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Colonization of the Streptomycin-Treated Mouse Large Intestine by a Human Fecal *Escherichia coli* Strain: Role of Growth in Mucus

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The relative colonizing abilities of *Escherichia coli* F-18, isolated from the feces of a healthy human, and *E. coli* F-18col$^-$, a strain derived from it which does not make the *E. coli* F-18 colicin, were studied. In a previous report, it was shown that when each strain was fed individually to streptomycin-treated mice, at approximately 10^{10} CFU per mouse, each colonized the large intestine at between 10^7 and 10^8 CFU/g of feces indefinitely. However, when simultaneously fed to mice, although *E. coli* F-18 colonized at about 10^8 CFU/g of feces, *E. coli* F-18col$^-$ dropped to a level of 10^3 CFU/g of feces within 3 to 5 days. In the present investigation, we show that when given enough time to establish a state of colonization, *E. coli* F-18col$^-$ persists in feces in high numbers despite subsequent challenge by *E. coli* F-18. Therefore, a major defect in the ability of *E. coli* F-18col$^-$ to colonize in the presence of *E. coli* F-18 appears to be in initiating that state. In addition, when mucus was scraped from the cecal wall and, without further treatment, was inoculated with *E. coli* F-18 or F-18col$^-$, both strains grew well. However, when cecal mucus was inoculated with both strains simultaneously, *E. coli* F-18 grew far more rapidly than *E. coli* F-18col$^-$. Moreover, neither strain grew in cecal luminal contents. Together, these data suggest the possibility that both *E. coli* F-18 and F-18col$^-$ must grow in mucus to colonize the streptomycin-treated mouse large intestine, that *E. coli* F-18 col$^-$ is eliminated by *E. coli* F-18 because it does not grow in mucus as well as *E. coli* F-18, and that *E. coli* F-18col$^-$ can resist elimination by *E. coli* F-18 if it is allowed enough time to establish itself within the mucus layer.

Electron microscopic evidence has shown that many of the resident microflora of the mammalian large intestine are found associated with the mucus layer on the intestinal wall (11, 26). Despite this, little is known about the role of mucus in bacterial colonization of the intestinal tract. It has been suggested that in some instances mucus may serve as a source of nutrients (13) and that in other instances it may enhance colonization by serving as an initial attachment site for bacteria (7) or as a matrix for permanent bacterial attachment (27). On the other hand, it has been suggested that the mucus layer may in some instances be an effective barrier, providing protection against penetration by invading microorganisms (7, 27).

Recently we reported that when *Escherichia coli* F-18, isolated from the feces of a healthy human, and its derivative *E. coli* F-18col$^-$, which lacks an 86-kilobase plasmid and no longer makes the *E. coli* F-18 colicin, were individually fed to streptomycin-treated mice, both strains colonized at between 10^7 and 10^8 CFU/g of feces (4). However, when simultaneously fed to streptomycin-treated mice, *E. coli* F-18 colonized at about 10^8 CFU/g of feces, whereas *E. coli* F-18col$^-$ colonized at a level of only 10^3 CFU/g of feces (4). In this study, we present evidence which suggests that growth in cecal mucus may be required for both *E. coli* F-18 and F-18col$^-$ colonization, that when the two strains are present in cecal mucus together *E. coli* F-18 grows better than *E. coli* F-18col$^-$, and that *E. coli* F-18col$^-$ can persist in the mouse large intestine despite its poor ability to grow in mucus if it is given enough time to establish the state of colonization before challenge with *E. coli* F-18.

MATERIALS AND METHODS

Bacteria. *E. coli* F-18 was isolated from the feces of a healthy University of Rhode Island student by the method of Dufour et al. (5) and was confirmed as *E. coli* by the API 20E series of biochemical tests (Analytab Products, Planview, N.Y.). The strain produces a trypsin-sensitive colicin, is motile, and contains seven plasmids (4). *E. coli* F-18col$^-$, isolated from *E. coli* F-18 as described previously (4), lacks an 86-kilobase plasmid and does not make the *E. coli* F-18 colicin (4). Although neither strain hemagglutinates human type A erythrocytes or is retained on phenyl Sepharose (4), suggesting that the strains do not contain type I pili (16), electron microscopic examination of *E. coli* F-18 and F-18col$^-$ revealed that both strains are piliated (30). A spontaneous double mutant of *E. coli* F-18 that is resistant to both streptomycin (Str^r) and rifampin (Rif^r) and a spontaneous double mutant of *E. coli* F-18col$^-$ that is both Str^r and resistant to nalidixic acid (Nal^r), isolated previously (4), were used in this study.

