Type 1 Pili Are Not Necessary for Colonization of the Streptomycin-Treated Mouse Large Intestine by Type 1-Piliated *Escherichia coli* F-18 and *E. coli* K-12

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Escherichia coli F-18, an excellent colonizer of the streptomycin-treated mouse large intestine, produces type 1 pili. *E. coli* F-18 FimA⁻, type 1 pilus negative, and *E. coli* F-18 FimH⁻, type 1 pilus positive but adhesin negative, were constructed by bacteriophage P1 transduction of defective *fimA* and *fimH* genes from the *E. coli* K-12 strains ORN151 and ORN133, respectively, into *E. coli* F-18. Adhesion of *E. coli* F-18 to an immobilized mannose-bovine serum albumin glycoconjugate was about sixfold greater than that of either *E. coli* F-18 FimA⁻ or *E. coli* F-18 FimH⁻, and adhesion of *E. coli* F-18 to immobilized cecal epithelial cell brush border membranes was between two- and threefold greater than that of *E. coli* F-18 FimA⁻ or *E. coli* F-18 FimH⁻. When either *E. coli* F-18 FimA⁻ or *E. coli* FimH⁻ was fed to streptomycin-treated mice together with *E. coli* F-18, the pilus-negative and adhesin-negative strains colonized as well as their type 1-piliated parent. Essentially the same result was observed when the type 1-piliated *E. coli* K-12 strain ORN152 was fed to streptomycin-treated mice together with a nearly isogenic K-12 FimA⁻ strain, ORN151. Furthermore, when streptomycin-treated mice were fed *E. coli* F-18 FimA⁻ or *E. coli* F-18 FimH⁻ together with *E. coli* F-18 Col⁻, which also makes type 1 pili but is a poor colonizer relative to *E. coli* F-18 because it grows poorly in mucus in the presence of *E. coli* F-18, the *E. coli* F-18 FimA⁻ and F-18 FimH⁻ strains colonized well (10^6 to 10^7 CFU/g of feces), whereas the number of *E. coli* F-18 Col⁻ in feces decreased rapidly to 10^2 CFU/g of feces. These data show that in streptomycin-treated mice, the inability to produce functional type 1 pili has no effect on the ability of *E. coli* F-18 and *E. coli* K-12 to colonize the large intestine.

At least 85% of *Escherichia coli* strains isolated from the feces of normal humans contain the *fim* operon (alternatively called the *pil* operon), which encodes the genes for type 1-pilus synthesis, and at least 58% express the pilus in broth (5). Type 1 pili mediate adhesion of *E. coli* strains to mannose-containing receptors present on a variety of epithelial cell types, including those in mammalian intestines (10, 35). It has been postulated by various workers that the roles of type 1 pili in the gastrointestinal tract are to bind to intestinal epithelial cells and thereby protect the bacteria from being swept away by normal cleansing mechanisms (e.g., peristalsis) (11, 48), to offer bacteria adhering to epithelial cells a growth advantage by being close to the food supply in the unstirred mucus layer near the epithelial cells (48), and to allow adhesion to large-intestine mucus (39). If any or all of these hypotheses are correct, *E. coli* strains bearing type 1 pili should be better colonizers of mammalian large intestines than their non-type 1-piliated counterparts.

E. coli F-18, isolated from the feces of a healthy human, is an excellent colonizer of the streptomycin-treated mouse large intestine (7, 33). It is found in both cecal mucus and cecal contents in vivo but grows only in cecal mucus in vitro (46), suggesting that it colonizes by growing in cecal mucus in vivo. Moreover, *E. coli* F-18 binds to specific glycoprotein receptors present in cecal and colonic mucus and cecal and colonic brush border membranes isolated from the respective epithelial cells (6, 47). Adhesion to these mucosal components is inhibitable by D-mannose and α-D-methyl mannoside (47), suggesting that type 1 pili may mediate adhesion (43). *E. coli* F-18 is, in fact, piliated (47). Here, we show that the *E. coli* F-18 pili are indeed type 1 but that type

1 pili are not necessary for maximal large-intestine colonizing ability of either *E. coli* F-18 or *E. coli* K-12 in streptomycin-treated mice.

