MSHA). Agglutination of human erythrocytes both in the presence and in the absence of mannose was defined as mannose-resistant hemagglutination (MRHA). Bacteria that did not agglutinate either of the erythrocyte species used were denoted as hemagglutination negative.

Growth curves. The four strains were grown on TSA plates overnight at 37°C, and a loopful of each strain was subcultured in nutrient broth for 2 h at 37°C. Each bacterial suspension was homogenized, and 2 ml of each homogenate was distributed into triplicate tubes which were incubated aerobically at 37°C with shaking. The optical density at 597 nm was measured with a Vitatron (Hugo Tillqvist, Göteborg, Sweden) every 15 to 20 min for 11 h, and a mean value was calculated from the readings of the three tubes for each time point and strain. A regression line was drawn (Macintosh Stat View), and a generation time was calculated according to the following formula:

$$\frac{\log N_i - \log N_o}{t\ (\log 2)}$$

where N_o represents the original population of bacteria and N_i is the bacterial population at time t (33).

Susceptibility to lysozyme. The resistance of the different mutant strains to the activity of lysozyme was tested by growing the bacteria in nutrient broth (pH 7) in the presence of 0, 1, 10, 25, 50, 100, and 200 μg of lysozyme (from human milk; 100,000 U/mg of protein; Sigma Chemical, St. Louis, Mo.) per ml (8). Growth was monitored by measuring the optical density at 597 nm and by performing viable counts 3 and 24 h after the addition of the enzyme.

Mucus adherence. The technique was modified from that of Cohen et al. (7). To obtain a crude mucus preparation, two conventional AGUS rats were given 5 g of streptomycin sulfate (Heyl Chemisch-Pharmazeutischen Fabrik, Berlin, Germany) per liter and 0.5 g of ampicillin (Doktacillin; Astra Läkemedel, Södertälje, Sweden) per liter in their drinking water to reduce facultative bacterial population densities. The next day, the rats were sacrificed and the small intestines were excised, placed in ice-cold PBS, cleared of feces, and opened longitudinally. The mucosa was scraped gently with a plastic loop, and the scrapings were suspended in a minimum amount of ice-cold Hanks' balanced salt solution with 0.01 M HEPES, pH 7.4. The mixture was centrifuged at 13,000 × *g* for 30 min at 4°C. After determination of its protein content by the Bio-Rad protein assay (Bio-Rad, Hercules, Calif.), the supernatant was kept frozen until used.

The wells of tissue culture plates (24-well flat bottomed; Nunc) were coated overnight at 4°C with 200 μl of rat mucus (0.3 mg of protein per ml) or commercial mucin (0.5 mg of protein per ml; type III partially purified mucin from porcine stomach; Sigma Chemical) per well or with a solution containing 20 mg of bovine serum albumin per ml to assess nonspecific binding (5, 24). The plates were washed twice with HEPES-Hanks' buffer, pH 7.4.

Bacteria were cultivated on TSA plates overnight at 37°C, and a loopful was suspended in 2 ml of Luria-Bertani broth into which 3 μl of [^{35}S]methionine (20 μCi/ml; Amersham Life Science, Solna, Sweden) was added. Bacteria were incubated at 37°C for 18 to 24 h, washed twice with PBS, and diluted to a concentration of 10^8 bacteria/ml. One hundred microliters of radiolabelled bacteria was added per well, in triplicate, and incubated for 1 h at 37°C. After being washed twice with HEPES-Hanks' buffer, pH 7.4, adherent bacteria were lysed by treatment with 500 μl of 5% (wt/vol) sodium dodecyl sulfate in distilled water for 1 to 2 h at 60°C. The released radioactivity was detected with a beta scintillation counter (Packard Tri-Carb; Ultima Gold scintillation fluid; Packard Instrument B.V.). The fraction of bacteria binding to mucus was calculated by subtracting the radioactivity bound to bovine serum albumin-coated wells from the radioactivity bound to mucin-coated wells and dividing this value by the total radioactivity added.

Statistical analysis. The differences in bacterial numbers for each mutant strain were evaluated by the Student *t* test. Translocation data were analyzed by Mann-Whitney's nonparametric test.