Mouse colonization experiments. The method used to distinguish the relative colonizing abilities of *E. coli* strains in mice has been described previously (19, 20). Briefly, CD-1 male mice (5 to 8 weeks old) were given drinking water containing streptomycin sulfate (5 g/liter). After 1 day of streptomycin treatment, the count of facultative bacteria dropped from approximately 10^8 to less than 10^2 CFU/g of feces, whereas the count of anaerobic bacteria remained constant at about 10^9 CFU/g of feces (19, 20). Mice were then starved from 18 to 24 h for food and water; depending on the experiment, one of the following protocols was employed.

(i) Challenge with 10^3 CFU of *E. coli* F-18 and 10^8 CFU of *E. coli* F-18col$^-$. After overnight starvation, three individually housed mice were fed 10^3 CFU of *E. coli* F-18 and 10^8 CFU of *E. coli* F-18col$^-$ simultaneously in 1 ml of sterile 20% (wt/vol) sucrose. The mice drank the bacterial suspension almost immediately and were then returned to their normal diet (Charles River Valley Rat, Mouse and Hamster Formula) including drinking water containing streptomycin

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sulfate. The following day and at 24- or 48-h intervals as indicated, fecal samples no older than 24 h were collected, homogenized, diluted, and plated (4, 19). In all colonization experiments, plates were incubated at 37°C from 18 to 24 h. Colonizing ability was assessed by the level at which a strain persisted in feces. Each colonization experiment reported in this study was performed at least twice with essentially identical results.

(ii) Precolonization with *E. coli* F-18col$^-$ followed by challenge with *E. coli* F-18. Three individually housed, starved mice were fed 10^{10} CFU of *E. coli* F-18col$^-$. They were then returned to their normal diets as described above. The next day and at 48-h intervals thereafter, 1 g of feces no older than 24 h was collected from each mouse, homogenized, diluted, and plated on selective agar media as described previously (4, 19). On day 10, the mice were again held overnight without food and water and the next morning were fed 10^{10} CFU of *E. coli* F-18. The mice were then returned to their normal diet, including drinking water containing streptomycin sulfate; the following day and at 48-h intervals, feces no older than 24 h were collected from each mouse, homogenized, diluted, and plated on selective agar media.

(iii) Individual or simultaneous challenge with 10^4 CFU of *E. coli* F-18 and *E. coli* F-18col$^-$. The morning after food and water were withheld, three individually housed mice were fed about 10^4 CFU of *E. coli* F-18 and *E. coli* F-18col$^-$ either one strain at a time or simultaneously. The animals were then returned to their normal diets including drinking water containing streptomycin; the next morning and at 24-h intervals thereafter fecal samples no older than 24 h were collected, homogenized, diluted, and plated.

Determination of *E. coli* on cecal epithelial cells and in cecal luminal contents. After food and water were withheld as described above, nine individually housed mice were fed 10^{10} CFU of *E. coli* F-18 and *E. coli* F-18col$^-$ either one stain at a time or simultaneously. The animals were then returned to their normal diet including drinking water containing streptomycin sulfate. At the times indicated, three animals were sacrificed and their entire colons and ceca were removed. Cecal luminal contents from each mouse were collected, homogenized, and plated on selective agar medium. Epithelial cells from three segments of the colon from each mouse, approximately 3 mm in length, and from the entire cecum were prepared by the method of Weiser (31). Microscopically, each epithelial cell was surrounded by mucus. The final suspension of each sample was in 3 ml of phosphate-buffered saline (pH 7.4). Typically, each segment from a colon yielded about 10^6 epithelial cells, whereas an entire cecum yielded about 10^7 epithelial cells. Epithelial cells were homogenized, diluted, and plated on selective agar media. The plates were incubated at 37°C from 18 to 24 h.

