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Role of *Escherichia coli* P Fimbriae in Intestinal Colonization in Gnotobiotic Rats

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Adherence via P fimbriae is associated with long-term persistence of *Escherichia coli* in the human large intestine, but a causal relationship has not been proven. In the present study, germfree rats were colonized with a mixture of two isogenic *E. coli* strains, one P fimbriated and the other type 1 fimbriated. Both types of fimbriae conferred adherence to rat colonic epithelial cells. With two mutant strains from a pyelonephritogenic isolate of serotype O75:K5:H$^-$, the P-fimbriated strain 824 attained much higher numbers than its type 1-fimbriated counterpart when colonized in vivo for 2 weeks (10^{10} versus 10^6 bacteria per g, respectively; $P < 0.0001$). The expression of P fimbriae by 824 was also retained during colonization. With transformant isogenic strains obtained from a normal fecal isolate incapable of phase variation, no benefit of P fimbriae was seen and most bacteria lost their plasmids during in vivo colonization. When the pyelonephritogenic mutant and fecal transformant strains were combined, the former colonized at high levels while the latter were suppressed. In contrast, no suppression was seen when the transformant *E. coli* strains colonized in combination with *Lactobacillus acidophilus* or *Peptostreptococcus* sp. The results indicate that P fimbriae, but also other bacterial traits linked to uropathogenicity, could play an important role for persistence in the gut normal microbiota. Neither P nor type 1 fimbriae seemed to contribute to the ability to translocate to the mesenteric lymph nodes.

In each individual, the colonic *Escherichia coli* population at any time consists of a limited number of strains, which can be identified by serotyping or other means. Some strains may persist over a long period of time (resident strains), whereas others are rapidly replaced (transient strains) (20–22). The role of bacterial adherence in the colonization of the colonic microbiota by *E. coli* has not yet been established. Human colonic epithelial cells express receptors for type 1 fimbriae (7, 31) and P fimbriae (31). In epidemiological studies, P fimbriae mediating the adherence to Galα1→4Galβ-containing receptors are more commonly expressed by resident than by transient colonic *E. coli* (26, 30). However, *E. coli* appears as stable clones exchanging very little genetic information with each other (6). Since each clone carries a number of virulence traits, such as particular O and K antigens, fimbriae mediating adherence, etc., epidemiological studies are unsuitable for pinpointing the roles of individual virulence traits for colonization. Experimental studies with isogenic strains that differ only in one trait, e.g., adhesin expression, are therefore needed to establish the role of adherence for colonization.

Translocation denotes the passage of viable bacteria from the gut to mesenteric lymph nodes (MLN) or other organs. This may be an important step in the development of bacteremia in debilitated patients (23, 25) and in newborn infants (19). Bacterial adherence to gut epithelial cells may play a role in this process. Thus, in neonatal septicemia caused by *E. coli*, strains expressing S and P fimbriae are increased in frequency compared with normal fecal strains (17, 27).

In this study, a gnotobiotic rat model for studying the contribution of isolated bacterial traits, e.g., adhesin expression, for intestinal colonization and translocation is described. Sterile rats were colonized with either of two pairs of isogenic strains differing only in adhesin expression. By measuring the ratio of the two isogenic strains in the gut contents, in gut mucosa, and in homogenates of MLN, the importance of type 1 and P fimbriae for intestinal colonization and translocation could be determined. One isogenic pair consisted of transformant strains from a nonpathogenic fecal isolate; the other pair consisted of mutants from the virulent pyelonephritogenic isolate GR-12.

MATERIALS AND METHODS

Rats. Male and female rats of the AGUS strain were 3 to 8 months of age when used in the experiments. They were reared germfree in stainless-steel isolators and checked weekly for sterility by culturing animal feces and isolator interiors for bacteria and yeasts and by checking the feces for protozoans by microscopy. Virus checks were performed at regular intervals throughout the year (10). After colonization, the animals were kept within the isolators for the whole course of the experiment.

Bacteria and substrates. Table 1 shows some characteristics of the bacterial strains used for colonization. The *E. coli* 506 transformant family was derived from a nonfimbriated human fecal isolate of the serotype O19,22:K1:H$^-$ (13). Strain 506 MS had been transformed with the vector pACYC184 containing the *pil* gene, encoding type 1 fimbriae with mannose-specific adhesins, inserted into the tetracycline resistance site. Strain 506 MR instead carried the vector with the *pap* gene, encoding P fimbriae with Galα1→4Galβ-specific adhesins, inserted into the chloramphenicol resistance site of the vector. The transformant strains constitutively express their respective adhesins as long as the plasmid, which also confers antibiotic resistance, is retained.

The second pair of isogenic *E. coli* transformants was derived from a lactose-negative mutant of the wild-type *E. coli* GR-12. *E. coli* GR-12 originated from a patient with acute pyelonephritis, expresses type 1 and P fimbriae, and has the serotype O75:K5:H$^-$ (24). From a spontaneously lactose-negative mutant of this strain, two isogenic strains differing in adhesin expression were obtained after *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine treatment (1). HU742 expresses mannose-specific adhesins and type 1 fimbriae, whereas HU824 expresses Galα1→4Galβ and P fimbriae. These GR-12 derived mutants are identical to the parent strain with regard to the following characteristics: the O:K:H serotype, the electrophoretic mobility of 13 chromosomally encoded enzymes, the carrying of three plasmid bands of the same size, the resistance to the bactericidal effect of serum, the lack of capacity to hemolyze horse erythrocytes, and the biotype as assayed by the Api 20E pattern (24). The GR-12-derived strains can modulate their adhesin expression by phase variation; i.e., they can turn on or off the expression of fimbriae depending on environmental conditions (8, 18).

Strain 742 expressed resistance to ampicillin (32 μg/ml), whereas strain 824 did

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TABLE 1. Bacterial strains and culture conditions used in this study

Family and bacterial strain	Serotype	Adhesin specificity	Metabolism	Differential substrate	Time (h)	Reference(s)
506 transformants						
E. coli 506 MS	O19,22:K1:H$^-$	Mannose	Facultative	TSA-chloramphenicol	24	13
E. coli 506 MR	O19,22:K1:H$^-$	Galα1→4Galβ	Facultative	TSA-tetracycline	24	13
GR-12-derived mutants						
E. coli 742	O75:K5:H$^-$	Mannose	Facultative	TSA-ampicillin[a]	24	24
E. coli 824	O75:K5:H$^-$	Galα1→4Galβ	Facultative	TSA		
				TSA-gentamicin[b]	24	24
Wild types						
L. acidophilus A10			Facultative[c]	MRS[d]	48	
Peptostreptococcus sp. strain N			Obligate anaerobe	Blood agar-nalidixic acid	72	11, 12

[a] Before colonization, the strain was passaged three times in static Luria broth with $CaCl_2$ to increase type 1 fimbrial expression.
[b] Used in colonization group E. For details, see Materials and Methods.
[c] Grows better under anaerobic conditions.
[d] MRS, de Man-Rugosa-Sharp agar.

not, permitting their differentiation on ampicillin-containing agar. To be able to further differentiate between the strains in a combined colonization with the 506 family (group E; see below), we selected for antibiotic resistance to gentamicin in 824 (the strain had shown intermediate resistance to this antibiotic). The procedure was done with gradient doses of gentamicin in agar plates. In this way, 824 achieved resistance to gentamicin (10 μg/ml) and this resistant strain was called 824R. All *E. coli* strains were cultivated aerobically at 37°C on tryptic soy agar (TSA) containing the proper antibiotic (Table 1). Before colonization, the expression of type-1 fimbriae in bacteria of strain 742 was increased by three passages in Luria-Bertani broth with 0.1% $CaCl_2$.