MATERIALS AND METHODS

Bacteria. *E. coli* K-12 strains were all derived from *E. coli* ORN115 (30). ORN152 contains a Tn*5* insertion in a silent region near the *fimH* (*pilE*) gene, making it kanamycin resistant but wild type for type 1 pili. *E. coli* ORN151 contains the tetracycline resistance gene from Tn*10* inserted in the *fimA* (*pilA*) gene, making it pilus negative. The strain will be referred to as ORN151 (FimA⁻). ORN133 (30) contains the neomycin phosphotransferase gene from Tn*5* inserted in the *fimH* (*pilE*) gene, making it adhesin negative and kanamycin resistant (29). It will be referred to as ORN133 (FimH⁻). *E. coli* F-18 was isolated from the feces of a healthy human in 1977 and is an excellent colonizer of the streptomycin-treated mouse large intestine (7, 33). The strain produces colicin V (Dwayne C. Savage, personal communication), and its serotype is rough:K1:H5 (Frits Ørskov and Ida Ørskov, personal communication). *E. coli* F-18 FimA⁻ and *E. coli* F-18 FimH⁻ were made by bacteriophage P1 transduction from ORN151 (FimA⁻) and ORN133 (FimH⁻), respectively. *E. coli* F-18 Col⁻ has the same serotype as *E. coli* F-18 but lacks an 86-kilobase plasmid, does not produce the *E. coli* F-18 colicin, and is a poor colonizer of the streptomycin-treated mouse large intestine (7).

P1 transduction. *E. coli* F-18 is resistant to streptomycin (Str^r) and rifampin (Rif^r) and grows well in minimal broth Davis. ORN151 (FimA⁻) and ORN133 (FimH⁻) are resistant to streptomycin, as well as to tetracycline (Tet^r) and kanamycin (Kan^r), respectively; however, the ORN strains are

polyauxotrophic and do not grow in minimal broth Davis. ORN151 and ORN133 were lysogenized with P1::Tn5 (41) and P1 clr 100 Cm (16), respectively. Lysates were induced at 42°C and used to infect E. coli F-18 at a multiplicity of infection of between 1.0 and 5.0. Putative transductants were selected on Luria agar plates containing 50 μg of rifampin per ml and 10 μg of tetracycline hydrochloride per ml (for E. coli F-18 FimA⁻) or on plates containing rifampin and 40 μg of kanamycin monosulfate per ml (for E. coli F-18 FimH⁻). All putative transductants were found to be lysogenized. Four putative E. coli F-18 FimA⁻ transductants were cured of P1::Tn5 and four putative E. coli F-18 FimA⁻ transductants were cured of P1::Tn5 and four putative E. coli F-18 FimH⁻ transductants were cured of P1 clr 100 Cm by growth at 37°C in the presence of sodium citrate (0.5 mM) to chelate calcium and prevent further P1 infection. All cured transductants were both Str^r and Rif^r, grew well in minimal broth Davis, and behaved as FimA⁻ or FimH⁻ both in binding to a mannose-bovine serum albumin (BSA) glyconjugate and in piliation (see Results). One E. coli F-18 FimA⁻ strain and one E. coli F-18 FimH⁻ strain were chosen for further study.

Mouse colonization experiments. The method used to distinguish the relative colonizing abilities of E. coli strains in mice has been described in detail previously (7, 46). Briefly, after 1 day of being fed streptomycin sulfate in their drinking water (5 g/liter), three male CD-1 mice were starved from 18 to 24 h for food (Charles River Valley Rat, Mouse, and Hamster Formula) and water and fed 10¹⁰ CFU each of the L broth-grown E. coli strains to be tested in 1 ml of sterile 20% (wt/vol) sucrose, as described previously (7). The mice drank the bacterial suspension almost immediately and were then given food and streptomycin-containing drinking water. 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 on selective media as described below. In all colonization experiments, plates were incubated at 37°C for 18 to 24 h. Colonizing ability was assessed by the level at which a strain persisted in feces. Each experiment was performed at least twice with essentially identical results.

To differentiate E. coli F-18 (Str^r Rif^r) from E. coli F-18 FimA⁻ (Str^r Rif^r Tet^r), fecal samples were plated on MacConkey agar containing 10 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, 50 μg of rifampin per ml, and 10 μg of tetracycline hydrochloride per ml. The numbers of E. coli F-18 FimA⁻ were determined directly from the plates containing tetracycline hydrochloride. The numbers of E. coli F-18 were determined each day by toothpicking 50 colonies from the plates without tetracycline hydrochloride to MacConkey agar plates containing tetracycline and determining the fraction of the total count that was tetracycline sensitive. The numbers of E. coli F-18 FimA⁻ per gram of feces calculated from the results of toothpicking agreed very well with the numbers determined by direct plating.