Growth in cecal mucus and cecal luminal contents. The ceca from 15 streptomycin-treated CD-1 male mice (5 to 8 weeks old) were removed, and their contents were collected. Routinely, the yield of cecal luminal contents was about 10 ml. To obtain mucus, the ceca were then rinsed briefly in sterile distilled water to remove fecal matter and debris and drained, and the walls of the tissue were gently scraped. Typically, 2 ml of crude extremely viscous mucus was obtained from 15 animals. Samples (2 ml) of cecal contents and cecal mucus preparations were each inoculated with 0.1 ml of HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid)–Hanks buffer (pH 7.4) containing about 10^4 CFU of *E. coli* F-18 and *E. coli* F-18col$^-$ either individually or simultaneously. They were then incubated at 37°C; at the times indicated, a 0.5-ml sample was removed, added to 0.5 ml of HEPES-Hanks buffer (pH 7.4), homogenized, diluted, and plated on selective agar media. All plates in growth studies were incubated at 37°C.

Media and antibiotics. L broth was made as described by Revel (25). L agar is L broth containing 12 g of Bacto-Agar (Difco Laboratories, Detroit, Mich.) per liter. MacConkey agar (Difco) was prepared according to package instructions. Streptomycin sulfate, rifampin, and nalidixic acid were purchased from Sigma Chemical Co., St. Louis, Mo.

Statistics. Where indicated, means derived from triplicate samples were compared by Student's *t* test (*P* values). Portions of the data in Fig. 1 and 2 were analyzed by regression analysis.

RESULTS

In vivo colonization. Recently, we reported that when 10^{10} CFU each of *E. coli* F-18 and F-18col$^-$ were simultaneously fed to streptomycin-treated mice, *E. coli* F-18 persisted in feces at 10^8 CFU/g, whereas *E. coli* F-18col$^-$ dropped from about 10^8 to about 10^3 CFU/g of feces within 3 to 5 days and remained at that level indefinitely (4). Each of the strains colonized at levels of between 10^7 and 10^8 CFU/g of feces when fed individually to mice (4). These results showed that *E. coli* F-18col$^-$ could colonize the mouse intestine alone but was a poor colonizer when fed to mice with its parent. To determine whether *E. coli* F-18col$^-$ was defective in its ability to either initiate or maintain the state of colonization, additional colonization studies were performed.

(i) Competition in mice fed 10^3 CFU of *E. coli* F-18 and 10^8 CFU of *E. coli* F-18col$^-$. Streptomycin-treated mice were simultaneously fed 10^3 CFU of *E. coli* F-18 and 10^8 CFU of *E. coli* F-18col$^-$. *E. coli* F-18col$^-$ remained at 10^7 CFU/g of feces for 4 days, during which time *E. coli* F-18 increased from 10^4 CFU/g of feces on day 1 to 10^7 CFU/g of feces on day 4 (Fig. 1). The level of *E. coli* F-18 remained constant at about 10^7 CFU/g of feces thereafter. In contrast, during the next week *E. coli* F-18col$^-$ dropped rapidly to about 10^3 CFU/g of feces and remained at that level thereafter (Fig. 1).

(ii) Precolonization of mice with *E. coli* F-18col$^-$ before challenge with *E. coli* F-18. Streptomycin-treated mice were fed 10^{10} CFU of the poor colonizer *E. coli* F-18col$^-$ and on day 10 postfeeding were challenged with 10^{10} CFU of *E. coli* F-18. For the next 12 days *E. coli* F-18col$^-$ remained in feces at between 10^7 and 10^8 CFU/g (Fig. 2), i.e., it was not eliminated as observed in Fig. 1. Therefore, at some time between days 4 and 10 *E. coli* F-18col$^-$ stabilized such that it became more resistant to challenge by *E. coli* F-18.

As a control, an *E. coli* F-18col$^-$ colony isolated from feces on day 17 (Fig. 2) was grown and simultaneously fed to mice along with *E. coli* F-18. This isolate was as poor a colonizer relative to *E. coli* F-18 as its parent (data not shown), suggesting that *E. coli* F-18col$^-$ did not persist in feces because the mouse intestine selected for revertants with better colonizing abilities.