Lactobacillus acidophilus A10 (kindly provided by the Norwegian Dairies Association, Oslo, Norway) was grown anaerobically at 37°C for 48 h on de Man-Rogosa-Sharpe agar (Oxoid, Basingstoke, England).

A *Peptostreptococcus* strain designated *Peptostreptococcus* sp. strain N CCUG no. 32127 (Culture Collection University of Göteborg) was isolated from normal rat microbiota (12). This strain is capable of degrading mucin from the rat intestine (11). It was cultivated anaerobically at 37°C for 48 to 72 h on blood agar plates (5% human blood) with 12 μg of nalidixic acid per ml, which inhibited the growth of the *E. coli* strains used.

Electromorphic typing. The electromorphic types of 742 and 824 have been determined to be identical (24). This method was also applied to 824R. The purpose of this test is to determine genetic variations of the same enzyme. The enzymes are separated by electrophoresis. Different migration patterns are obtained if amino acid substitutions have taken place. If the strains have the same electrophoretic mobility for all the enzymes studied they are said to have the same electromorphic type (5). The enzymes studied were adenylate kinase, phosphoglucose isomerase, glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, mannose-6-phosphate isomerase, malate dehydrogenase, leucyl-glycyl-glycine peptidase, and phenylalanyl-leucine peptidase (Sigma, St. Louis, Mo.).

Growth curves. Growth curves for all GR-12-derived *E. coli* strains used in the experiments were obtained. Bacteria were grown on TSA plates. A loopful of each bacterium was cultivated on nutrient broth. After 1 to 2 h, the bacterial suspension was homogenized and 5 ml of each suspension was distributed into three tubes, which were cultivated aerobically at 37°C with shaking. The optical density was measured every 15 to 20 min up to about 11 h, and an average was calculated for the three tubes. Growth curves were plotted, and regression lines were calculated with linear regression for each strain.

In vitro adherence to epithelial cells isolated from rat intestine. The in vitro adherence of *E. coli* 506 MR, 506 MS, 742, and 824 to rat intestinal epithelial cells was tested by a procedure previously used for human colonic epithelial cells (31). A fecal normal *E. coli* strain of serotype O25 expressing neither type 1, P, nor S fimbriae was used as a negative control. Pieces of cecum and small and large intestine were obtained from conventional AGUS rats and were turned inside-out and rinsed free of intestinal contents. A piece of intestine was added to a suspension of bacteria (bacterial concentration, 10^9/ml), in Weiser B solution (29), with 1.5 mM dithiothreitol (Sigma). The tubes were incubated at 4°C with end-over-end rotation for 1 h. The piece of remaining intestine was removed, and detached cells were washed two to three times with cold phosphate-buffered saline (PBS) and fixed with neutral buffered formaline (Histofix; Histolab Products AB, Västra Frölunda, Sweden). Adhering bacteria were observed by interference-contrast microscopy (Nikon UFX-II Optiphot; magnification, 500×). The viabilities of the bacteria were shown not to be affected by exposure to Weiser B solution (data not shown).

Bacterial colonization. Altogether, five groups of rats were used. Groups A, B, and C were colonized with a mixture of 506 MS and 506 MR with or without the addition of *L. acidophilus* and *Peptrostreptococcus* spp. (Table 2). Group D was colonized with the GR-12-derived mutant strains 742 and 824, and group E was colonized with a mixture of the 506 transformant strains (506 MS and 506 MR) and the GR-12-derived mutant strains, 742 and 824R, the latter one selected for resistance to gentamicin.

The bacteria were grown overnight on the proper substrates, harvested, and adjusted to a bacterial density of approximately 10^9/ml by optical density measurement. A mixture of the different bacteria was prepared, aiming at equal concentrations of the strains and at a total bacterial concentration of 10^8 to 10^9/ml. The bacterial suspensions were poured into 10- to 20-ml glass ampoules, which were sterilized on the outside with chromosulfuric acid and transferred into the germfree isolators. One milliliter of the bacterial suspension was given by gastric intubation to each rat. The actual bacterial concentration in the suspension given to the rats was determined by viable counts performed on plates containing the appropriate antibiotics (Table 2).

Determination of bacterial counts in intestinal contents and in association with the gut wall. After different periods of time, the rats were anesthetized with ether and exsanguinated by heart puncture. In group A, two rats were sacrificed after 6 h and two rats each were sacrificed on days 1, 2, 3, and 14 (Table 2). In groups B, C, D, and E, all rats were sacrificed between days 11 and 15.

Samples of the gut contents from the cecum and large intestine were obtained with a calibrated loop. Serial dilutions in sterile saline were performed and distributed on bacterial plates appropriate for each bacterial strain or species. The plates were incubated under optimal conditions for each strain (Table 1),

TABLE 2. Profile of experimental groups

Group	Bacterium	No. of bacteria given	Days of sacrifice	No. of rats sacrificed each day
A	*E. coli* 506 MS	2.7×10^7	0.25, 1, 2, 3, 14	2, 2, 2, 2, 2
	E. coli 506 MR	2.4×10^7		
B	*E. coli* 506 MS	9.2×10^7	14, 15	2, 2
	E. coli 506 MR	4.0×10^7		
	L. acidophilus	5.9×10^7		
C	*E. coli* 506 MS	1.6×10^8	14, 15	2, 2
	E. coli 506 MR	1.4×10^8		
	L. acidophilus	6.0×10^7		
	Peptostreptococcus sp.	1.0×10^7[a]		
D	*E. coli* 742	3.1×10^8	11, 12	3, 3
	E. coli 824	5.6×10^8		
E	*E. coli* 742	6.8×10^8	13, 14	1, 2
	E. coli 824R	7.4×10^8		
	E. coli 506 MS	3.2×10^9		
	E. coli 506 MR	5.6×10^8		

[a] Approximate number determined from gradient dilutions (1:10) in tubes.