To differentiate E. coli F-18 (Str^r Rif^r) from E. coli F-18 FimH⁻ (Str^r Rif^r Kan^r), fecal samples were plated on MacConkey 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, 50 μg of rifampin per ml, and 40 μg of kanamycin monophosphate per ml. The numbers of E. coli F-18 FimH⁻ were determined directly from the plates containing kanamycin monosulfate. The numbers of E. coli F-18 were determined each day by

toothpicking 50 colonies from the plates without kanamycin monosulfate to MacConkey agar containing kanamycin and determining the fraction of the total count that was kanamycin sensitive. The numbers of E. coli F-18 FimH⁻ per gram of feces calculated from the results of toothpicking agreed very well with the numbers determined by direct plating.

To differentiate E. coli F-18 FimA⁻ (Str^r Rif^r Tet^r) from E. coli F-18 Col⁻ (Str^r Nal^r), fecal samples were plated on MacConkey agar containing 100 μg of streptomycin sulfate per ml, 50 μg of rifampin per ml, and 10 μg of tetracycline hydrochloride per ml and on MacConkey agar containing 100 μg of streptomycin sulfate per ml and 50 μg of nalidixic acid per ml.

To differentiate E. coli F-18 FimH⁻ (Str^r Rif^r Kan^r) from E. coli F-18 Col⁻ (Str^r Nal^r), fecal samples were plated on MacConkey agar containing 100 μg of streptomycin sulfate per ml, 50 μg of rifampin per ml, and 40 μg of kanamycin monosulfate per ml and on MacConkey agar containing 100 μg of streptomycin sulfate per ml and 50 μg of nalidixic acid per ml.

To differentiate E. coli ORN152 (Str^r Kan^r) from the FimA⁻ strain E. coli ORN151 (Str^r Tet^r), fecal samples were plated on MacConkey agar plates containing 100 μg of streptomycin sulfate per ml and 40 μg of kanamycin monosulfate per ml and on MacConkey agar plates containing 100 μg of streptomycin sulfate per ml and 10 μg of tetracycline hydrochloride per ml.

Cecal brush border membrane preparation. Cecal epithelial cells were isolated from 15 mice as described previously (47). The final suspensions contained 4.0 × 10⁶ epithelial cells per ml in 24.5 mM HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid) buffer (pH 7.5) containing 2.5 mM monosodium phosphate and 98 mM sodium chloride. Brush border membranes were prepared from cecal epithelial cells as described previously (47). Final suspensions, containing brush borders from 10⁷ epithelial cells per ml, were in HEPES-Hanks buffer (pH 7.4).

Radioactive labeling of E. coli strains. Cultures of the E. coli F-18 strains were labeled in modified Davis minimal medium as described previously (6, 47). ³⁵SO₄-labeled cultures were washed twice in HEPES-Hanks buffer (pH 7.4) at 4°C and suspended in the same buffer at 37°C at about 10⁹ cells per ml. The specific activity was routinely between 2 × 10⁻³ and 5 × 10⁻³ cpm/CFU, depending on the age of the ³⁵SO₄. The E. coli ORN strains were labeled in modified Davis minimal medium supplemented with 2 μg of thiamine per ml and 50 μg each of L-arginine, L-leucine, and L-threonine per ml.

Electron microscopy. Bacterial preparations were grown overnight in stationary culture at 37°C in brain heart infusion broth, diluted back 100-fold, grown identically in fresh brain heart infusion broth 4 days in succession for maximal type 1-pilus expression, and stained for 1 min with 1% phosphotungstic acid (pH 7.0) on copper grids coated with Formvar and carbon. The samples were examined in a JEOL 1200 SX microscope.