(iii) Colonization of *E. coli* F-18 (10^4 CFU) and *E. coli* F-18col$^-$ (10^4 CFU) fed to mice individually and simultaneously. When streptomycin-treated mice were fed 10^4 CFU of *E. coli* F-18, it grew such that a level of 10^8 CFU/g of feces was reached within 24 h (data not shown). When 10^4 CFU of *E. coli* F-18col$^-$ were fed to mice, they also grew to a level of about 10^8 CFU/g of feces (Table 1). However, when mice were simultaneously fed 10^4 CFU each of *E. coli* F-18 and F-18col$^-$ (Table 1), *E. coli* F-18 grew to about 10^8 CFU/g of feces within 24 h, whereas *E. coli* F-18col$^-$ was found at

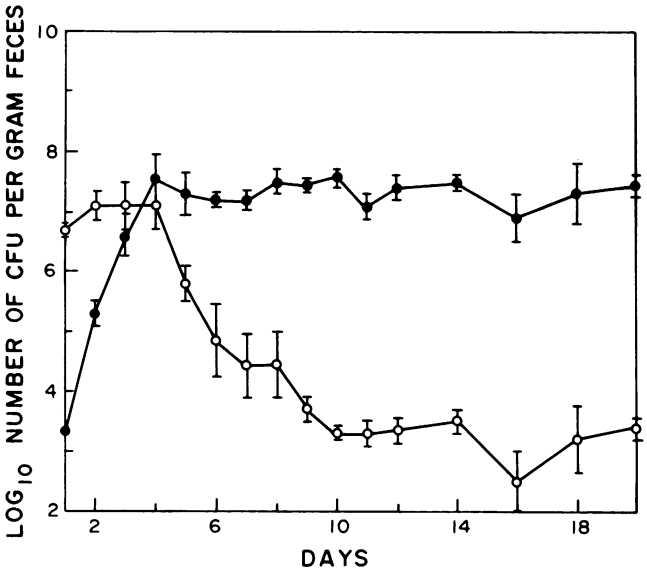

FIG. 1. Competition in mice fed *E. coli* F-18 and F-18col$^-$. Three streptomycin-treated mice were simultaneously fed 10^3 CFU of *E. coli* F-18 (Str^r Rif^r) and 10^8 CFU of *E. coli* F-18col$^-$ (Str^r Nal^r). At the times indicated, fecal samples were plated on L agar containing 100 μg of streptomycin sulfate per ml and 50 μg of rifampin per ml and on MacConkey agar containing 100 μg of streptomycin sulfate per ml and 50 μg of nalidixic acid per ml. The bar at each data point represents the standard error of the geometric mean of the CFU per gram of feces. Symbols: ●, *E. coli* F-18; ○, *E. coli* F-18col$^-$. The slope of the regression line for *E. coli* F-18col$^-$ between days 4 and 10 ± 95% confidence limits around the line is −0.65 ± 0.16 order of magnitude in CFU per gram of feces per day.

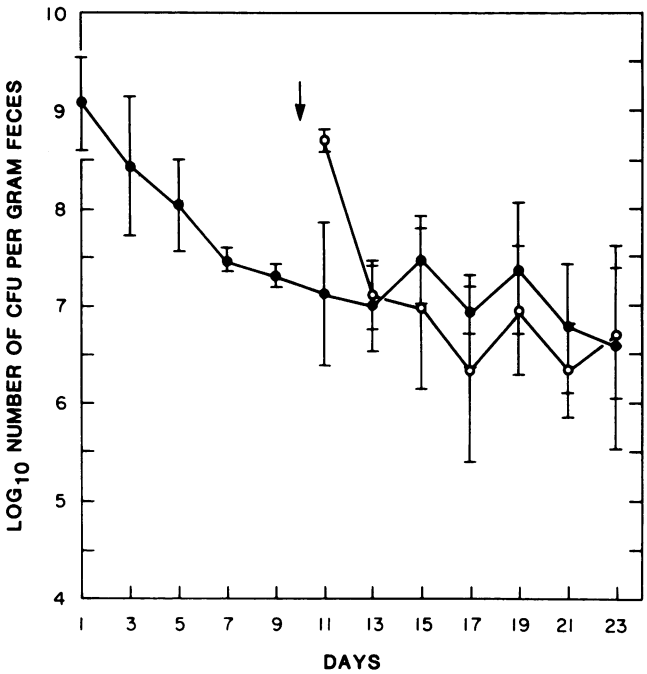

FIG. 2. Stability of *E. coli* F-18col$^-$ in vivo when fed to mice before *E. coli* F-18. Three streptomycin-treated mice were fed 10^{10} CFU of *E. coli* F-18col$^-$ (Str^r Nal^r) (●). At the indicated time (arrow), the same mice were fed 10^{10} CFU of *E. coli* F-18 (Str^r Rif^r) (○). At the times indicated, fecal samples were plated on L agar containing 100 μg of streptomycin sulfate per ml and 50 μg of rifampin per ml or on MacConkey agar containing 100 μg of streptomycin sulfate per ml and 50 μg of nalidixic acid per ml. The bar at each data point represents the standard error of the geometric mean of the CFU per gram of feces. The slope of the regression line for *E. coli* F-18col$^-$ between days 11 and 23 ± 95% confidence limits around the line is −0.036 ± 0.054 order of magnitude in CFU per gram of feces per day.