RESULTS

Bacterial numbers in luminal contents. The first group of four rats were colonized with a mixture of mutants 972 (O^- $K5^+$) and 998 (O^- K^-). The viable counts performed on the initial mixture showed that the inoculum contained 6.8×10^8 972 cells and 4.4×10^8 998 cells. The rats were sacrificed on day 11 or 12. In the intestinal contents, the $K5^+$ mutant reached numbers that were 3.8 log units higher than those of the $K5^-$ mutant, as determined by growth on antibiotic-con-

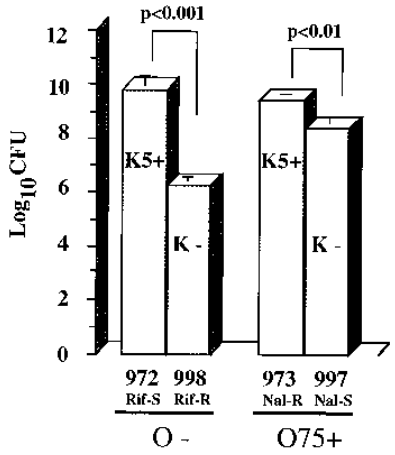

FIG. 1. Bacterial density ($\log_{10}$ CFU per gram) in large intestinal contents after 11 to 15 days of colonization in mutants differing in K5 expression. In the first colonization group, both mutants were defective in O75 expression (O−), whereas in the second colonization group, both mutants expressed the O75 phenotype (O75+). The identities of the strains were determined by cultivation on antibiotic-containing media (rifampin for distinguishing strain 998, which is rifampin-resistant [Rif-R], from strain 972, which is rifampin sensitive [Rif-S], and nalidixic acid for distinguishing strain 973, which is nalidixic acid sensitive [Nal-S], from strain 997, which is nalidixic acid resistant [Nal-R]). The bars represent the means for the four rats in each group, and the error bars denote the standard deviations.

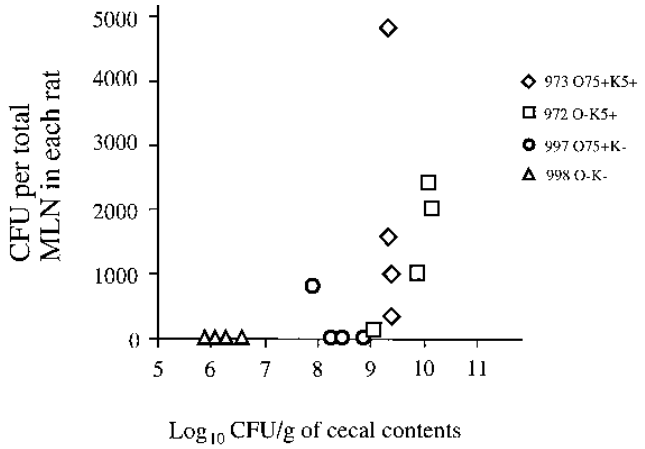

FIG. 2. Relationship between numbers of bacteria translocated to mesenteric lymph nodes (MLN) and the number of bacteria in intestinal contents in individual rats. Each symbol denotes the density of one *E. coli* mutant in one individual rat. The identity of the strains was determined by cultivation on antibiotic-containing media. *E. coli* 972 (rifampin sensitive) and 998 (rifampin resistant) were distinguished by growth on rifampin-TSA plates. Strains 973 (nalidixic acid sensitive) and 997 (nalidixic acid resistant) were distinguished by growth on nalidixic acid-containing TSA.

taining agar plates ($P < 0.001$) (Fig. 1). The results were confirmed by O and K antigen typing of 160 colonies obtained from the cultivation of intestinal contents on unsubstituted TSA plates, which permits the growth of both mutant strains. All colonies analyzed expressed K5, and none was specifically agglutinated by an anti-O75 antiserum.

The second group of four rats were given a mixture of 4.4×10^7 CFU of 973 ($O75^+$ $K5^+$) and 4.4×10^8 CFU of 997 ($O75^+$ K^-). In this case as well, there was a higher level of establishment of the strain which expressed the K5 capsule than of the $K5^-$ mutant, the difference being 1.3 log units ($P < 0.01$) (Fig. 1). Serology performed on 70 colonies from the unsubstituted TSA plates confirmed the results; 93% of the colonies tested expressed the K5 antigen, while five (7%) did not. All colonies expressed O75.

Relationship between colonization in the lumen and in the epithelium. To determine if the bacterial population present in association with the gut mucosa differed from that of the luminal contents, viable counts were performed on homogenates of the intestinal wall. The ratio between each pair of mutant strains in the gut wall was similar to the corresponding ratio in the lumen (data not shown).

Translocation. Translocation was measured as the number of viable bacteria obtained from homogenates of the mesenteric lymph nodes. In the colonization with the pair lacking the O75 side chain, strain 972 ($O75^-$ $K5^+$), which colonized at high levels, translocated in all four rats, while 998 ($O75^-$ $K5^-$), which colonized at low levels, did not translocate in any of the four rats. In accordance, all colonies recovered from mesenteric lymph nodes expressed K5. In the colonization with the $O75^+$ pair, strain 973 ($O75^+$ $K5^+$), which colonized at a higher density in the intestine, translocated in all four rats while 997 ($O75^+$ K^-) translocated in only one of the four rats.