Adhesion to cecal brush membranes. The assay of adhesion to immobilized brush border membranes isolated from 2.5 × 10⁶ epithelial cells has been described in detail previously (47). All assays were performed in triplicate. Briefly, prewarmed ³⁵SO₄-labeled E. coli cells (0.2 ml) were added to wells containing immobilized brush borders. The plates were incubated for 1 h at 37°C, and the wells were then washed twice with HEPES-Hanks buffer (pH 7.4) to remove unbound bacteria. Adherent bacteria were released by adding 0.5 ml of 5% sodium dodecyl sulfate to each well and then
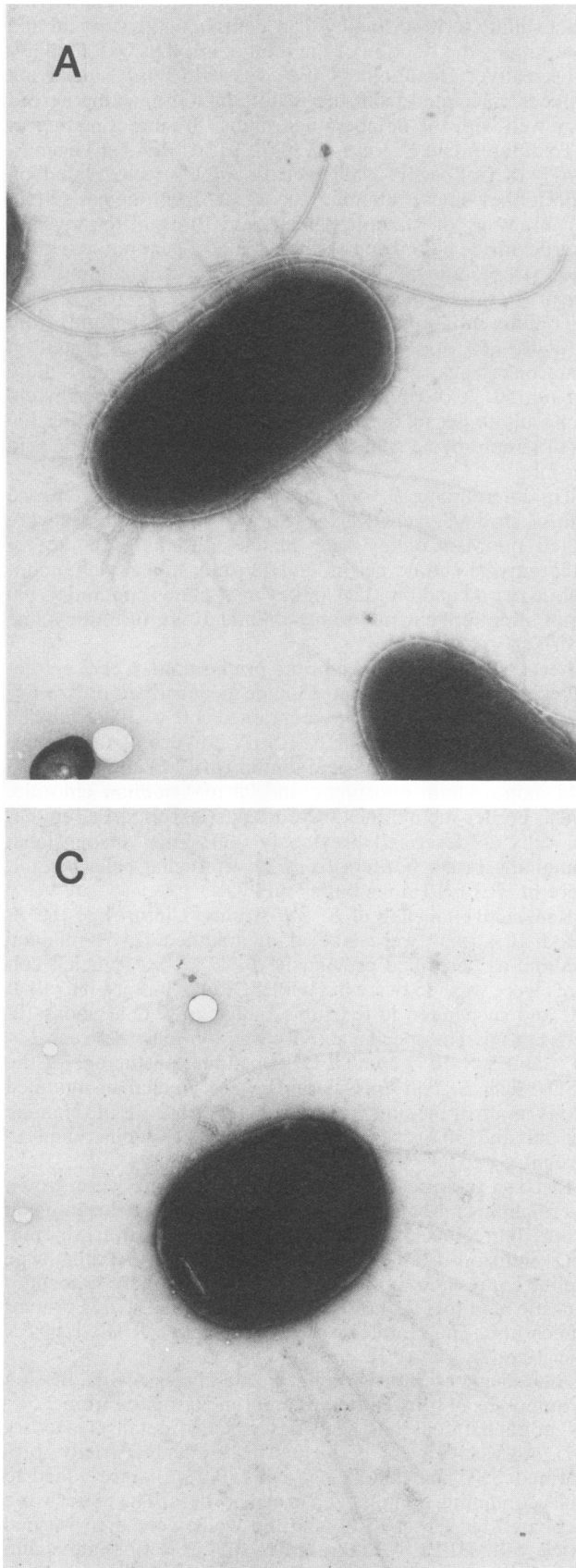
FIG. 1. Electron micrographs of *E. coli* F-18 (A), *E. coli* F-18 FimA⁻ (B), and *E. coli* F-18 FimH⁻ (C).

incubating the plates for 3 h at 37°C. The sodium dodecyl sulfate was removed from each well, and the level of radioactivity was determined by scintillation counting.

Radioactive bacteria released less than 0.5% of the total incorporated label during the 1-h incubation period. Furthermore, subsequent incubation of supernatants failed to produce significant levels of radioactivity adhering to epithelial cells or brush borders (<250 cpm).

Adhesion to BSA glycoconjugates. (Mannose-α-O-CETE)$_n$-BSA, hereafter called Man-BSA, and (CH$_3$-O-CETE)$_n$-BSA, hereafter called CETE-BSA, were immobilized in polystyrene tissue culture wells as described previously (47) [CETE is 2-(2-carbomethoxyethylthio)ethyl]. Adhesion assays using ^{35}SO$_4$-labeled *E. coli* were then performed as described above.

Media and antibiotics. L broth was made as described by Revel (42). L agar is L broth containing 12 g of Bacto-Agar (Difco Laboratories, Detroit, Mich.) per liter. MacConkey agar (Difco) and brain heart infusion broth (Scott Laboratories, Inc., Fiskeville, R.I.) were prepared according to package instructions. Streptomycin sulfate, rifampin, tetracycline hydrochloride, and kanamycin monosulfate were purchased from Sigma Chemical Co., St. Louis, Mo.

Chemicals. All chemicals were reagent grade. Man-BSA and CETE-BSA were purchased from Carbohydrates International, Inc., Arlov, Sweden.

Statistics. When indicated in the text, means derived from triplicate samples were compared by using Student's *t* test (*P* values).

TABLE 1. Adhesion of E. coli F-18 strains

Adhesion to:	Adhesion (cpm [mean ± SE])[a]		
	E. coli F-18	E. coli F-18 FimA⁻	E. coli F-18 FimH⁻
Man-BSA	32,517 ± 781	5,270 ± 704	4,920 ± 640
CETE-BSA	6,911 ± 737	6,013 ± 837	5,738 ± 151
Brush borders	17,151 ± 241	7,851 ± 764	5,267 ± 357
BSA	6,559 ± 131	8,528 ± 513	6,217 ± 450

[a] All adhesion assays were performed in triplicate. The specific activities of the three strains were essentially identical at 6.0×10^{-3} cpm/CFU.