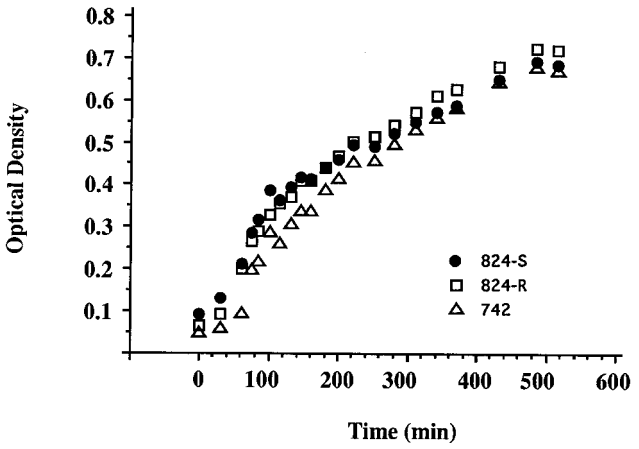

FIG. 1. Growth curves of the mutant strains obtained after cultivation on shaking broth cultures. The optical densities were determined at 597 nm.

and the numbers of colonies were counted and expressed as colony-forming units per gram of gut contents.

To assess the composition of the bacterial population closely associated with the gut wall, pieces of intestinal wall were excised, rinsed, and homogenized. The homogenate was diluted and cultivated on agar plates as described above. The ratio between the bacterial strains in this fraction was calculated and compared with the ratio from the lumenal contents.

Quantification of bacterial translocation. The MLN were aseptically removed and homogenized in sterile saline. Viable counts were performed on the adequate media, as described above.

Hemagglutination patterns. The hemagglutination patterns for the *E. coli* isolates were determined to confirm their adhesin specificities, both on cultures performed from the original mixture given to the rats and on colonies recovered from viable counts performed on gut contents after colonization. The following erythrocyte suspensions (3%, vol/vol) were made: human erythrocytes in PBS, human erythrocytes in 2.5% methyl-α-D-mannoside in PBS, guinea pig erythrocytes in PBS, and guinea pig erythrocytes in 2.5% methyl-α-D-mannoside in PBS. A drop of each of these suspensions was mixed on a microscope slide with a loopful of bacteria from the 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 a mannose-sensitive hemagglutination (MSHA), while 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.

Statistical analyses. Differences in bacterial numbers were evaluated by Student's *t* test on the base-10 logarithm of bacterial concentration values. Translocation data were analyzed by Mann-Whitney's nonparametric test.

RESULTS

Strain characteristics. 824R showed the same pattern by electromorphic typing as did 742 and 824. The mutant strains also showed similar growth curves (Fig. 1).

In vitro adherence of *E. coli* with different adhesin expression to rat intestinal epithelial cells. The capacities of the *E. coli* strains to adhere to the mucosa were assessed with epithelial cells isolated from the small intestine, cecum, and large intestine. Both the type 1-fimbriated strains, 506 MS and 742, and the P-fimbriated strains, 506 MR and 824, adhered to intestinal epithelial cells, whereas the hemagglutination-negative strain *E. coli* O25 did not adhere (Fig. 2). As with human colonic cells, we observed that adherence occurred not only directly to the cell surface but also to material loosely associated with it (not shown).

Colonization with the 506 transformant strains. To establish when the intestinal microbiota reached a steady level, the rats in group A were colonized with a mixture of 506 MS and 506 MR and sacrificed after 6 h or on day 1, 2, 3, or 14. As shown in Fig. 3, the *E. coli* strains rapidly established a level of approximately 10^9 bacteria per g of gut content and remained stable at this density during 14 days of colonization. However, a majority of the bacteria lost their plasmids with time; the number of bacteria retaining the 506 MS antibiotic resistance pattern was 1 log unit lower than that of *E. coli* growing on TSA plates. The number of bacteria retaining the 506 MR resistance pattern was even lower (by approximately 2 log units) than the total *E. coli* level.

To see whether the addition of another bacterial species would affect the *E. coli* population density, the rats in group B were colonized with the two bacteria from group A plus *L. acidophilus*. Although the *Lactobacillus* strain colonized to a level of 10^9 bacteria per g of cecal content, the total level of *E. coli* was not reduced and the relationship between 506 MS and 506 MR was not altered (Fig. 4). Group C was colonized with the three strains mentioned above plus a mucin-degrading bacterium, *Peptostreptococcus* sp. strain N. Also in this case, the numbers of total *E. coli* organisms, as well as the relation between 506 MS and 506 MR, remained practically unaltered. The growth of bacteria in the large intestine was very similar to that in the cecum (data not shown).

After each colonization, a number of colonies were tested for their hemagglutination patterns. As expected, all bacteria growing on TSA plates with tetracycline displayed an MRHA pattern, whereas colonies from chloramphenicol-TSA plates all showed an MSHA pattern. Of 216 colonies of bacteria tested from unsubstituted TSA plates, 9.7% showed MSHA, 1.9% showed MRHA, and 88% were hemagglutination negative, confirming the findings obtained from the antibiotic resistance pattern of a loss of the plasmid in a majority of the bacteria, especially from 506 MR.

Colonization with the GR-12-derived mutant family (742 and 824). Group D was colonized with the GR-12-derived mutant strains 742 and 824. Since 742 was ampicillin resistant, this antibiotic was used to differentiate between the two strains. The number of 824 colonies was obtained by subtracting the number of 742 colonies on the ampicillin plates from the number of colonies on unsubstituted TSA plates permitting the growth of both strains. The fimbrial expression of the GR-12-derived mutant strains is subject to phase variation; i.e., it can be turned on or off depending on the environmental conditions. The GR-12-derived strains therefore differ from the 506 transformants in that growth on an antibiotic-containing agar does not assure the expression of their fimbriae. Consequently, the expression of fimbriae had to be tested by hemagglutination.

On days 11 to 12 the P-fimbriated strain 824 showed much higher numbers than the type 1-fimbriated strain 742, the mean difference being 4.25 log units ($P < 0.0001$) (Fig. 5). The viable count performed on the mixture of bacteria given to the rats showed similar amounts of each of the strains, and hemagglutination of these colonies demonstrated the presence of both MRHA and MSHA adhesins but also some hemagglutination-negative colonies (Table 3). After 11 to 12 days of intestinal colonization, almost all colonies expressed P fimbriae, few were hemagglutination negative, and none expressed type 1 fimbriae, in accordance with the small numbers of 742 bacteria present (Table 3). Thus, P fimbriae were still expressed by 824 to a large extent after in vivo colonization for 11 to 12 days.