only 10^4 CFU/g of feces ($P < 0.001$). By the next day, *E. coli* F-18col$^-$ had dropped to an undetectable number (i.e., less than 10^2 CFU/g of feces), whereas *E. coli* F-18 persisted at about 10^8 CFU/g of feces (Table 1). Therefore, although *E. coli* F-18col$^-$ was able to grow in the intestine when 10^4 CFU were fed alone to mice, it was unable to grow well enough to maintain itself in the intestine when fed to mice along with 10^4 CFU of *E. coli* F-18.

Location of *E. coli* F-18 and F-18col$^-$ in the mouse cecum. Nine streptomycin-treated mice were individually or simultaneously fed 10^{10} CFU each of *E. coli* F-18 and F-18col$^-$ (Tables 2 and 3). Three mice were sacrificed at the times indicated, and cecal luminal contents were collected and assayed for viable counts (see Materials and Methods). Colonic and cecal epithelial cells were also isolated and assayed for viable counts. The numbers of either strain found on cecal epithelial cells were at least 10 times greater than the number found on epithelial cells from the proximal, middle, and distal sections of the colon. Furthermore, the ratios of the two strains on colonic epithelial cells at all times mimicked the cecal data. Therefore, only the results from cecal contents and cecal epithelial cells are presented.

When *E. coli* F-18 was fed to streptomycin-treated mice alone, the number of CFU on epithelial cells was between 10^7 and 10^8 CFU per cecum throughout the 72-h period after feeding (Table 2). When *E. coli* F-18col$^-$ was fed to streptomycin-treated mice alone, the number of CFU on epithelial cells was between about 5 and 20 times less than the number of *E. coli* F-18 CFU on epithelial cells during the same time period, i.e., not very different (Table 2). Similarly, between about 5 and 20 times as many *E. coli* F-18 as *E. coli* F-18col$^-$ were found in cecal contents (Table 2). In contrast, when mice were simultaneously fed *E. coli* F-18 and F-18col$^-$ (Table 3), although the numbers of *E. coli* F-18 on cecal epithelial cells was again between 10^7 and 10^8 CFU over a 60-h period, the numbers of *E. coli* F-18col$^-$ on epithelial cells declined sharply such that by 60 h the ratio of *E. coli* F-18 to *E. coli* F-18col$^-$ was greater than 3,000 to 1 ($P < 0.001$). The increasing ratio of *E. coli* F-18 to *E. coli* F-18col$^-$ on cecal epithelial cells was also reflected in cecal luminal contents (Table 3).

Growth of *E. coli* F-18 and F-18col$^-$ in cecal mucus and cecal luminal contents. Cecal mucus and cecal luminal con-

TABLE 1. Colonizing abilities of 10^4 CFU of *E. coli* F-18 and F-18col$^-$

Day	Log_{10} no. of CFU/g of feces (mean ± SE)		
	E. coli F-18col$^-$		*E. coli* F-18
	Fed to mice alone[a]	Fed to mice with *E. coli* F-18[b]	Fed to mice with *E. coli* F-18col$^-$[b]
1	7.54 ± 0.73	3.90 ± 0.02	8.14 ± 0.32
2	8.15 ± 0.68	<2.0	7.72 ± 0.22

[a] Three streptomycin-treated mice were each fed 10^4 CFU of *E. coli* F-18col$^-$. Fecal samples were diluted and plated as described in Materials and Methods.

[b] As in footnote *a*, except that three mice were fed 10^4 CFU each of *E. coli* F-18 and *E. coli* F-18col$^-$.