RESULTS

Electron microscopy of E. coli F-18, E. coli F-18 FimA⁻ and E. coli F-18 FimH⁻. E. coli F-18 was bacteriophage P1 transduced with E. coli ORN151 (P1::Tn5) and E. coli ORN133 (P1 clr 100 Cm) lysates. E. coli F-18 FimA⁻ and E. coli F-18 FimH⁻ transductants were selected (see Materials and Methods) and viewed by transmission electron microscopy (Fig. 1). Similarly, an E. coli F-18 Col⁻ FimA⁻ strain selected by P1 transduction was viewed by transmission electron microscopy (not shown). E. coli F-18 FimA⁻ had no pili (Fig. 1B), as reported previously for fimA mutants (22, 37), showing clearly that the pili observed on E. coli F-18 (Fig. 1A) were, in fact, type 1. Similarly, E. coli F-18 Col⁻ FimA⁻ had no pili, showing that its pili (47) are also type 1. Furthermore, pili were observed on E. coli F-18 FimH⁻ (Fig. 1C), as reported previously for E. coli K-12 fimH (i.e, adhesion-negative) mutants (24, 29).

Adhesion of the E. coli F-18 strains and the E. coli ORN strains to Man-BSA and CETE-BSA. E. coli strains bearing type 1 pili bind specifically to receptors containing mannose (22, 37, 43), whereas type 1 pilus-negative (FimA⁻) and pilus-positive but adhesin-negative (FimH⁻) mutants do not (22, 37). To determine whether E. coli F-18, E. coli F-18 FimA⁻, and E. coli F-18 FimH⁻ behaved as expected for type 1-containing, type 1-negative, and adhesin-negative strains, respectively, the E. coli F-18 strains and their E. coli ORN counterparts were tested for their abilities to bind to immobilized Man-BSA and CETE-BSA. As expected for type 1 pilus-bearing strains, adhesion of E. coli F-18 (Table 1) to Man-BSA was about fourfold greater than to CETE-BSA (P < 0.001) and adhesion of E. coli ORN152 (Table 2) to Man-BSA was about threefold greater than to CETE-BSA (P < 0.001). Similarly, as expected for type 1 pilus-negative and adhesin-negative strains, adhesion of E. coli F-18 FimA⁻ and F-18 FimH⁻ (Table 1) and E. coli ORN151 (FimA⁻) and ORN133 (FimH⁻) (Table 2) to Man-BSA and CETE-BSA was about equal (P > 0.10 in each case); i.e., no mannose specificity was observed.

Since the colonization model used in this study requires treating mice with streptomycin, E. coli F-18 and E. coli F-18

TABLE 2. Adhesion of E. coli ORN strains

Adhesion to:	Adhesion (cpm [mean ± SE])[a]		
	E. coli ORN152	E. coli ORN151 (FimA⁻)	E. coli ORN133 (FimH⁻)
Man-BSA	22,752 ± 953	16,469 ± 418	5,823 ± 958
CETE-BSA	7,967 ± 152	6,745 ± 267	5,350 ± 555
Brush borders	12,084 ± 116	4,434 ± 101	1,958 ± 24
BSA	7,019 ± 158	6,549 ± 239	5,531 ± 220

[a] All adhesion assays were performed in triplicate. The specific activities of the three strains were essentially identical at 3.6×10^{-3} cpm/CFU.
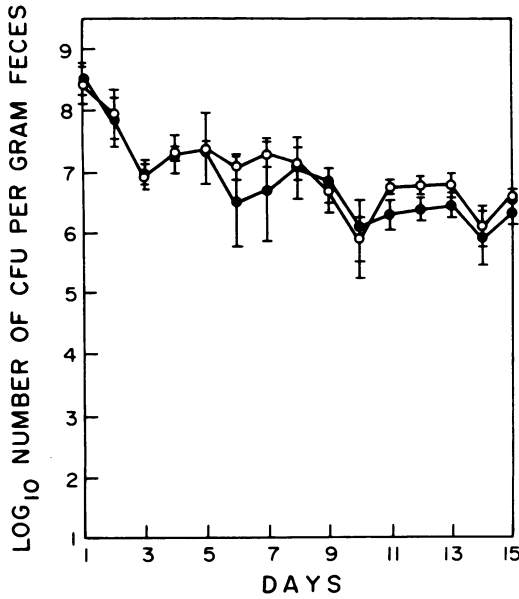
FimA⁻ were grown in the presence of streptomycin sulfate (100 μg per ml), examined microscopically, and tested for their abilities to bind to Man-BSA and CETE-BSA in both the presence and absence of streptomycin sulfate (100 μg/ml). The data obtained were no different from those presented in Fig. 1 and Table 1; i.e., growing E. coli F-18 in streptomycin did not affect type 1-pilus expression nor did the presence of streptomycin alter type 1-pilus adhesive function (data not shown).