Group E was colonized with 742, 824R, and the 506 transformants, 506MS and 506 MR. The gentamicin-resistant strain 824R was used to differentiate it from the 506 transformants and 742, since all four bacteria would grow on TSA plates without antibiotics. The MRHA of 824R had been weakened when antibiotic resistance to gentamicin was introduced, as judged from the results obtained from the hemagglutination

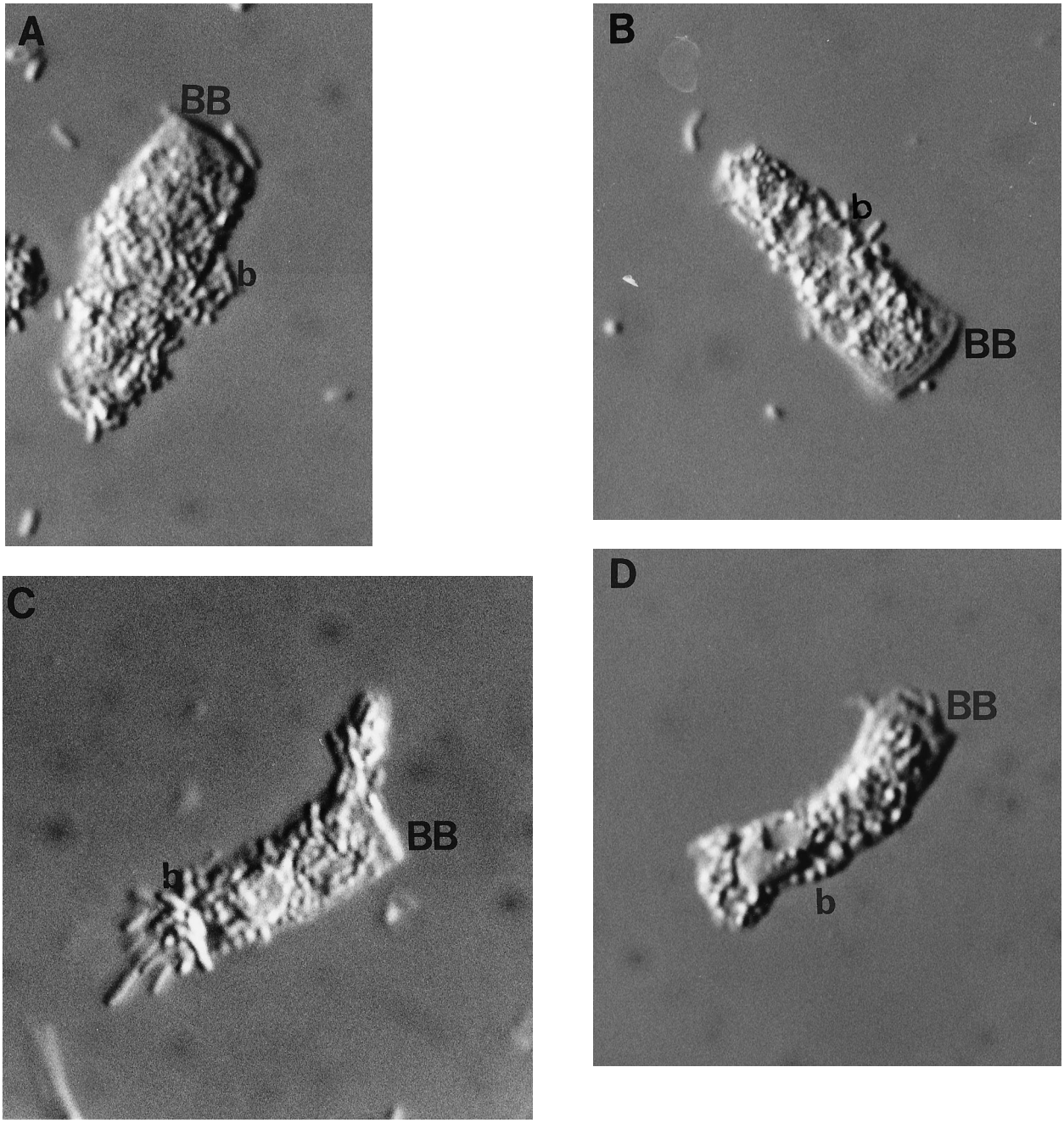

FIG. 2. In vitro adherence of *E. coli* strains to intestinal cells of AGUS rats. (A) *E. coli* 506 MS adherence to a cecal enterocyte (the brush borders are not easily identified because of the load of bacteria); (B) *E. coli* 506 MR binding to a cecal enterocyte; (C) *E. coli* 742 adhering to a cecal enterocyte; (D) *E. coli* 824 binding to a colonic enterocyte; (E) a cecal enterocyte showing no bacterial binding of the hemagglutination-negative *E. coli* control strain. Magnification, ×1,200 (interference-contrast microscopy). BB, epithelial cell brush border; b, bacteria.

analysis of the colonies isolated at the initial viable count (Table 4). After 14 to 15 days, the rats were sacrificed and viable counts from the different organs were performed. The bacterial density of the 506 transformants decreased about 2 log units compared with the groups in which they were the only *E. coli* strains (Fig. 5 [group A is added in the figure for comparison]), whereas the bacterial densities of GR-12-derived mutant strains reached high numbers. The P-fimbriated 824R did not reach numbers as high as did 824 in group D, as judged by counts, from gentamicin-containing plates, maybe due to a growth disadvantage imposed by the gentamicin resistance. Approximately half of the colonies growing on unsubstituted TSA expressed MRHA. These probably represented the 824R strain with its resistance to gentamicin lost since the contribution of the other strain capable of MRHA, 506 MR, was very low (Fig. 5 and Table 4). In contrast, as in colonization group D, no MSHA colonies were found.