TABLE 2. Determination of *E. coli* F-18 and F-18col$^-$ on cecal epithelial cells and in cecal luminal contents after strains were fed individually to streptomycin-treated mice

Sample	Time (h)	Log_{10} CFU (mean ± SE)		F-18/F-18col$^-$ ratio
		F-18	F-18col$^-$	
Epithelial cells	24	7.76 ± 0.20	6.56 ± 0.15	15.5
	48	6.98 ± 0.28	6.37 ± 0.18	4.0
	72	7.65 ± 0.41	6.29 ± 0.28	22.6
Luminal contents	24	9.62 ± 0.40	9.14 ± 0.05	3.0
	48	9.15 ± 0.24	8.38 ± 0.17	5.8
	72	9.49 ± 0.05	8.31 ± 0.41	15.1

[a] Strains F-18 and F-18col$^-$ (10^{10} CFU of each) were fed individually to nine streptomycin-treated mice. At the indicated times, three mice were sacrificed, and cecal epithelial cells and cecal luminal contents were plated as described in Materials and Methods.

tents were inoculated with either *E. coli* F-18col$^-$ alone (between 10^3 and 10^4 CFU/ml) or simultaneously with *E. coli* F-18 and F-18col$^-$ (between 10^3 and 10^4 CFU of each per ml). Alone, *E. coli* F-18col$^-$ grew in cecal mucus to a level of about 10^8 CFU/ml (Table 4), i.e., as well as *E. coli* F-18 (data not shown). However, in the presence of *E. coli* F-18, *E. coli* F-18col$^-$ grew poorly, such that by 42 h the ratio of *E. coli* F-18 to *E. coli* F-18col$^-$ was about 10,000 to 1 (Table 5). Neither strain grew in cecal luminal contents (Tables 4 and 5).

DISCUSSION

The large and small intestinal walls consist of an epithelium composed of brush border epithelial cells and mucus-secreting goblet cells (2, 21). The mucus layer is a thick (up to 400 μm), viscous covering that contains mucin, a 2-megadalton glycoprotein and many smaller components, including glycoproteins, glycolipids, and lipids (1, 6, 17, 22, 23, 28, 29). Many of the smaller components of mucus are presumably shed from the epithelial cells (22, 23, 32). The mucus layer is in a state of equilibrium, continuously synthesized by the goblet cells and actively degraded by the indigenous microflora (13, 21). Degraded mucus components are shed into the lumen and eventually find their way into feces (13, 14, 29, 33).

The data presented here are consistent with the possibility that, to colonize the streptomycin-treated mouse large intestine, both strains must grow in cecal mucus since neither strain grows to any great extent in cecal luminal contents in vitro, yet both grow very well in vitro in cecal mucus (Tables 4 and 5). If so, when the strains are fed individually to mice, the CFU found in cecal luminal contents are CFU contained in sloughed cecal mucus. Furthermore, *E. coli* F-18col$^-$ is clearly at a disadvantage in its ability to grow in cecal mucus when placed in direct competition with *E. coli* F-18 in vitro (Table 5). This defect may explain the poor colonizing ability of *E. coli* F-18col$^-$ relative to *E. coli* F-18 when mice are fed either high or low numbers of both strains simultaneously (Fig. 1 and Table 1) and why *E. coli* F-18col$^-$ is found in low numbers relative to *E. coli* F-18 on the cecal wall in vivo (Table 3).

The question remains, however, as to how *E. coli* F-18col$^-$ can resist elimination when challenged by *E. coli* F-18 10 days after mice are initially fed *E. coli* F-18col$^-$ (Fig. 2). We have shown that this is not because the mouse intestine selects for better-colonizing strains of *E. coli* F-18col$^-$, and it does not appear to be because *E. coli* F-18col$^-$ grows in cecal mucus as well as *E. coli* F-18col$^-$ once it adapts to the intestine, since *E. coli* F-18col$^-$ is almost completely eliminated by *E. coli* F-18 as late as 5 days postfeeding, i.e., at a time when it is growing such that for 4 days it was able to maintain itself at about 10^7 CFU/g of feces (Fig. 1). One possibility is that when given enough time alone (e.g., more than 4 but fewer than 10 days), *E. coli* F-18col$^-$ grows through the mucus layer to the underlying epithelial and mucus-secreting goblet cells, where it has a continuous supply of fresh nutrients. If it also takes several days postfeeding for *E. coli* F-18 to penetrate through the mucus layer, *E. coli* F-18col$^-$ would have no competition during that time and would continue to replicate, colonize in high numbers, and resist elimination. A second possibility is that if given enough time alone *E. coli* F-18col$^-$ can grow through