Adhesion of the E. coli F-18 strains and the E. coli ORN strains to cecal brush borders. Adhesion of E. coli F-18 to immobilized cecal brush border membranes (Table 1) was about two- and threefold greater than that of E. coli F-18 FimA⁻ and E. coli F-18 FimH⁻, respectively, to immobilized brush border membranes (P < 0.001 in each case). These data suggest that E. coli F-18 type 1 pili bind specifically to cecal brush border receptors and in doing so give E. coli F-18 an advantage in binding relative to E. coli F-18 FimA⁻ and E. coli F-18 FimH⁻. In addition, similar adhesion experiments using the E. coli K-12 strains (Table 2) showed that the type 1-piliated E. coli ORN152 bound approximately three- and sixfold better to cecal brush border membranes than did ORN151 (FimA⁻) and ORN133 (FimH⁻), respectively (P < 0.001 in each case). Also, the presence of streptomycin sulfate (100 μg/ml) did not alter the ability of E. coli F-18 to bind to cecal brush borders (data not shown).

Relative colonizing abilities of E. coli F-18, E. coli F-18 FimA⁻, and E. coli F-18 FimH⁻. The system used to assess the relative colonizing abilities of the E. coli strains tested is a competitive streptomycin-treated mouse model. In this model the normal mouse facultative gram-negative flora is reduced and the relative numbers of the different anaerobic flora are altered by streptomycin treatment (18, 32). Without such treatment, exogenously introduced E. coli strains do not colonize, but with streptomycin treatment, most E. coli strains colonize indefinitely at about 10⁷ CFU/g of feces when fed to mice individually (33, 45). Therefore, we normally feed streptomycin-treated mice two nearly isogenic streptomycin-resistant strains and continue streptomycin treatment. If the two strains are equally fit in the intestine, then both will colonize the large intestine equally well (i.e., both will persist in feces at between 10⁶ and 10⁷ CFU/g) (33). However, if one strain has an advantage over the other, then it will become the dominant organism and persist in feces while the other strain will decrease in feces with time (6, 7, 33). With this in mind, streptomycin-treated mice were fed either E. coli F-18 and E. coli F-18 FimA⁻ (Fig. 2) or E. coli F-18 and E. coli F-18 FimH⁻ (Fig. 3). Under these conditions both E. coli F-18 FimA⁻ and E. coli F-18 FimH⁻ were as good colonizers as their parent, E. coli F-18. That is, each strain colonized at between 10⁶ and 10⁷ CFU/g of feces indefinitely (Fig. 2 and 3). Furthermore, when mice were fed 10⁴ CFU each of E. coli F-18 and E. coli F-18 FimA⁻ or 10⁴ CFU each of E. coli F-18 and E. coli F-18 FimH⁻ (instead of 10¹⁰ CFU), each strain grew equally well in vivo from 10⁴ CFU/g of feces to between 10⁷ and 10⁸ CFU/g of feces within 48 h and colonized at that level indefinitely (data not shown). These data suggest that at any dose E. coli F-18 FimA⁻ and E. coli FimH⁻ colonize the streptomycin-treated mouse large intestine as well as E. coli F-18.

E. coli F-18 and E. coli F-18 FimA⁻ were also fed to streptomycin-treated mice at 10¹⁰ CFU each and allowed to colonize for 17 days. At that time, streptomycin treatment was discontinued and the numbers of each strain in feces were determined at 24-h intervals for the next 11 days.

FIG. 2. Colonization by *E. coli* F-18 (O) and *E. coli* F-18 FimA⁻ (●).

FIG. 4. Colonization by *E. coli* F-18 FimA⁻ (●) and *E. coli* F-18 Col⁻ (O).

During that time, the numbers of both strains dropped from 10^7 CFU/g of feces to 10^4 CFU/g of feces at identical rates (data not shown), suggesting that streptomycin treatment did not prevent *E. coli* F-18 interactions in vivo which would give it an advantage over *E. coli* F-18 FimA⁻ in untreated mice.