When the number of translocating bacteria was related to the level of the particular strain in the intestinal contents, translocation was seen to occur above a level of 10^7 CFU/g in intestinal contents and increased thereafter, regardless of strain characteristics (Fig. 2). This indicated that the differences in translocation between the strains was a function of the differences in their levels in the intestinal contents.

Adhesin expression. All mutant strains had the ability to express type 1 as well as P fimbriae. Before colonization, the strains were passaged three times in Luria broth in order to maximize the expression of type 1 fimbriae (12). The bacteria present in the inoculate expressed type 1 fimbrial MSHA when the broth culture was tested directly for hemagglutination, but when tested individually from the viable-count agar overnight culture, only one-third of the colonies obtained displayed MSHA (Table 2). P fimbriae, on the other hand, were expressed by all colonies. After 2 weeks of colonization, almost all colonies still expressed P fimbriae (Table 2). The expression of type 1 fimbriae, as evidenced by MSHA, decreased in the colonization with the $O75^-$ pair and was practically unchanged compared with the initial inoculum in the colonization with the $O75^+$ pair (Table 2).

Growth curves. The generation times (doubling times) obtained from the growth curves were 38 min for strain 998, 30 min for strains 972 and 973, and 32 min for strain 997.

Susceptibility to lysozyme. Lysozyme did not affect bacterial viability at any of the concentrations used. Thus, bacterial

TABLE 2. Hemagglutination patterns of colonies isolated from the viable count of the mixture given to the rats and from colonies obtained from intestinal contents

Strain pair	No. of colonies tested	% of colonies exhibiting	
		MRHA	MSHA
972-998			
Initial inoculum	10	100	30
After colonization	80	93	6
973-997			
Initial inoculum	14	100	29
After colonization	83	99	20

TABLE 3. In vitro adherence of mutant *E. coli* to crude rat intestinal mucus and porcine mucin[a]

Strain	Serotype	Adhesion (% of added radioactivity ± SD) to:	
		Rat mucus	Porcine mucin
972	O75$^-$ K5$^+$	5.7 ± 0.18	4.1 ± 2.09
998	O75$^-$ K5$^-$	0.2 ± 0.10	0.6 ± 0.17
973	O75$^+$ K5$^+$	2.0 ± 0.40	2.7 ± 1.18
997	O75$^+$ K5$^-$	2.5 ± 0.91	5.1 ± 0.58

[a] Bacterial binding was measured as the amount of radioactivity (in cpm) obtained from labelled bacteria remaining in the plate (after washings) divided by the total radioactivity (amount of [^{35}S]methionine incorporated in each strain). Three determinations were done for each sample.

growth in lysozyme-containing medium did not differ from growth in unsubstituted medium (results not shown).

Mucus and mucin adhesion in vitro. The results of the mucus binding assay are shown in Table 3. In general, the *E. coli* strains did not bind to mucus, apart from a weak interaction of strain 972 with rat mucus and similarly weak interactions of strains 972 and 997 with porcine mucin.

DISCUSSION

The results of the present study suggest that the expression of the K5 capsule significantly enhances *E. coli* persistence in the rat large intestinal microflora. An advantage of the K5$^+$ phenotype was seen in two different combinations of isogenic strains, in which one pair expressed the native O75 phenotype and the other pair had a mutant rough phenotype. The expression of a capsule has previously been considered important only in invasive disease, as a means to avoid complement activation and phagocytosis. Recently, however, capsular expression by *Streptococcus pyogenes* (*Streptococcus* group A) was shown to enhance not only invasive disease but also mucosal colonization in the upper respiratory tract (47).

Neither the facts that one of the strains was antibiotic resistant and the other one was antibiotic sensitive nor the minor differences in generation time between the strains are likely to have been responsible for the differences in colonization. First, in the colonization with the first pair, the rifampin-sensitive strain (972) was a better colonizer than its rifampin-resistant counterpart (998), but in the colonization with the second pair, the nalidixic acid-resistant strain (973) was superior to the nalidixic acid-sensitive strain (997). Second, resistant bacteria in this study reached levels in the intestinal contents comparable to those previously obtained for non-antibiotic-resistant strains (between 10^8 and 10^{10} organisms). Third, strain 998, which grew the slowest, was able to compete favorably with other mutant *E. coli* also lacking K5 (unpublished results). The generation time of *E. coli* in vivo in the intestine has been reported to range from 40 to 80 min in the mucus layer, whereas the bacteria remain static in the luminal contents (32).

