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Colonization of the Streptomycin-Treated Mouse Large Intestine by a Human Fecal *Escherichia coli* Strain: Role of Adhesion to Mucosal Receptors

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***Escherichia coli* F-18, a normal fecal isolate, was previously shown to be an excellent colonizer of the streptomycin-treated CD-1 mouse large intestine, whereas *E. coli* F-18col$^-$, a derivative of *E. coli* F-18 that no longer makes the *E. coli* F-18 colicin, was shown to be a poor mouse colonizer. It was also shown that *E. coli* F-18 bound two to three times more soluble colonic mucus protein than did *E. coli* F-18col$^-$ and that a major receptor in CD-1 mouse colonic mucus was a 50.5-kilodalton glycoprotein. In the present investigation, an additional *E. coli* F-18 colonic mucus glycoprotein receptor (66 kilodaltons) and three cecal mucus glycoprotein receptors (94, 73, and 66 kilodaltons) were identified. Numerous colonic and cecal brush border protein receptors specific for *E. coli* F-18 were also identified. Furthermore, *E. coli* F-18col$^-$ was found to bind to the same mucus and brush border receptors as *E. coli* F-18, although to a far lesser extent. Adhesion of both *E. coli* F-18 and F-18col$^-$ was inhibited by D-mannose and α-methyl-D-mannoside, and both strains were shown to bind specifically to the mannose moiety of a mannose-bovine serum albumin glycoconjugate, although again *E. coli* F-18col$^-$ bound to a lesser extent. Finally, both *E. coli* F-18 and F-18col$^-$ were shown to be piliated. The possible role of pilus mediated adhesion in *E. coli* F-18 colonization of the streptomycin-treated mouse large intestine is discussed.**

It has long been recognized that many pathogenic bacteria form close associations with the intestinal mucosa and that the inability to do so results in the rapid elimination of the organism from the gut (9, 32). Mucosal adherence is therefore an early stage of colonization and is necessary for virulence of many microorganisms (17, 21, 32, 38). For example, *Vibrio cholerae* is capable of attachment to brush border surfaces and also possess the ability to penetrate and grow in intestinal mucus (10, 13, 14, 22, 23). Nonchemotactic and nonmotile mutants are unable to penetrate mucus and are far less virulent than wild-type *V. cholerae* (10, 14). Further, enterotoxigenic *Escherichia coli* strains have been shown to bind to brush border preparations (28, 29), and nonbinding mutants derived from them have been shown to be far less virulent (7, 16, 24, 28, 29, 33, 34). Also, *Shigella flexneri* is more virulent for monkeys than guinea pigs, and guinea pig colonic mucus but not monkey colonic mucus has been shown to inhibit the ability of *S. flexneri* to invade HeLa cells in vitro (4).

In contrast to pathogens, little information is available on the role of adhesion in colonization of the mammalian large intestine by normal fecal *E. coli* strains, although it is known that many of the resident microflora are associated with the mucus layer (19, 36). Recently Freter and co-workers, using in vivo experiments, in vitro continuous-flow culture techniques, and mathematical modeling, suggested that when two nearly isogenic strains are able to grow equally well in the lumen of the mouse intestine, under limiting growth conditions, the adherent strain will be the better colonizer since it will be protected against washout in feces (11, 12, 15). Therefore the ability of a bacterium to adhere to intestinal mucosa and grow in the intestine may influence its colonizing ability.

In a recent report, we showed that *E. coli* F-18, isolated from the feces of healthy human, is a far better colonizer of the mouse large intestine than *E. coli* F-18col$^-$, a strain derived from *E. coli* F-18 which no longer makes the *E. coli* F-18 colicin and which lacks an 86-kilobase plasmid, when both strains are fed simultaneously to streptomycin-treated male CD-1 mice (2). In the accompanying manuscript (40), we demonstrate that it is highly likely that both *E. coli* F-18 and F-18col$^-$ colonize the mouse intestine by growing in cecal mucus; i.e., both strains grow well when inoculated alone into cecal mucus, but neither grows in cecal luminal contents in vitro (24). However, although both strains grow well alone in cecal mucus together, *E. coli* F-18 grows well but *E. coli* F-18col$^-$ grows poorly (40). Although this finding explains why *E. coli* F-18 is a better colonizer than *E. coli* F-18col$^-$ when both strains are fed to mice simultaneously, it does not explain the additional finding that *E. coli* F-18col$^-$ resists elimination in the presence of *E. coli* F-18 when given 10 days alone in the mouse intestine before challenge with *E. coli* F-18 (40). One explanation for this result is that, when given enough time alone, *E. coli* F-18col$^-$ binds irreversibly to specific receptors deep within the intestinal mucosa (e.g., to receptors on epithelial cells), which allows it to resist competition from *E. coli* F-18 despite the fact that it grows slower than its parent. In the present investigation, we identify cecal and colonic mucus and brush border receptors specific for *E. coli* F-18. Moreover, we show that although *E. coli* F-18col$^-$ binds to the same receptors, its ability to bind to them is far less.

MATERIALS AND METHODS

Bacteria. The spontaneous double mutant of *E. coli* F-18, resistant to both streptomycin and rifampin, and the spontaneous double mutant of *E. coli* F-18col$^-$, resistant to both streptomycin and nalidixic acid, employed in the accompanying manuscript (40) were used in the present study.