Relationship between colonization in the gut lumen and epithelium. Adhering bacteria may preferentially grow in close association to the mucosa, making quantification of bacteria in

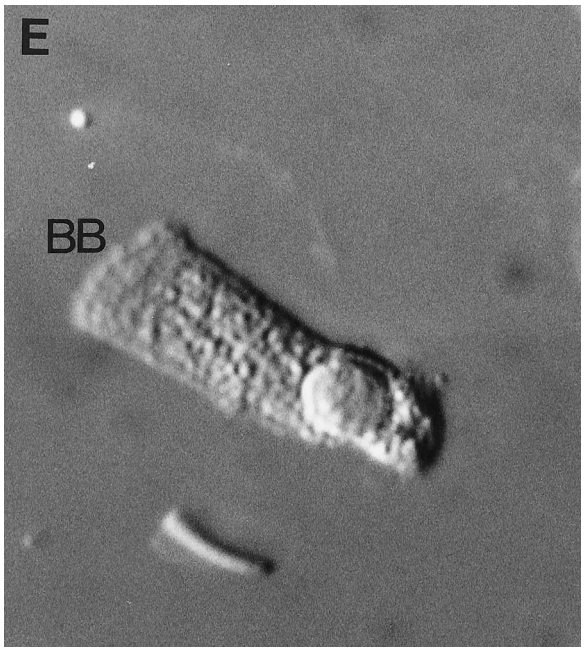

FIG. 2 — *Continued.*

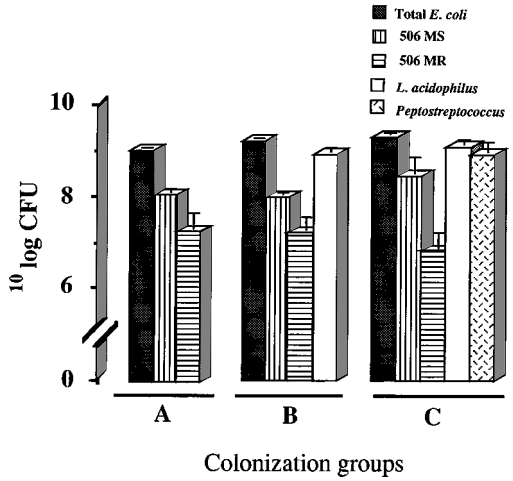

FIG. 4. Bacterial densities of *E. coli*, *L. acidophilus*, and *Peptostreptococcus* sp. strain N in cecal contents of rats sacrificed on days 14 to 15. A, rats colonized with 506 MS and 506 MR; B, rats colonized with 506 MS, 506 MR and *L. acidophilus*; C, rats colonized with 506 MS, 506 MR, *L. acidophilus*, and *Peptostreptococcus* sp. strain N. Error bars show standard deviations. The total *E. coli* count represents bacteria growing on unsubstituted TSA, 506 MS represents bacteria growing on TSA-chloramphenicol, and 506 MR represents bacteria growing on TSA-tetracycline.

the intestinal lumen inadequate. Therefore, pieces of intestinal mucosa were homogenized and viable counts were performed from this homogenate. We measured the ratio between *E. coli* 506 MS and 506 MR and between 742 and 824. The ratios between both pairs of strains were almost identical to those observed in the intestinal content for groups A, B, C, and D (data not shown). However, in the experiments with the gentamicin-resistant 824R, the ratio of 824R to 742 was significantly higher in the intestinal wall than in the lumen (the ratio was on average 5.6 times higher in the intestinal wall than in the lumen; $P < 0.05$ [six rats]). Thus, whereas the presence of different adhesins did not affect the preferred niche for the bacteria, the presence of resistance to gentamicin made the bacterium more apt to survive close to the intestinal mucosa than in the lumen.

Translocation to MLN. Translocation was measured as the number of viable bacteria cultivated from homogenates of MLN. In the first three colonization groups, all rats exhibited bacterial translocation of *E. coli* and *L. acidophilus*, whereas *Peptostreptococcus* sp. did not translocate (Table 5). Translocation of both *E. coli* and *L. acidophilus* was, however, reduced when *Peptostreptococcus* sp. was included in the colonization, although the difference was not statistically significant (Table 5).

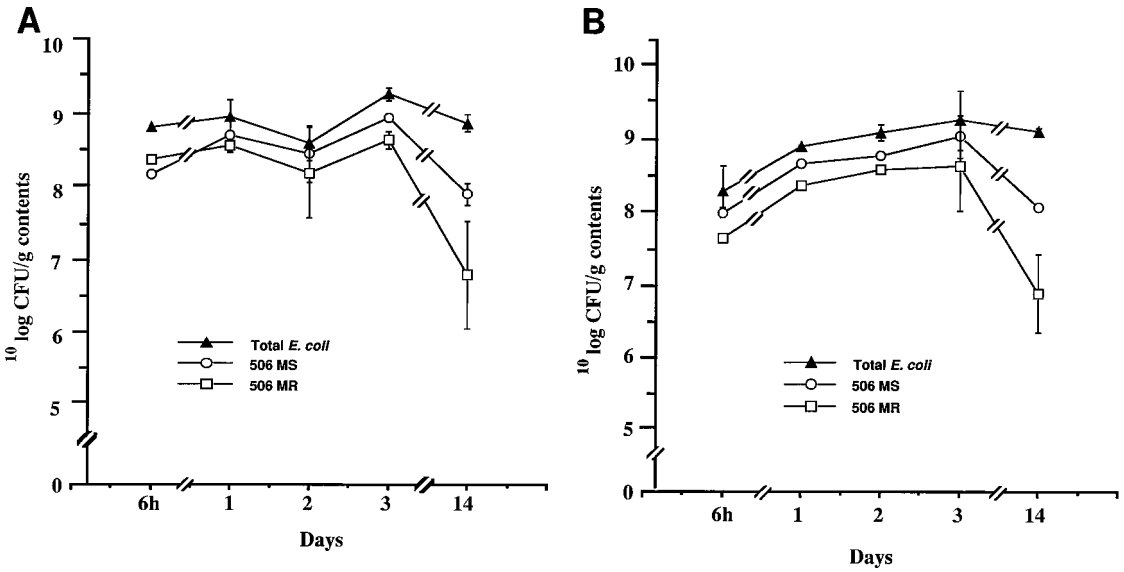

FIG. 3. Bacterial densities in cecal (A) and large intestine (B) contents of rats sacrificed at different times after colonization. Error bars show standard deviations. The total *E. coli* count represents bacteria growing on unsubstituted TSA, 506 MS represents bacteria growing on TSA-chloramphenicol, and 506 MR represents bacteria growing on TSA-tetracycline.

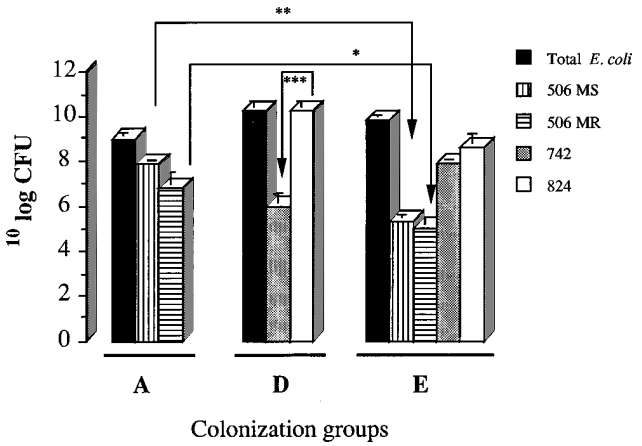

FIG. 5. Bacterial densities of *E. coli* in cecal contents on days 11 to 15. A, rats colonized with 506 MS and 506 MR (from Fig. 2; included for comparison); D, rats colonized with the mutant strains 742 (type 1 fimbriated) and 824 (P fimbriated); E, rats colonized with the two pairs of isogenic strains, 506 MS and 506 MR and 742 and 824R, the latter strain selected to be resistant to gentamicin. Error bars show standard deviations. The numbers of total *E. coli* organisms were assessed from unsubstituted TSA plates, numbers of 742 organisms were assessed from ampicillin-containing TSA plates, and numbers of 824 organisms were the difference between those for the total *E. coli* and 742. 824R was assessed from growth on TSA with gentamicin. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$

Figure 6 shows the relationship between translocation and cecal bacterial numbers from the colonizations in which only *E. coli* strains were used to limit confounding factors that could be provided by the presence of the other strains. No translocation was seen with a cecal population level below 10^7 to 10^8; at higher levels, translocation seemed to increase in an approximately linear way, regardless of fimbrial specificity.