TABLE 3. Determination of *E. coli* F-18 and F-18col$^-$ on cecal epithelial cells and in cecal luminal contents after simultaneous feeding of both strains to streptomycin-treated mice[a]

Sample	Time (h)	Log_{10} CFU (mean ± SE)		F-18/F-18col$^-$ ratio
		F-18	F-18col$^-$	
Epithelial cells	12	7.36 ± 0.34	6.71 ± 0.38	4.46
	36	7.36 ± 0.16	4.84 ± 0.44	331.00
	60	8.16 ± 0.19	4.62 ± 0.26	3,467.00
Luminal contents	12	9.41 ± 0.16	8.88 ± 0.21	3.39
	36	9.22 ± 0.28	6.95 ± 0.50	186.00
	60	9.87 ± 0.02	6.42 ± 0.03	2,817.00

[a] Nine mice were simultaneously fed 10^{10} CFU each of *E. coli* F-18 and F-18col$^-$. At the indicated times, three mice were sacrificed, and cecal epithelial cells and cecal luminal contents were plated as described in Materials and Methods.

TABLE 4. Growth of *E. coli* F-18col$^-$ in cecal mucus and cecal luminal contents[a]

Time (h)	CFU/ml in:	
	Mucus	Luminal contents
0	1.1×10^4	5.3×10^3
18	4.4×10^7	5.4×10^2
42	1.8×10^8	$<1.0 \times 10^1$

[a] Cecal mucus and cecal luminal contents were inoculated with *E. coli* F-18col$^-$ as described in Materials and Methods; at the indicated times samples were plated on MacConkey agar containing 100 μg of streptomycin sulfate per ml and 50 μg of nalidixic acid per ml.

TABLE 5. Growth of *E. coli* F-18 and F-18col$^-$ together in cecal mucus and cecal luminal contents

Sample	Time (h)	CFU/ml		F-18/F-18col$^-$ ratio
		F-18	F-18col$^-$	
Mucus	0	1.1×10^4	4.5×10^3	2.4
	18	1.1×10^6	4.8×10^3	229
	42	1.7×10^8	1.6×10^4	10,625
Luminal contents	0	6.3×10^3	7.3×10^2	
	18	6.5×10^3	7.0×10^2	
	42	2.5×10^3	$<1.0 \times 10^1$	

[a] Cecal mucus and cecal luminal contents were inoculated with both *E. coli* F-18 and F-18col$^-$. At the indicated times samples were plated on L-agar containing 100 μg of streptomycin sulfate per ml and 50 μg of rifampin per ml and on MacConkey agar containing 100 μg of streptomycin sulfate per ml and 50 μg of nalidixic acid per ml.

the mucus layer, form a more stable association there through adhesion to specific receptors on underlying epithelial cells, and thereby resist elimination after challenge by *E. coli* F-18 despite a lower growth rate in mucus. Each of these possibilities are consistent with the model of Freter et al. for bacterial colonization of the large intestine (8–10).

We have shown elsewhere that *E. coli* F-18 does indeed bind specifically to a 50.5-kilodalton glycoprotein receptor present in colonic mucus in vitro (4a). In the accompanying manuscript, several glycoprotein receptors present in colonic and cecal mucus as well as numerous colonic and cecal epithelial cell brush border receptors specific for *E. coli* F-18 are identified (30). Furthermore, we show that *E. coli* F-18col$^-$ also binds to the same receptors as *E. coli* F-18 but to a far lesser degree (30). Therefore, it appears possible that adhesion of *E. coli* F-18 and F-18col$^-$ to specific mucosal receptors may also play an important role in the relative abilities of these strains to colonize the large intestines of streptomycin-treated mice.

At the present time, we do not know why *E. coli* F-18col$^-$ grows poorly in cecal mucus in the presence of *E. coli* F-18, although it is unlikely that it is caused by the *E. coli* F-18 colicin (15). That is, although *E. coli* F-18col$^-$ no longer makes the *E. coli* F-18 colicin, it remains resistant to it (4). Finally, conditions in cecal luminal contents which have been reported to inhibit bacterial growth and which might be contributing factors here in preventing both *E. coli* F-18 and F-18col$^-$ growth include the presence of hydrogen sulfide (8), bacteriocins (9), short-chain fatty acids (12, 24) and low E_h and pH (3, 18). Whether any of these factors is indeed responsible for the observed poor growth of the strains in cecal luminal contents is presently under investigation.

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