Mucus isolation. Mucus was isolated from the colons or

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ceca of male CD-1 mice (5 to 8 weeks old) as described previously (1, 3). Briefly, the walls of the excised colons or ceca from eight mice were gently scraped into 5 ml of HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) plus Hanks balanced salt solution (HEPES-Hanks buffer), pH 7.4, and contaminating epithelial cells and membranes were centrifuged away once at 12,000 × *g* for 10 min and once at 27,000 × *g* for 15 min. The protein concentration of crude mucus was determined by the method of Lowry et al. (30).

Mucus fractionation. Cecal mucus (20 mg of protein) in approximately 3 ml of HEPES-Hanks buffer (pH 7.4) was fractionated on a Bio-Rad A.5m column (1.5 by 37 cm) as described previously (1, 29). The column was standardized before mucus fractionation as described previously (1, 29).

Preparation of brush borders. Routinely, intestinal cells from the colons and ceca from 12 male CD-1 mice were prepared by the method of Weiser (41). At 24 h before cell isolation, the animals were deprived of food and given sterile water containing streptomycin sulfate (5 g/liter). Brush borders were isolated as described previously (8, 29), and final suspension was in 7 ml of HEPES-Hanks buffer (pH 7.4).

Radioactive labeling of *E. coli* F-18 and F-18col$^-$. Cultures of the *E. coli* strains were labeled in modified Davis minimal medium (MDMM) as described previously (1). $^{35}SO_4$-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 10^9 cells per ml. The specific activity was routinely between 2×10^{-3} and 5×10^{-3} cpm/CFU, depending on the age of the $^{35}SO_4$.

Electron microscopy. Bacterial preparations grown in MDMM without $^{35}SO_4$ as described above were 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 assay. The adhesion assay to immobilized mucosal components employed in this study has been described in detail previously (1, 3). All assays were performed in triplicate. Briefly, prewarmed $^{35}SO_4$-labeled *E. coli* cells (0.2 ml) were added to wells containing immobilized colonic or cecal mucus or colonic or cecal 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 (SDS) to each well and then incubating the plates for 3 h. The SDS 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 mucus or brush borders (<250 cpm).

Proteolytic enzyme treatment. Trypsin or pronase (0.2 ml of a 1-mg/ml solution in HEPES-Hanks buffer [pH 7.4]) was added directly to wells containing immobilized crude mucus or brush borders, and plates were incubated for 1 h at 37°C. The plates were then washed twice with HEPES-Hanks buffer (pH 7.4), and the adhesion assay was performed. Control experiments with bovine serum albumin (BSA)-treated rather than enzyme-treated mucosal preparations showed no inhibition of adhesion.

Sodium metaperiodate oxidation. Immobilized mucus or brush borders were subjected to sodium metaperiodate oxidation by the method of Izhar et al. (20). Wells were incubated with 0.4 ml of 10 mM sodium iodate or 10 mM sodium metaperiodate, both in 0.2 M sodium acetate buffer (pH 4.5), for 2 h in the dark at 37°C. After the cells were washed twice with HEPES-Hanks buffer (pH 7.4), adhesion assays were performed.

Mannose inhibition. Wells containing immobilized mucosal components were simultaneously incubated with radioactive *E. coli* F-18 or F-18col$^-$ and 0.1 M D-mannose or α-methyl-D-mannoside in HEPES-Hanks buffer (pH 7.4) for 1 h at 37°C. The wells were washed twice with HEPES-Hanks buffer (pH 7.4), and then adherent bacteria were counted as described above.

Assay of adhesion to BSA glycoconjugates. BSA glycoconjugates (0.2 ml of a 50-g/ml solution in HEPES-Hanks buffer [pH 7.4]) were immobilized in polystyrene tissue culture wells as described above for mucus and brush border preparations. Adhesion assays were then performed with $^{35}SO_4$-labeled *E. coli* as described above.

SDS-PAGE. SDS-polyacrylamide gel electrophoresis (PAGE) was performed on 10% polyacrylamide gels as described previously (26, 31). The following protein standards were used: phosphorylase *b*, 92.5 kilodaltons (kDa); BSA, 66.2 kDa; ovalbumin, 45 kDa; carbonic anhydrase, 31 kDa; soybean trypsin inhibitor, 21.5 kDa; and lysozyme, 14.4 kDa. After electrophoretic separation, the proteins were stained with Coomassie blue. The SDS-PAGE gels were also periodic acid-Schiff (PAS) stained to identify glycoproteins by the method of Glossmann and Neville (18).

Western blotting (immunoblotting) for receptor identification. Samples were separated by SDS-PAGE and electroblotted onto nitrocellulose membranes (Bio-Rad Laboratories, Richmond, Calif.) by the method of Towbin (39). Membranes were then incubated overnight at 4°C in 0.01 M phosphate-buffered saline (pH 7.4) containing 1% BSA. The membranes were then incubated with $^{35}SO_4$-labeled *E. coli* cells in HEPES-Hanks buffer (pH 7.4) for 1 h at room temperature for receptor identification (3, 29). The bacterial suspensions employed were grown as described above, except that $Na_2{}^{35}SO_4$ was added to a final concentration of 5.0 μCi/ml. After incubation, the membranes were washed extensively in phosphate-buffered saline containing 0.05% Tween 20. Each wash was for approximately 5 min. The membranes were fixed with phosphate-buffered saline containing 4% formaldehyde for 15 min at room temperature and then incubated for 20 min in NEF-974 Enlightening rapid autoradiography enhancer (New England Nuclear Corp., Boston, Mass.) at room temperature. Membranes were autoradiographed for 18 h, and X-ray films were developed by the method of Laskey and Mills (27).