DISCUSSION

The purpose of the present study was to determine the importance of type 1 and P fimbriae for colonization and persistence in the intestinal tract of the rat, as well as for translocation over the intestinal wall. This was done with isogenic *E. coli* strains which differed only in their adhesin specificities, expressing either type 1 fimbriae with mannose-specific adhesins, or P fimbriae expressing Galα1→4Galβ-specific adhesins. Both specificities conferred the capacity to adhere to rat intestinal epithelial cells, similar to the situation in humans (31).

With the 506 transformants, the type 1-fimbriated strain 506 MS reached higher numbers than did 506 MR in the gut contents, but the majority of the bacteria lost their plasmids conferring both fimbriae and antibiotic resistance. This could be due to a large energy cost to therefore synthesize the plasmid. However, a third transformant strain of the 506 family (506 P), which carries the plasmid pACYC184 with no gene coding for any fimbriae, colonized at approximately the same rate as 506 MS (unpublished observations). Thus, in the 506 transformants, fimbriation could not be demonstrated to be of advantage for the bacteria.

With the GR-12-derived strains, adherence to Galα1→4Galβ-containing receptors mediated by P fimbriae indeed seemed to be beneficial for colonization of the rat intestine. The P-fimbriated strain 824 attained numbers that were, on average, 4 log units higher than those of its type-1-fimbriated counterpart in the intestinal contents. Further, the MRHA was retained by strain 824 after 11 to 12 days of colonization of the rat intestine. In accordance with these results, with a mutant *E. coli* strain expressing both type-1 and P fimbriae for colonization almost all colonies expressed MRHA after 2 weeks of colonization whereas a minority expressed MSHA (unpublished results). Thus, P fimbriae seem to confer a colonizing ability on *E. coli*. The reason why this was not evident with the 506 transformants may relate to the fact that these transformants cannot regulate their fimbrial expression by phase variation as wild-type strains and the mutants of the GR-12 family can. However, since the two families differ in a number of other properties, other explanations may be possible. For example, P fimbriae may be important only as a colonizing factor in combination with other virulence traits, such as certain O or K antigens. In a study of colonization and bacteremia in newborn rats, P fimbriae seemed to be beneficial for colonization of the rat intestine, a result similar to ours. In this study, wild-type K1-positive *E. coli*, deriving either from blood cultures or fecal specimens (32), was used.

In contrast to P fimbriae, which were retained during colonization, type 1 fimbriae were downregulated. The reason for this downregulation, though both P fimbriae and type 1 fimbriae conferred adherence to rat intestinal epithelial cells, is not known. This resembles the human situation in which receptors for both P and type 1 fimbriae are found on colonic epithelial cells but in which only P fimbriae seem to be asso-

TABLE 3. Hemagglutination patterns observed for colonization of group D[a]

Hemagglutination pattern[b]	Result for sample taken at:			
	Initial count		11–12 days after colonization	
	No. of colonies	% of colonies	No. of colonies	% of colonies
MSHA	8	25	0	0
MRHA	13	41	214	89
Negative	11	34	26	11

[a] Group D comprises mutant strains 742 and 824. Patterns were determined after the initial viable count from TSA plates of the bacterial mixture fed to the rats and after 11 to 12 days of in vivo colonization.

[b] MSHA denotes agglutination of guinea pig erythrocytes in the presence but not in the absence of methyl-α-D-mannoside. MRHA denotes the agglutination of human erythrocytes in the presence and absence of methyl-α-D-mannoside. Negative implies no agglutination with human or guinea pig erythrocytes.

TABLE 4. Hemagglutination patterns observed for the colonization of group E[a]

Hemagglutination pattern[b]	Result for sample taken at:			
	Initial count		14–15 days after colonization	
	No. of colonies	% of colonies	No. of colonies	% of colonies
MSHA	10	56	0	0
MRHA	0	0	23	46
Negative	8	44	27	54

[a] Group E comprises a mixture of transformant strains 506 MS and 506 MR and the GR-12-derived strains 742 and 824. Patterns were determined after the initial viable count from TSA plates of the bacterial mixture fed to the rats and after 14 to 15 days of in vivo colonization.

[b] MSHA denotes agglutination of guinea pig erythrocytes in the presence but not in the absence of methyl-α-D-mannoside. MRHA denotes the agglutination of human erythrocytes in the presence and absence of methyl-α-D-mannoside. Negative implies no agglutination with human or guinea pig erythrocytes.

TABLE 5. Frequency and growth of total bacteria that translocated to MLN in the five colonization groups

Group (strains used)	Translocation results for bacterium					
	E. coli		*L. acidophilus*		*Peptostreptococcus* sp.	
	Freq.[a]	CFU	Freq.	CFU	Freq.	CFU
A (*E. coli* 506 transformants)	2/2	4,400 220				
B (*E. coli* 506 transformants + *L. acidophilus*)	4/4	8,200 7,200 100 40	4/4	18,600 12,400 12,000 200		
C (*E. coli* 506 transformants + *L. acidophilus* + *Peptostreptococcus* sp.)	4/4	480 460 280 160	4/4	380 360 260 100	0/4	0 0 0 0
D (*E. coli* GR-12-derived mutants)	6/6	660 630 520 520 400 400				
E (*E. coli* 506 transformants + *E. coli* GR-12-derived mutants)	3/3	1,100 190 170				

[a] Freq., frequency, i.e., number of rats demonstrating translocation/number of rats in group.

ciated with long-term colonization (26, 30). Our results disagree with those of Krogfelt et al., who found upregulation of type 1 fimbriae upon in vivo colonization of the mouse large intestine (16).