Although the present strains were not knockout mutants with defined genetic lesions but rather were spontaneous K5$^-$ mutants selected by K5 phage resistance, we consider the difference in K5 expression to be the most likely source of the difference in colonization capacity. This is specially true for the O$^-$ mutant pair 972-998, in which case the K5$^-$ mutant derived directly from the K5$^+$ parent strain; the probability of more than one spontaneous mutation occurring at the same time after K5 phage-resistant colonies were selected is low.

The mechanism by which K5 increases *E. coli* virulence in extraintestinal infections is not known. The K5 capsule consists of repeating *N*-acetylglucosamine and glucuronic acid units. Its low immunogenicity long hampered its detection and, thus, the understanding of its involvement in disease (45); K5 was classified as K nontypeable until the early 1980s, when a K5-specific bacteriophage was found that could be used as a tool for typing this capsule (15). The K5 antigen confers a negative charge and hydrophilic properties on the bacterium, but it does not in itself seem to confer resistance to phagocytosis (9, 20). When the four strains used in this study were tested for sensitivity to phagocytosis, only the double-negative mutant (O75$^-$ K5$^-$) was sensitive (40).

Even if the capsule, in combination with LPS, could offer protection from phagocytosis, it is difficult to understand how this would help a bacterial strain become established in the large intestinal environment, where phagocytes are scarce under normal circumstances. It is also not likely that resistance to complement is of importance for survival in the intestinal milieu. Even if some complement components are synthesized by intestinal epithelial cells (27), complement-mediated lysis is suppressed in intestinal contents (14), perhaps due to the synthesis of α-1 antitrypsin by the intestinal epithelium (30). Moreover, with the *E. coli* strains used in the present study, complement-mediated lysis seemed to depend on the possession of a complete O75 antigen and not on capsular expression (40).

So far, we have not found any mechanism likely to explain the advantage of K5 for intestinal colonization. All the mutants were resistant to lysozyme, and they all interacted only very weakly with mucus either from the rat intestine or the porcine stomach. This suggests that specific receptors for type 1 and P fimbriae might not be present in mucin: bacteria which interact via specific binding mechanisms show higher binding activities than those found in the present study (5). In contrast, all the mutants bound well to rat intestinal epithelial cells in vitro (unpublished observations), as could be expected by their expression of type 1 and P fimbriae which bind to rat (17) as well as human (1) epithelial cells.

The importance of P fimbrial expression for intestinal colonization was confirmed in the present study. Here, all strains used had the capacity to express both P and type 1 fimbriae. Before colonization, type 1 fimbriae were expressed by only a minority of the bacteria. Still, after 2 weeks of colonization in vivo, the fraction of bacteria expressing type 1 fimbriae was unchanged or had further decreased. In contrast, 96% of the colonies recovered from the rat intestine after 2 weeks expressed P fimbriae. In contrast, Krogfelt et al. showed an upregulation of type 1 fimbriae by *E. coli* colonizing the mouse large intestine (21). The reason for the divergent results is not known. We have recently shown that in the human bowel, type 1 fimbrial expression is enhanced in the presence of secretory immunoglobulin A (SIgA) (13), probably due to an interaction between carbohydrate receptors on IgA and type 1 fimbrial lectin (48). Possibly, such an interaction could also take place in the mouse intestine, where SIgA is formed after passage of dimeric IgA over the intestinal epithelial cells and therefore reaches high concentrations in the mucus layer. In the rat, intestinal epithelial cells transport very little dimeric IgA, and most SIgA is delivered via the bile (10). The SIgA molecules may consequently be located primarily in the lumen, giving less advantage to bacteria binding to these molecules.

Translocation increases with increasing population densities in the intestine (39). Apart from this, no bacterial factors that are in themselves favorable for translocation have been defined. The results of the present study suggest that expression of the K5 capsule favors translocation only indirectly, second-

arily to the increased population levels in the intestine. The same results were previously found for P fimbriae (17).

The results of the present study confirm that bacterial virulence factors, like P fimbriae and the K5 capsule, confer colonization advantages in the intestinal milieu and suggest that they may, in fact, have evolved to increase colonization of the colon. The epidemiological association of capsule with disease may therefore, in part, reflect the fact that capsule expression enables the bacteria to reach high numbers in the intestinal environment and to persist for extended periods of time, which increases the probability of their spreading to other sites (31). Once in the tissues, after bacteria have spread from the intestine, the capsule may further add to virulence by protecting the organisms from phagocytosis and complement-mediated lysis. The $O75^+$ $K5^+$ *E. coli* mutant had an advantage over the $O75^+$ K^- mutant in experimental pyelonephritis in the mouse (40).

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