Chemicals. All chemicals were reagent grade. The BSA glycoconjugates used were $(CH_3$-*O*-CETE$)_n$-BSA, (Man-α-*O*-CETE$)_n$-BSA, (Gal-β-*O*-CETE$)_n$-BSA, and (Gal-α-1→4 Gal-β-*O*-CETE$)_n$-BSA and are referred to below as BSA, Man-BSA, Gal-BSA, and Gal-Gal-BSA, respectively. CETE stands for 2-(2-carbomethoxyethylthio)ethyl. The BSA glycoconjugates were purchased from Carbohydrates International, Inc., Arlov, Sweden.

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

RESULTS

Adhesion of *E. coli* F-18 and F-18col$^-$ to immobilized colonic and cecal mucus and colonic and cecal brush borders. The ability of *E. coli* F-18 to adhere to immobilized colonic mucus has been reported previously (1, 3). At that time, we

TABLE 1. Adhesion of *E. coli* F-18 and F-18col$^-$ to immobilized colonic and cecal mucus and brush borders

Expt no. and prepn	Adhesion (cpm ± SE)[a]	
	F-18	F-18col$^-$
1		
Colonic mucus[b]	17,937 ± 896	10,298 ± 538
Cecal mucus	8,314 ± 135	4,813 ± 179
BSA	3,141 ± 173	3,072 ± 92
2		
Colonic brush borders[c]	4,265 ± 19	1,672 ± 6
Cecal brush borders	4,829 ± 309	1,806 ± 82
BSA	1,309 ± 44	1,227 ± 73

[a] In all experiments, data are presented as the means ± standard errors of triplicate samples.
[b] In this experiment, the specific activity of both strains was essentially identical at 4.2×10^{-3} cpm/CFU.
[c] In this experiment, the specific activity of both strains was essentially identical at 1.8×10^{-3} cpm/CFU.

demonstrated that adhesion of *E. coli* F-18 to colonic mucus was two- to sixfold greater than that to either BSA or to polystyrene, depending on the experiment. In the present study (Table 1), adhesion of *E. coli* F-18 to cecal mucus and to colonic mucus and to cecal and colonic brush borders was also anywhere between two- and sixfold greater than adhesion to BSA ($P < 0.001$). Interestingly, *E. coli* F-18col$^-$ also bound to the mucosal preparations (Table 1) but only about half as well as *E. coli* F-18 ($P < 0.001$).

Inhibition of adhesion. Treatment of immobilized cecal and colonic mucus with pronase or trypsin (Table 2) resulted in a marked inhibition of *E. coli* F-18 adhesion ($P < 0.001$). However, cecal and colonic brush border preparations were completely resistant to trypsin and far less sensitive to pronase than were the mucus preparations (Table 3). Treatment with either enzyme similarly inhibited adhesion of *E. coli* F-18col$^-$ to colonic and cecal preparations (data not shown).

Immobilized mucus and brush borders were also subjected to treatment with sodium metaperiodate to oxidize sugars. Control wells were treated with sodium iodate. A marked reduction of *E. coli* F-18 adhesion was observed after treatment of colonic and cecal mucus ($P < 0.01$), colonic brush borders ($P < 0.02$), and cecal brush borders ($P < 0.01$) with sodium metaperiodate but not with sodium iodate (Tables 4 and 5). Similarly, treatment of mucus and brush borders with sodium metaperiodate inhibited adhesion of *E. coli* F-18col$^-$ (data not shown). Together, these data suggest that colonic and cecal mucus receptors specific for *E. coli* F-18 and F-18col$^-$ are, at least in part, glycoprotein in nature

TABLE 2. Effect of protease treatment of immobilized crude colonic and cecal mucus on adhesion of *E. coli* F-18[a]

Pretreatment	Colonic mucus		Cecal mucus	
	Adhesion (cpm ± SE)[b]	% of control	Adhesion (cpm ± SE)[b]	% of control
None	3,494 ± 95	100	4,010 ± 108	100
Pronase	1,338 ± 74	38.3	1,697 ± 177	42.3
Trypsin	1,231 ± 95	35.2	1,475 ± 38	36.7

[a] Pretreatments were conducted with 0.2 ml of enzyme (1 mg/ml) in HEPES-Hanks buffer (pH 7.4). After the wells were washed twice with 1 ml of HEPES-Hanks buffer (pH 7.4), adhesion assays were performed.
[b] Adhesion of *E. coli* F-18 to BSA in this experiment was 1,502 ± 109 cpm. The specific activity of *E. coli* F-18 was 2.2×10^{-3} cpm/CFU.