L. acidophilus and/or *Peptostreptococcus* sp. was introduced together with *E. coli* in some of the colonization groups. The purpose of this was to see if an increased ecological pressure could alter the balance between the isogenic *E. coli* strains. In addition, the *Peptostreptococcus* strain used, which is a mucin-degrading one (4, 12), could increase the access of *E. coli* to glycosidic receptors on the mucosa. The levels of mucin are enormously increased in germfree rats (11, 14) and could possibly interfere with the adherence. However, the relation between 506 MS and 506 MR remained largely the same despite the introduction of the other two species, suggesting that the latter strains do not compete with *E. coli* for the same ecological niche. In contrast, when the two *E. coli* families (the transformant strains 506 MR and 506 MS and the GR-12-derived mutants 742 and 824) were introduced at the same time, the 506 transformants were markedly suppressed. These results suggest that different *E. coli* strains compete for the same ecological niche, whereas this is not true for distantly related bacteria. The competition could relate either to the availability of metabolic substrates or to competition for the same glycoconjugate receptors.

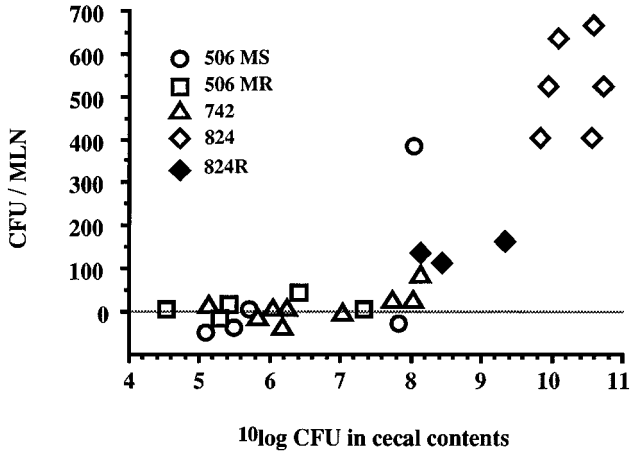

FIG. 6. Relationship between the number of translocated bacteria and the number of bacteria in cecal contents for different *E. coli* strains. Only colonization groups A, D, and E are included to avoid interference from other bacterial species.

The 506 transformants were derived from a nonadhering fecal isolate of serotype O19,22:K1:H$^-$, into which adherence was introduced by transformation. This serotype has not been associated with long-term persistence in the human intestine (26, 30). In contrast, the GR-12 strains were derived from a virulent pyelonephritic isolate of the O75:K5:H$^-$ serotype. This O75 serotype is enriched in resident colonic strains, which resemble uropathogenic *E. coli* both in their serotypes and in their common expression of P fimbriae (30). Thus, traits other than adherence may be as important as adherence in determining the capacity for long-term colonization of the large intestine.

The population of bacteria growing in close association with the gut mucosa seemed to be indistinguishable from that present in the lumen with respect to the ratio of strains with different adhesins. In contrast, 824R, the gentamicin-resistant variety of 824, was selectively enriched close to the mucosa. The preferred position of the gentamicin-resistant strain, close to the epithelium instead of the lumen, could be related to the availability of nutrients. Lumenal contents in the cecum or

large intestine are a poorer bacterial growth substrate than the mucous layer covering the epithelium (9, 28), and a close association with eukaryotic cells gives access to nutrients leaking out from them (33).

Translocation of indigenous bacteria, e.g., *E. coli*, over the epithelial barrier in the intestine represents a major threat to patients undergoing cytostatic or immunosuppressive treatments and to premature newborn infants. Apart from the total numbers of bacteria in the intestine, no special bacterial traits have been linked to the capacity to translocate (25). Our findings did not support any direct contribution of the two adhesin specificities tested (type 1 and P fimbriae) for bacterial translocation. However, translocation is a very variable process, especially if the levels of bacteria are not very high. The number of animals used in these experiments may have been too few to discern small or moderate differences in the translocation capacity. At any rate, the presence of P fimbriae could give an indirect advantage for translocation if it permits the establishment of large numbers of bacteria in the intestine, thus increasing the chances for passage over the intestinal epithelium. Other adhesins such as S fimbriae may, however, directly promote translocation, but this remains to be proven. The levels of these fimbriae are increased in *E. coli* strains, causing septicemia (15), and they mediate adherence to human colonic epithelial cells (2).

A decrease of anaerobic bacteria due to antibiotic treatment permits *E. coli* or other enterobacteria to increase in numbers and facilitates the passage to MLN or other organs. *Peptostreptococcus* sp. decreased the number of bacteria translocating to MLN, which agrees with the studies showing that indigenous anaerobic bacteria are primarily responsible for antagonism of the translocating bacteria (3). However, the explanation usually afforded for this protection—a reduction of the numbers of *E. coli* organisms in the cecal population—did not seem to apply to our results. In our study the numbers of *E. coli* organisms in the cecum remained the same after the introduction of *Peptostreptococcus* as when *E. coli* alone colonized the cecum. Thus, other factors besides, or in addition to, a reduction of *E. coli* numbers seemed to contribute to the capacity of *Peptostreptococcus* sp. to decrease translocation.

The pattern of adherence to rat intestinal epithelial cells was parallel to that of human colonocytes, in that rat epithelium expressed receptors for P-fimbriated as well as type 1-fimbriated *E. coli* (31). Because of this resemblance to the human situation and since the gnotobiotic rat model proved very stable and reproducible, this model may be used in future studies to delineate the role of factors associated with intestinal colonization. If such studies are made with the purpose of investigating the benefit of adherence, strains with the natural capacity for phase variation should be used.