Relative colonizing abilities of *E. coli* F-18 FimA⁻, *E. coli* F-18 FimH⁻, and *E. coli* F-18 Col⁻. *E. coli* F-18 Col⁻ is a far worse colonizer of the streptomycin-treated mouse large intestine than its parent, *E. coli* F-18, because it grows poorly in cecal mucus in the presence of *E. coli* F-18 (46). However, *E. coli* F-18 Col⁻ does make type 1 pili and binds specifically to Man-BSA (47). Therefore, to determine whether type 1 pili confer an advantage to *E. coli* F-18 Col⁻,
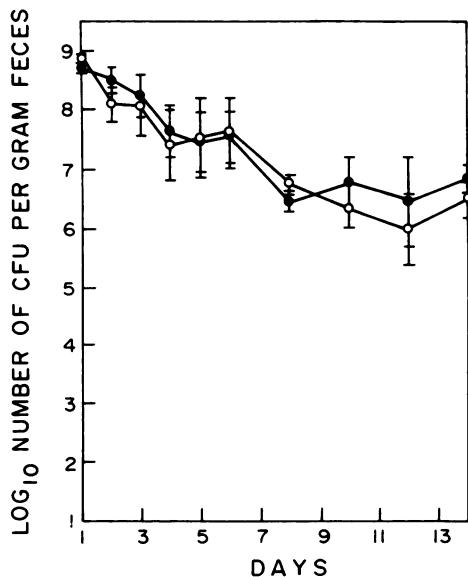
making it a better colonizer relative to the pilus-negative and adhesin-negative strains than it is relative to *E. coli* F-18, streptomycin-treated mice were fed either *E. coli* F-18 FimA⁻ and *E. coli* F-18 Col⁻ (Fig. 4) or *E. coli* F-18 FimH⁻ and *E. coli* F-18 Col⁻ (Fig. 5). Both *E. coli* F-18 FimA⁻ and *E. coli* F-18 FimH⁻, like *E. coli* F-18 (7, 46), were found to be far better colonizers than *E. coli* F-18 Col⁻ (Fig. 4 and 5).

Relative colonizing abilities of the K-12 strains, *E. coli* ORN152 and *E. coli* ORN151 (FimA⁻). Streptomycin-treated mice were fed both the type 1-piliated *E. coli* ORN152 and the nonpiliated *E. coli* ORN151 (FimA⁻). The nonpiliated strain colonized at about 10^6 CFU/g of feces, whereas the

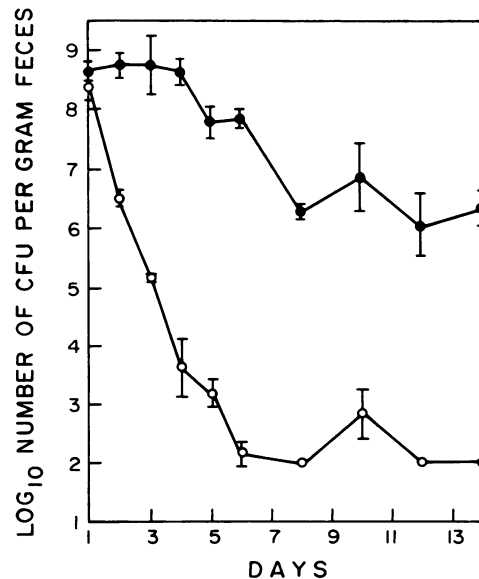FIG. 3. Colonization by *E. coli* F-18 (O) and *E. coli* F-18 FimH⁻ (●).

FIG. 5. Colonization by *E. coli* F-18 FimH⁻ (●) and *E. coli* F-18 Col⁻ (O).
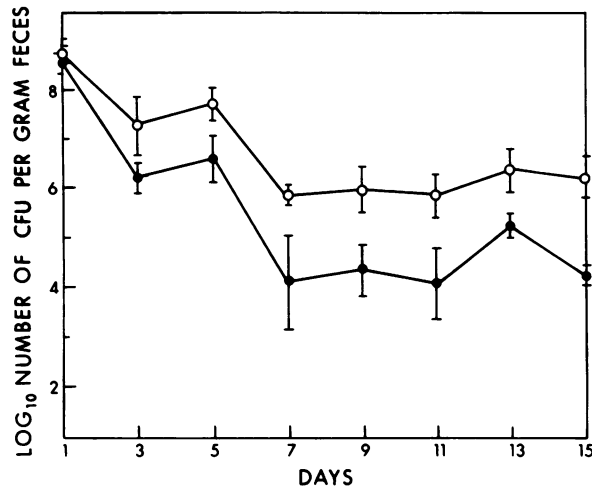
FIG. 6. Colonization by *E. coli* ORN152 (●) and *E. coli* ORN151 (FimA⁻) (○).

type 1-piliated strain colonized at between 10^4 and 10^5 CFU/g of feces (Fig. 6).