TABLE 3. Effect of protease treatment of immobilized colonic and cecal brush borders on adhesion of *E. coli* F-18[a]

Pretreatment	Colonic brush borders		Cecal brush borders	
	Adhesion (cpm ± SE)[b]	% of control	Adhesion (cpm ± SE)[b]	% of control
None	4,404 ± 252	100	4,012 ± 246	100
Pronase	2,902 ± 75	65.8	3,124 ± 125	77.8
Trypsin	4,099 ± 199	93.1	5,606 ± 138	139

[a] Pretreatments were conducted with 0.2 ml of enzyme (1 mg/ml) in HEPES-Hanks buffer (pH 7.4). After the wells were washed with 1 ml of HEPES-Hanks buffer (pH 7.4), adhesion assays were performed.
[b] Adhesion of *E. coli* F-18 to BSA in this experiment was 1,506 ± 256 cpm. The specific activity of *E. coli* F-18 was 2.1×10^{-3} cpm/CFU.

and that the receptors present in brush border preparations contain one or more oxidizable sugars.

In a previous study, mouse colonic mucus was fractionated; the 125- to 250-kDa fraction contained a large amount of receptor activity (1). The 125- to 250-kDa region also contained about 50% protein and 50% sugar (wt/wt), and the sugars were those normally found in mucus and brush border preparations (25). Of these sugars, 100 mM D-mannose and its nonmetabolizable derivative α-methyl-D-mannoside (100 mM) significantly inhibited adhesion of *E. coli* F-18 to colonic and cecal mucus and brush borders (Table 6). None of the other sugars inhibited adhesion (data not shown). Essentially identical results were observed for *E. coli* F-18col$^-$ (data not shown).

Adhesion of *E. coli* F-18 and F-18col$^-$ to BSA glycoconjugates. To determine directly whether *E. coli* F-18 and F-18col$^-$ recognize and bind to the mannose moiety, BSA glycoconjugates were immobilized on polystyrene wells, and *E. coli* F-18 and F-18col$^-$ were then tested for adhesion (Table 7). Adhesion of *E. coli* F-18 to Man-BSA was about 10-fold greater than that to BSA alone ($P < 0.001$) or to either Gal-BSA ($P < 0.001$) or Gal-Gal-BSA ($P < 0.001$). Adhesion of *E. coli* F-18col$^-$ to Man-BSA was less than twofold greater than adhesion to BSA alone ($P < 0.01$), and about twofold greater than adhesion to either Gal-BSA ($P < 0.002$) or Gal-Gal-BSA ($P < 0.002$). In addition, adhesion of *E. coli* F-18 to Man-BSA was about twice that of *E. coli* F-18col$^-$ ($P < 0.001$).

Visualization of *E. coli* F-18 and F-18col$^-$ by electron microscopy. That adhesion of both *E. coli* F-18 and F-18col$^-$ to mucosal components was inhibited by D-mannose and that both strains bound specifically to Man-BSA suggested that both strains contain type 1 pili (37). Indeed, upon

TABLE 4. Effect of sodium metaperiodate treatment of immobilized crude colonic and cecal mucus on adhesion of *E. coli* F-18[a]

Pretreatment	Colonic mucus		Cecal mucus	
	Adhesion (cpm ± SE)[b]	% of control	Adhesion (cpm ± SE)[b]	% of control
None	4,681 ± 241	100	3,499 ± 348	100
Sodium iodate	5,255 ± 884	112	3,272 ± 16.5	93.5
Sodium metaperiodate	2,538 ± 232	54.2	1,357 ± 104	38.8

[a] Pretreatments were for 2 h in the dark at a concentration of 10 mM sodium iodate or sodium metaperiodate in 0.2 M sodium acetate buffer (pH 4.5). Assays were performed after the wells were washed twice with 1 ml of HEPES-Hanks buffer (pH 7.4).
[b] In this experiment, adhesion of *E. coli* F-18 to BSA was 1,412 ± 210 cpm.

TABLE 5. Effect of sodium metaperiodate treatment of immobilized colonic and cecal brush borders on adhesion of *E. coli* F-18[a]

Pretreatment	Colonic brush borders		Cecal brush borders	
	Adhesion (cpm ± SE)[b]	% of control	Adhesion (cpm ± SE)[b]	% of control
None	4,354 ± 374	100	3,689 ± 63	100
Sodium iodate	4,085 ± 286	93.8	4,394 ± 126	119
Sodium metaperiodate	2,211 ± 141	50.8	2,442 ± 258	66.1

[a] Pretreatments were for 2 h in the dark at a concentration of 10 mM in 0.2 M sodium acetate buffer (pH 4.5). Assays were performed after the wells were washed twice with 1 ml of HEPES-Hanks buffer (pH 7.4).

[b] In this experiment, adhesion of *E. coli* F-18 to BSA was 1,501 ± 123 cpm. The specific activity of *E. coli* F-18 was 1.71×10^{-3} cpm/CFU.

examination of MDMM-grown *E. coli* F-18 and F-18col$^-$ by electron microscopy, both strains were found to be piliated (Fig. 1).

Gel filtration of cecal mucus. We recently reported that after gel filtration the majority of *E. coli* F-18 colonic mucus receptor activity was found in the 125- to 250-kDa range, although significant activity was found at the lower molecular sizes as well (1). To characterize the cecal mucus receptors for *E. coli* F-18, cecal mucus was fractionated by gel filtration on a Bio-Rad A.5m column. A large percentage of total *E. coli* F-18 adhesion was routinely found in the 125- to 250-kDa region, although significant activity was also consistently observed in the lower-molecular-size ranges and at times in the void volume (Fig. 2).