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REFERENCES

1. **Adelberg, E. A., M. Mandel, and G. C. Cheu.** 1965. Optimal conditions for mutagenesis by N-methyl-N-nitro-N-nitroguanidine in *E. coli* K-12. Biochem. Biophys. Res. Commun. **18:**788–795.
2. **Adlerberth, I., L. Å. Hanson, C. Svanborg, A.-M. Svennerholm, S. Nordgren, and A. E. Wold.** 1995. Adhesins of *Escherichia coli* associated with extraintestinal pathogenicity confer binding to colonic epithelial cells. Microb. Pathog. **18:**373–385.
3. **Berg, R. D.** 1980. Inhibition of *Escherichia coli* translocation from the gastrointestinal tract by normal cecal flora in gnotobiotic or antibiotic-decontaminated mice. Infect. Immun. **29:**1073–1081.
4. **Carlstedt-Duke, B., T. Midtvedt, C. E. Nord, and B. E. Gustafsson.** 1986. Isolation and characterization of a mucin-degrading strain of *Peptostreptococcus* from rat intestinal tract. Acta Pathol. Microbiol. Immunol. Scand. **94:**293–300.
5. **Caugant, D. A.** 1983. Enzyme polymorphism in *Escherichia coli*: genetic structure of intestinal populations: relationships with urinary tract infection strains and with *Shigella.*
6. **Caugant, D. A., B. R. Levin, and R. K. Selander.** 1981. Genetic diversity and temporal variation in the *E. coli* population of a human host. Genetics **98:**467–490.
7. **Duguid, J. P., and R. R. Gillies.** 1957. Fimbriae and adhesive properties in dysentery bacilli. J. Pathol. Bacteriol. **74:**397–411.
8. **Eisenstein, B. I.** 1981. Phase variation of type 1 fimbriae in *Escherichia coli* is under transcriptional control. Science **214:**337–339.
9. **Guiot, H. F. L.** 1982. Role of competition for substrate in bacterial antagonism in the gut. Infect. Immun. **38:**887–892.
10. **Gustafsson, B. E.** 1959. Light weight stainless steel systems for rearing germfree animals. Ann. N. Y. Acad. Sci. **78:**17–28.
11. **Gustafsson, B. E., and B. Carlstedt-Duke.** 1984. Intestinal water-soluble mucin in germfree, exgermfree and conventional animals. Acta Pathol. Microbiol. Immunol. Scand. **92:**247–252.
12. **Gustafsson, B. E., B. Carlstedt-Duke, and C. E. Nord.** 1981. Mucosa related intestinal bacteria and host metabolism, p. 249–254. *In* S. Sasaki, A. Ozawa, and K. Hashimoto (ed.), Recent advances in germfree research. Tokai University Press, Tokyo.
13. **Hagberg, L., R. Hull, S. Hull, S. Falkow, R. Freter, and C. Svanborg Edén.** 1983. Contribution of adhesion to bacterial persistence in the mouse urinary tract. Infect. Immun. **40:**265–272.
14. **Hoskins, L. C., and N. Zamcheck.** 1968. Bacterial degradation of gastrointestinal mucins. I. Comparison of mucus constituents in the stools of germfree and conventional rats. Gastroenterology **54:**210–217.
15. **Korhonen, T. K., M. V. Valtonen, J. Parkkinen, V. Väisänen-Rhen, J. Finne, F. Ørskov, I. Ørskov, S. B. Svenson, and P. H. Mäkelä.** 1985. Serotypes, hemolysin production, and receptor recognition of *Escherichia coli* strains associated with neonatal sepsis and meningitis. Infect. Immun. **48:**486–491.
16. **Krogfelt, K. A., B. A. McCormick, R. L. Burghoff, D. C. Laux, and P. S. Cohen.** 1991. Expression of *Escherichia coli* F-18 type 1 fimbriae in the streptomycin-treated mouse large intestine. Infect. Immun. **59:**1567–1568.
17. **Parkkinen, J., K. Korhonen, A. Pere, J. Hacker, and S. Sonila.** 1988. Binding sites in the rat brain for *Escherichia coli* S fimbriae associated with neonatal meningitis. J. Clin. Invest. **81:**860–865.
18. **Rhen, M., P. H. Mäkelä, and T. K. Korhonen.** 1983. P fimbriae of Escherichia coli are subject to phase variation. FEMS Microbiol. Lett. **19:**267–261.
19. **Sarff, L. D., G. H. McCracken, M. S. Schiffer, M. P. Glode, J. R. Robbins, I. Ørskov, and F. Ørskov.** 1975. Epidemiology of *Escherichia coli* K1 in healthy and diseased. Lancet **i:**1099–1104.
20. **Sears, H. J., and I. Brownlee.** 1951. Further observations on the persistence of individual strains of *Escherichia coli* in the intestinal tract of man. J. Bacteriol. **63:**47–57.
21. **Sears, H. J., I. Brownlee, and J. K. Uchiyama.** 1949. Persistence of individual strains of *Escherichia coli* in the intestinal tract of man. J. Bacteriol. **59:**293–301.
22. **Sears, H. J., H. Janes, R. Saloum, I. Brownlee, and L. F. Lamoreaux.** 1956. Persistence of individual strains of *Escherichia coli* in man and dog under varying conditions. J. Bacteriol. **71:**370–372.
23. **Steffen, E. K., R. D. Berg, and E. A. Deitch.** 1988. Comparison of translocation rates of various indigenous bacteria from the gastrointestinal tract to the mesenteric lymph node. J. Infect. Dis. **157:**1032–1038.
24. **Svanborg-Edén, C., R. Freter, L. Habgerg, R. Hull, S. Hull, H. Leffler, and G. Schoolnik.** 1982. Inhibition of experimental ascending urinary tract infection by receptor analogues. Nature (London) **298:**560–562.
25. **Tancrède, C.** 1992. Role of human microflora in health and disease. Eur. J. Clin. Microbiol. Infect. Dis. **11:**1012–1015.
26. **Tullus, K., I. Ørskov, F. Ørskov, and R. Möllby.** 1992. The importance of P and type 1 fimbriae for the persistence of *Escherichia coli* in the human gut. Epidemiol. Infect. **108:**415–421.
27. **Tullus, K., and P. Sjöberg.** 1986. Epidemiological aspects of P-fimbriated *Escherichia coli*. II. Variations in incidence of *E. coli* infections in children attending a neonatal ward. Acta Paediatr. Scand. **75:**205–210.
28. **Wadolkowski, E. A., D. C. Laux, and P. S. Cohen.** 1988. Colonization of the streptomycin-treated mouse large intestine by a human fecal *Escherichia coli* strain: role of growth in mucus. Infect. Immun. **56:**1030–1035.
29. **Weiser, M. M.** 1973. Intestinal epithelial cell surface membrane glycoprotein synthesis. I. An indicator of cellular differentiation. J. Biol. Chem. **248:**2536–2541.
30. **Wold, A. E., D. A. Caugant, G. Lidin-Janson, P. de Man, and C. Svanborg.**

1992. Resident colonic *Escherichia coli* strains frequently display uropathogenic characteristics. J. Infect. Dis. **165:**46–52.
31. **Wold, A. E., M. Thorssén, S. Hull, and C. Svanborg Eden.** 1988. Attachment of *Escherichia coli* via mannose- or Galα1→4Galβ-containing receptors to human colonic epithelial cells. Infect. Immun. **56:**2531–2537.
32. **Wullenweber, W., L. Beutin, S. Zimmermann, and C. Jonas.** 1993. Influence of some bacterial and host factors on colonization and invasiveness of *Escherichia coli* K1 in neonatal rats. Infect. Immun. **61:**2138–2144.
33. **Zafiri, D., Y. Oron, B. I. Eisenstein, and I. Ofek.** 1987. Growth advantage and enhanced toxicity of *Escherichia coli* adherent to tissue culture cells due to restricted diffusion of products secreted by the cells. J. Clin. Invest. **79:**1210–1216.

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