DISCUSSION

The data presented here show that functional type 1 pili are not necessary for *E. coli* F-18 to colonize the streptomycin-treated mouse large intestine. That is, transduction of a defective *fimA* gene into *E. coli* F-18 resulted in bald F-18 cells (Fig. 1B), proving that *E. coli* F-18 pili are indeed type 1; but colonizing ability was retained by *E. coli* F-18 FimA⁻ (Fig. 2) despite a twofold drop in its ability to bind to cecal brush border membranes in vitro (Table 1). Furthermore, *E. coli* F-18 FimH⁻, i.e., lacking the type 1 adhesin protein in its pili (2, 3, 25), also retained the colonizing ability of its parent (Fig. 3), despite a threefold drop in ability to bind to cecal brush border membranes in vitro (Table 1). In addition, the FimA⁻ *E. coli* K-12 strain ORN151 colonized the streptomycin-treated mouse large intestine at about an order of magnitude better than its type 1-piliated counterpart, ORN152 (Fig. 6), despite a threefold drop in ability to bind to cecal brush border membranes in vitro (Table 2). We cannot rule out the possibility that this difference reflects an effect of the expression of the tetracycline resistance gene present in ORN151 (FimA⁻) or the kanamycin resistance gene present in ORN152 rather than a slight adverse effect of type 1 pilus expression on the colonizing ability of *E. coli* ORN152. In any case, these data again suggest that type 1 pili do not increase large-intestine colonizing ability. These results are in sharp contrast to the role type 1 pili appear to play in ascending urinary tract infection of mouse and rat bladders (4, 14, 17, 20, 21).

The data reported here do not rule out the possibility that adhesion to cecal brush border membranes is important in the ability of *E. coli* F-18 to colonize the streptomycin-treated mouse large intestine. Human fecal *E. coli* strains often make more than one adhesin (5, 11). Some *E. coli* adhesin genes are on plasmids (11, 13, 15, 38, 40, 44), and others, including those for type 1 pili, are on the chromosome (5, 15, 19, 26–28). Therefore, *E. coli* F-18, which contains seven plasmids (7), may make one or more adhesins other than type 1 pili in vivo that are not made in vitro. If so, adhesion to cecal epithelial cells might still be important for colonization in vivo; i.e., *E. coli* F-18 FimA⁻ and *E. coli*

F-18 FimH⁻ might both make epithelial cell-specific adhesins in vivo which allow them to compete effectively with *E. coli* F-18. However, while this may be a strong possibility for *E. coli* F-18, it is a less likely, although still possible, explanation as to why *E. coli* ORN152 and *E. coli* ORN151 (FimA⁻) colonize about equally well (Fig. 6). That is, K-12 strains are less likely to contain operons for additional adhesins since they are plasmidless and their chromosomes do not contain nucleotide sequences related to the *pap*, *prs*, and *afaI* operons, which encode chromosomal genes for adhesins commonly found in uropathogenic *E. coli* strains (34; Richard Goldstein, personal communication).

If *E. coli* F-18 and *E. coli* ORN152 make no other adhesin in vivo, there are still other possibilities to explain why type 1 pili play no role in their ability to colonize the streptomycin-treated mouse large intestine. For example, it is known that transcription of the *fimA* gene is regulated by the orientation of an invertable 300-base-pair DNA segment which contains the *fimA* promoter (1, 12, 23, 36). If the transcription-off orientation is favored in vivo, *E. coli* F-18 would have no type 1 pili in vivo and therefore no advantage over *E. coli* F-18 FimA⁻ and *E. coli* FimH⁻. Alternatively, it is possible that *E. coli* F-18 expresses type 1 pili in vivo but does not bind to mannose-containing receptors present in epithelial cell border membranes because it binds first to type 1-specific receptors present in cecal mucus (47). If so, *E. coli* F-18, *E. coli* F-18 FimA⁻, and *E. coli* F-18 FimH⁻ might be identical in their abilities to grow in mucus, resist washout, and colonize the large intestine. Specific receptors for various *E. coli* and *Salmonella typhimurium* strains in mucus have, in fact, been implicated in inhibiting adhesion to epithelial cells (8, 9, 31). Experiments designed to test whether *E. coli* F-18 expresses type 1 pili in vivo and, if so, whether type 1-specific receptors present in cecal mucus prevent *E. coli* F-18 from binding to cecal epithelial cells are in progress.

ACKNOWLEDGMENTS

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