SDS-PAGE of fractionated colonic and cecal mucus and colonic and cecal brush borders. SDS-PAGE was conducted on colonic and cecal brush borders and three pooled molecular-size ranges of colonic and cecal fractionated mucus: the 125- to 250-kDa region, the 25- to 125-kDa region, and the range of less than 25 kDa. Colonic and cecal mucus fractions in the 125- to 250-kDa region were clearly different from one another (Fig. 3, lanes 1 and 5). Colonic mucus contained five major proteins in the 125- to 250-kDa region, whereas cecal mucus contained numerous proteins. Similarly, differences in colonic and cecal mucus proteins in the 25- to 125-kDa range (Fig. 3, lanes 2 and 6) and in the <25-kDa range (Fig. 3, lanes 3 and 7) were observed. Differences in colonic and cecal brush border proteins were also observed (Fig. 4). PAS staining of SDS-PAGE gels showed that the 50.5- and 66-kDa proteins present in colonic mucus and the 94- and 66-kDa proteins present in cecal mucus were heavily glycosylated (data not shown). None of the brush border proteins could be identified as PAS positive.

TABLE 6. Effect of D-mannose and α-methyl-D-mannoside on the adhesion of *E. coli* F-18 to immobilized colonic and cecal mucus and brush borders[a]

Inhibitor	Adhesion (% of control ± SE)			
	Colonic mucus	Cecal mucus	Colonic brush borders	Cecal brush borders
None	100.0 ± 2.5	100.0 ± 7.7	100.0 ± 3.1	100.0 ± 5.4
D-Mannose	61.7 ± 7.9[b]	36.8 ± 3.3[c]	30.0 ± 2.0[d]	25.1 ± 2.0[d]
α-Methyl-D-mannoside	53.3 ± 3.7[d]	30.2 ± 2.5[d]	ND[e]	ND

[a] Bacteria and sugars at a concentration of 100 mM were added to wells simultaneously. Control (100%) values were as follows: colonic mucus, 4,940 ± 127 cpm; cecal mucus, 4,636 ± 360 cpm; colonic brush borders, 9,603 ± 293 cpm; cecal brush borders, 8,037 ± 433 cpm.

[b] Significance of inhibition, $P < 0.01$ (Student's t test).

[c] Significance of inhibition, $P < 0.002$ (Student's t test).

[d] Significance of inhibition, $P < 0.001$ (Student's t test).

[e] ND, Not done.

TABLE 7. Adhesion of *E. coli* F-18 and F-18col$^-$ to bovine serum albumin glycoconjugates

BSA glycoconjugate	Adhesion (cpm ± SE)[a]	
	F-18	F-18col$^-$
Man-BSA	16,207 ± 573	7,734 ± 403
Gal-BSA	1,577 ± 42	3,299 ± 442
Gal-Gal-BSA	1,700 ± 121	3,986 ± 173
BSA	1,680 ± 181	4,513 ± 438

[a] The specific activity of the two strains was identical at 3.1×10^{-3} cpm/CFU.

Identification of *E. coli* F-18 and F-18col$^-$ colonic and cecal mucus and brush border receptor proteins. Fractionated colonic and cecal mucus proteins and brush border proteins separated by SDS-PAGE were Western blotted onto nitrocellulose and the ability of $^{35}SO_4$-labeled *E. coli* F-18 (Fig. 5) or F-18col$^-$ (Fig. 6) to adhere to individual proteins was assessed by autoradiography. Both *E. coli* F-18 and F-18col$^-$ bound to the 50.5-kDa protein present in the 125- to 250-kDa range of colonic mucus (Fig. 5 and 6, lanes 1), although the extent of *E. coli* F-18 adhesion was routinely far greater. *E. coli* F-18 and F-18col$^-$ also bound to two other proteins of 94 and 73 kDa, present in the 125- to 250-kDa cecal mucus fraction (Fig. 5 and 6, lanes 5). Again, the extent *E. coli* F-18 binding was clearly greater. The two lower-molecular-size fractions of colonic and cecal mucus contained a 66-kDa protein receptor for *E. coli* F-18 to which *E. coli* F-18col$^-$ barely bound (Fig. 5 and 6, lanes 2, 3, 6, and 7). Furthermore, both *E. coli* F-18 and F-18col$^-$ bound to numerous colonic and cecal brush border proteins, but again the extent *E. coli* F-18 binding was routinely greater (Fig. 5 and 6, lanes 4 and 8). Finally, 100 mM α-methyl-D-mannoside completely inhibited adhesion of *E. coli* F-18 and F-18col$^-$ to all Western blotted mucosal receptors mucosal (data not shown).

DISCUSSION

In the accompanying manuscript, we demonstrated that it is likely that *E. coli* F-18col$^-$ is a poor colonizer of the streptomycin-treated mouse large intestine when fed to mice with its parent, *E. coli* F-18, because in its parent's presence it is unable to grow well in cecal mucus (40). However, we also demonstrated that if given enough time to complete the colonization process, *E. coli* F-18col$^-$ resists elimination in the face of subsequent challenge by *E. coli* F-18 (40). According to Freter's model of large intestine colonization, one possible way for a poor colonizer to colonize is to form an association with the intestinal mucosa such that despite challenge with a better colonizer it remains permanently bound to stable mucosal components (e.g., epithelial cells) (11, 12, 15). Under these conditions, even though the poor colonizer replicates more slowly than its challenger, the fact that it is permanently bound to the mucosa and replicates from there allows it to resist washout in feces (11, 12, 15).

Recently, we identified a major 50.5-kDa glycoprotein receptor in CD-1 mouse colonic mucus specific for *E. coli* F-18 (3). In the present investigation, an additional *E. coli* F-18 glycoprotein colonic receptor (66 kDa), which also

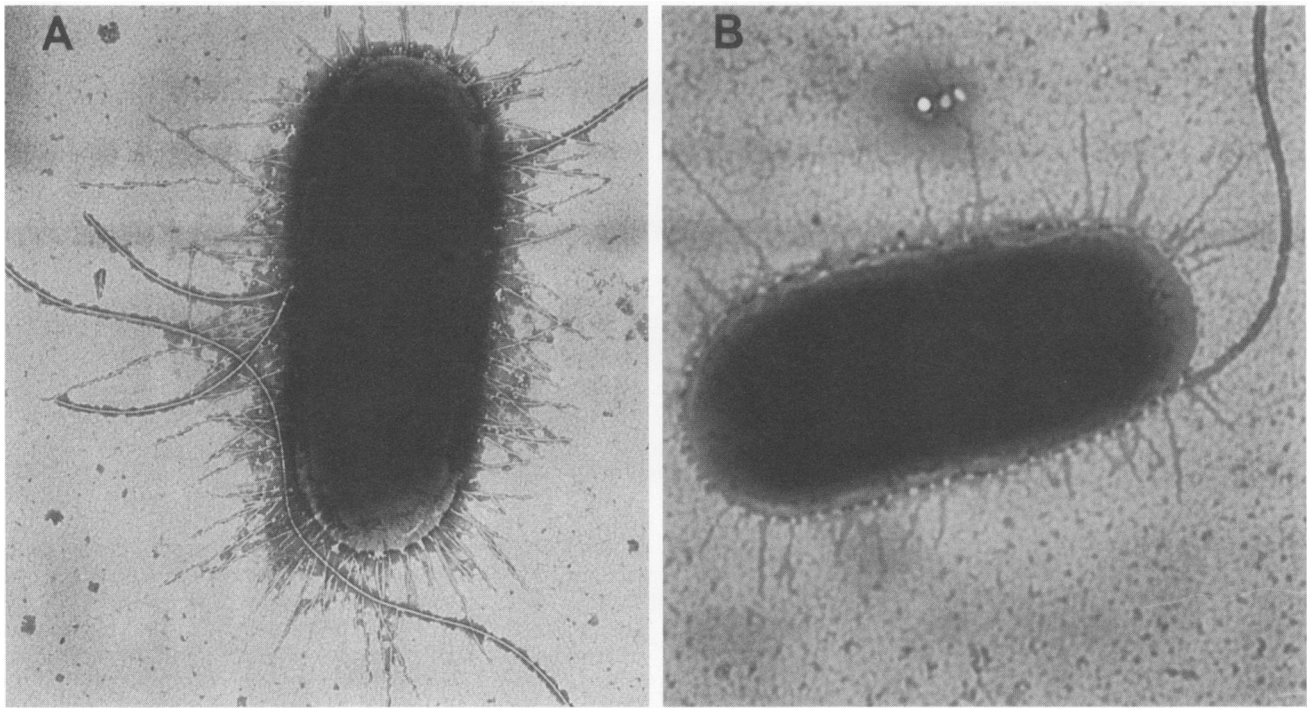

FIG. 1. Electron micrographs of *E. coli* F-18 (A) and *E. coli* F-18col$^-$ (B) grown in MDMM at 37°C for 18 h.

appears to be present in mouse cecal mucus, and two other cecal mucus receptors (94 and 73 kDa, Fig. 5) were identified. Also, numerous colonic and cecal brush border protein receptors specific for *E. coli* F-18 (Fig. 5) were identified. The brush border receptors could not be positively identified as glycoproteins because they were not PAS positive on SDS-PAGE; however, they may still be glycoproteins, since adhesion to brush borders was inhibited by sodium metaperiodate (Table 6) and since relatively large amounts of oxidizable sugar are required for PAS-positive tests on gels (18). Possibly, the brush border protein receptors were resistant to proteolytic enzyme treatment (Table 3) because they are embedded in the brush border lipid matrix.

It is also important to emphasize that it is possible that the mucus and brush border receptors we have identified may all have the same active site specific for the *E. coli* F-18 adhesin (e.g., an oligosaccharide) associated with a variety of proteins of different molecular weight and therefore in the conventional sense, that there is only one intestinal receptor for *E. coli* F-18.

Adhesion of both *E. coli* F-18 and F-18col$^-$ to mucus and brush border receptors was mannose sensitive (Table 6).

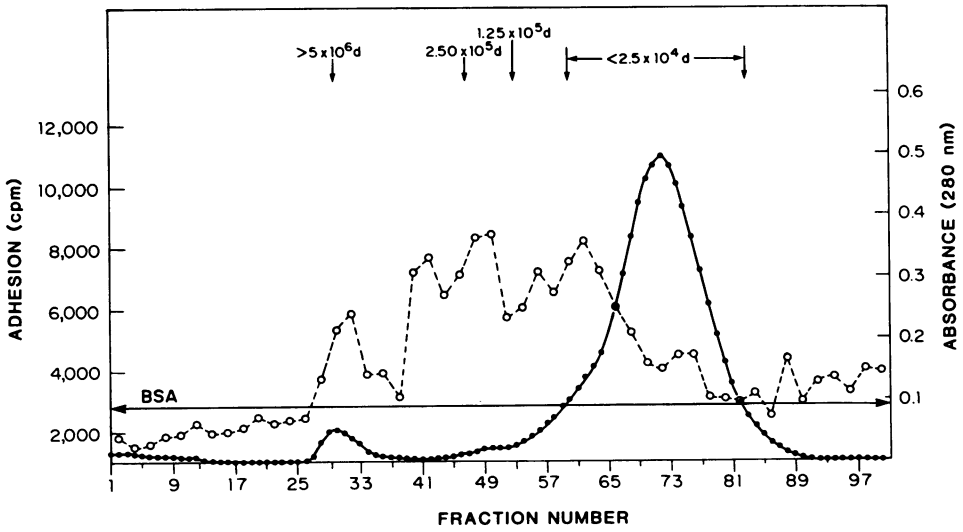

FIG. 2. Adhesion of *E. coli* F-18 to fractionated cecal mucus. Crude cecal mucus was fractionated and immobilized as described in the text. Symbols: ○, adhesion; ●, A_{280}. The line designated BSA is the level of adhesion of *E. coli* F-18 to BSA in this experiment. The specific activity of *E. coli* F-18 was 2.9×10^{-3} cpm/CFU.

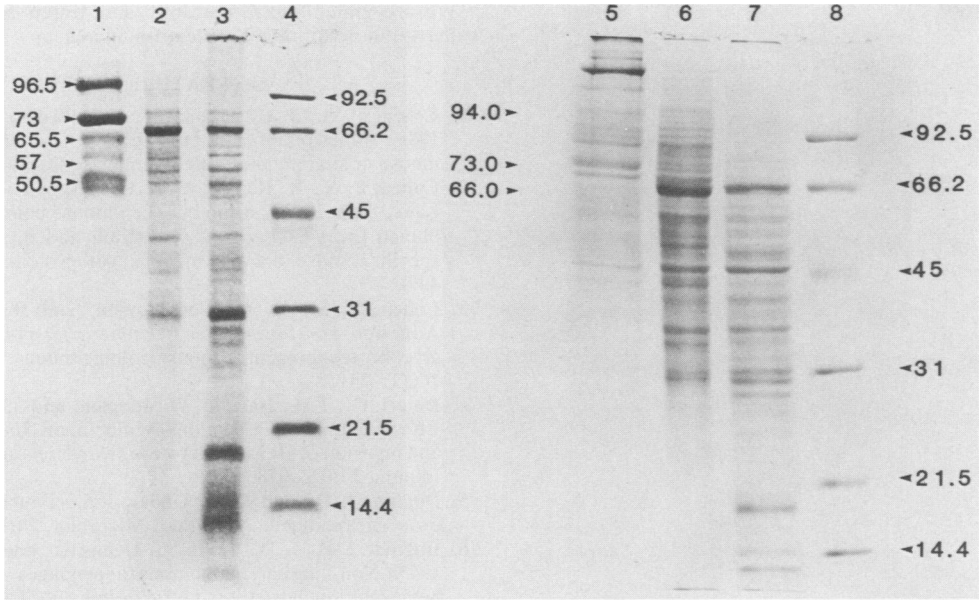

FIG. 3. SDS-PAGE of fractionated colonic and cecal mucus. Lanes: 1 through 3, colonic mucus; 1, 125- to 250-kDa range, 15 μg of protein; 2, 25- to 125-kDa range, 15 μg of protein; 3, <25-kDa range, 15 μg of protein; 5 through 7, cecal mucus; 5, 125- to 250-kDa range, 15 μg of protein; 6, 25- to 125-kDa range, 15 μg of protein; 7, <25-kDa range, 15 μg of protein; 4 and 8, protein standards. Molecular sizes are indicated in kDa. Proteins were stained with Coomassie blue.

Furthermore, both strains were found to be piliated (Fig. 1). Moreover, both strains bound specifically to immobilized Man-BSA glycoconjugate (Table 7). These data suggest that both *E. coli* F-18 and F-18col$^-$ specifically bind to a mannose or mannose-like moiety present in mucosal glycoprotein receptors through type 1 pili (5, 6, 35, 37). Experiments designed to test this possibility more directly and the role of type 1 pili in *E. coli* F-18 colonization are presently in progress.

We recently reported lipopolysaccharide to be a major *E. coli* F-18 adhesin for binding to mouse colonic mucus (1, 2), and it now appears that type 1 pili may be an important *E. coli* F-18 adhesin. Lipopolysaccharide is very difficult to

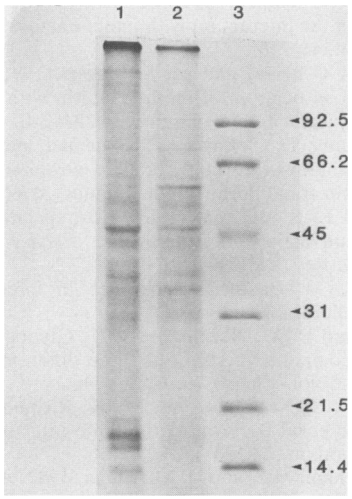

FIG. 4. SDS-PAGE of colonic and cecal brush borders. Lanes: 1, colonic brush borders, 20 μg of protein; 2, cecal brush borders, 20 μg of protein; 3, protein standards in kDa. Proteins were stained with Coomassie blue.

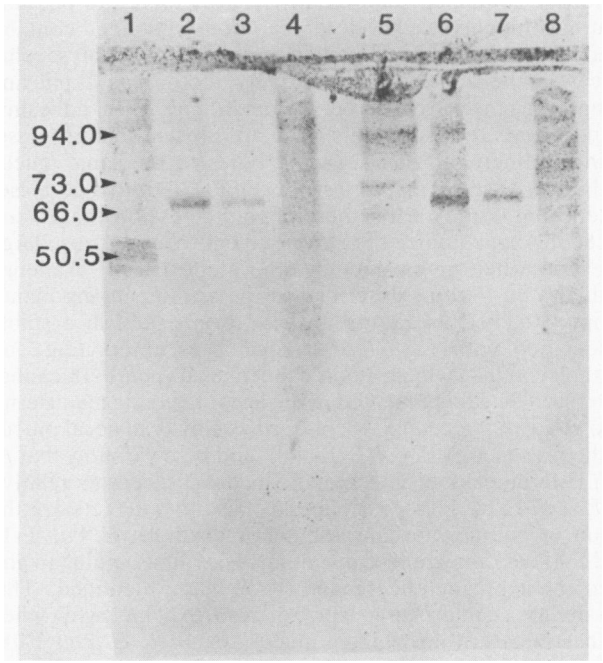

FIG. 5. Autoradiogram of *E. coli* F-18 adhesion to a Western blot of fractionated mucus and brush borders. Lanes: 1 through 3, colonic mucus; 1, 125- to 250-kDa range, 20 μg of protein; 2, 25- to 125-kDa range, 20 μg of protein; 3, <25-kDa range, 20 μg of protein; 4, colonic brush borders, 20 μg of protein; 5 through 8, cecal mucus; lane 5, 125- to 250-kDa range, 20 μg of protein; 6, 25- to 125-kDa range, 20 μg of protein; 6, <25-kDa range, 20 μg of protein; 8, cecal brush borders, 20 μg of protein. Molecular sizes of receptors (kDa) are indicated on the left. The specific activity of *E. coli* F-18 was 5 × 10^{-3} cpm/CFU.

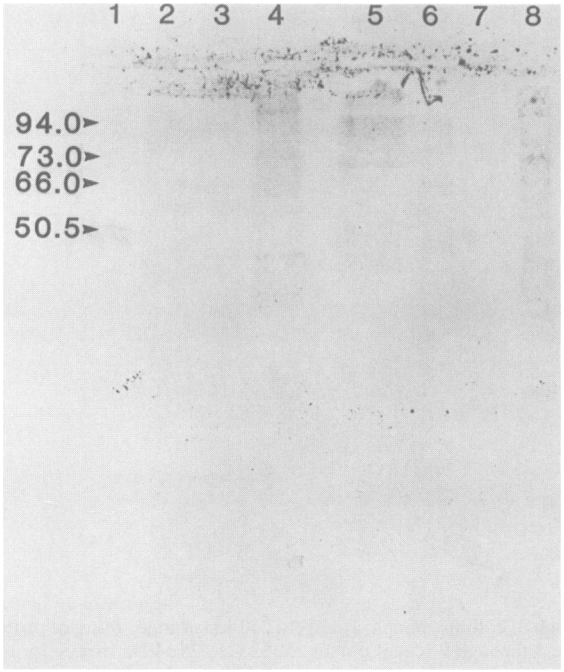

FIG. 6. Autoradiogram of *E. coli* F-18col$^-$ adhesion to a Western blot of fractionated mucus and brush borders. Lane assignments are identical to those in Fig. 5. The specific activity of *E. coli* F-18col$^-$ was 5×10^{-3} cpm/CFU.

purify free of contaminating protein, and our preparations routinely contained 2% protein (1). It is therefore possible that our lipopolysaccharide preparations contained contaminating type 1 pili which made it appear that lipopolysaccharide was the adhesin. Alternatively, both type 1 pili and lipopolysaccharide may be major *E. coli* F-18 adhesins. Experiments to resolve this issue are presently in progress.

Interestingly, *E. coli* F-18col$^-$ binds to the same mucus and brush border receptors as *E. coli* F-18 but to a far lesser extent (Fig. 5 and 6). It is therefore quite possible that *E. coli* F-18col$^-$ can colonize the streptomycin-treated mouse large intestine when given enough time alone before challenge with *E. coli* F-18 as described in the accompanying manuscript (40), because alone it can slowly establish a stable association with *E. coli* F-18-specific receptors (e.g., on epithelial cells), which, upon challenge, *E. coli* F-18 cannot reverse. The data presented in the accompanying manuscript also show that *E. coli* F-18col$^-$ grows slowly in cecal mucus in the presence of *E. coli* F-18 (40), and here we show that *E. coli* F-18 binds poorly to specific mucosal receptors relative to *E. coli* F-18. It is possible that these two defects are the result of one genetic alteration. For example, *E. coli* F-18 and F-18col$^-$ may both grow in mucus by first binding to and then metabolizing the receptors we have identified. This possibility is interesting, since it also explains why when both strains are growing in mucus together; *E. coli* F-18, because it binds to the receptors far better than *E. coli* F-18col$^-$, is at a decided growth advantage (40). Experiments to resolve these issues are presently in progress and should help us to understand more fully how *E. coli* F-18 colonizes the streptomycin-treated mouse large intestine.

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