Enhanced Virulence of *Escherichia coli* Bearing a Site-Targeted Mutation in the Major Structural Subunit of Type 1 Fimbriae

ADDISON K. MAY,^{1*} CRAIG A. BLOCH,² ROBERT G. SAWYER,¹ MICHAEL D. SPENGLER,¹
AND TIMOTHY L. PRUETT¹

Department of Surgery, Surgical Infectious Diseases Laboratory, University of Virginia Health Sciences Center, Charlottesville, Virginia 22908,¹ and the Department of Pediatrics, Division of Pediatric Infectious Diseases, University of Michigan, Ann Arbor, Michigan 48109²

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Type 1 fimbriae promote enterobacterial adherence to a variety of mammalian cells and are thought to play an important role in the establishment of various extraintestinal infections. Whether or not this adhesin has a role in the pathogenesis of peritoneal *Escherichia coli* infections, such as those initiated by bowel leakage during intraabdominal surgery, is unclear. By using two genetically engineered *E. coli* strains, each bearing an antibiotic resistance element inserted at a different site within the type 1 fimbria operon, we examined the role of type 1 fimbriation in intraperitoneal infection in rats. A permanently nonfimbriated insertion mutant was compared with an analogously constructed normally fimbriated one. After intraperitoneal inoculation of adult rats, the permanently nonfimbriated mutant produced mortality more rapidly and resulted in a greater number of culturable organisms from both the peritoneum and the blood. Moreover, the differences between these two insertion mutants were dramatically enhanced by preinoculation growth conditions favoring fimbrial expression. After growth under these conditions, 10^3 CFU of the fimbriation-proficient strain inoculated intraperitoneally caused no mortality; in sharp contrast, the permanently nonfimbriated insertion mutant resulted in death in 60% of the animals inoculated. Notwithstanding evidence that type 1 fimbriae mediate enterobacterial adherence to mammalian oropharyngeal and bladder mucosae, the results presented here demonstrate that type 1 fimbrial expression can lead to diminution of the number of *E. coli* organisms within the peritoneum.

Intraabdominal infections continue to cause significant morbidity and mortality in patients despite the introduction of new antibiotics and the use of aggressive therapy (8, 12, 29). Surprisingly, relatively little is known about the mechanisms that bacteria employ to evade host defenses and establish infection within the peritoneum. Significant improvement in the outcome of patients with such infections will likely require new approaches to therapy. The development of these approaches will demand a greater understanding of the interactions between the host and the pathogen within the peritoneum.

Surgical intraperitoneal infections generally result from a breach in the integrity of the gastrointestinal tract, introducing a broad mixture of both aerobic and anaerobic bacteria into the peritoneum. Of the numerous aerobic bacteria inoculated, *Escherichia coli* quickly emerges as the predominant pathogen, isolated in greater than 60% of intraperitoneal infections (10, 16). *E. coli* is therefore particularly adept at making the transition from the environment of the gastrointestinal tract to that of the peritoneum. The characteristics that allow this organism to so effectively evade host defenses, proliferate, and establish infection within the peritoneum remain unknown.

Significant heterogeneity exists among strains of *E. coli*, apparently altering their ability to produce infection. Infections such as cystitis, pyelonephritis, neonatal meningitis, septicemia, and gastroenteritis are more often associated with bacteria that produce certain virulence factors that are thought to provide a competitive advantage to these strains

(19, 24, 26, 28). The typical intraabdominal infection differs from most other infectious models in that colonization prior to infection is nonexistent and infection results from a rapid introduction of numerous strains of bacteria. The environment presented to the bacteria differs significantly from the gastrointestinal tract and subsequently undergoes a rapid and continuous change (25). As in infections in other sites, *E. coli* strains differ in their ability to adapt to the peritoneal environment. We have previously demonstrated that, after induction of fecal peritonitis in rats with a fecal inoculum in which hemolytic *E. coli* strains constitute only 5% of the total *E. coli* strains, the hemolytic strains outnumber the nonhemolytic strains in less than 24 h (18). The characteristics that allow one strain of *E. coli* to gain selective advantages and proliferate within the peritoneum are not known and are the focus of this research.

Bacterial adherence plays a critical role in a number of extraperitoneal infections by promoting colonization and enhancing toxin delivery (2). Adherence to the surface of mammalian cells by bacteria is frequently associated with the expression of fimbrial adhesins on the bacterial cell surface. These adhesins have structural binding specificities that, in part, account for the organ selectivity demonstrated by some strains (2). One particular adhesin, the type 1 fimbria, is widely distributed among *E. coli* strains and promotes attachment to a number of mammalian cells, including neutrophils and macrophages (1, 4, 5, 23). This fimbria has been shown to influence colonization of the proximal gastrointestinal tract, phagocytosis by macrophages and neutrophils, and intravascular clearance of *E. coli*. Additionally, it may enhance colonization of the lower urinary tract as well as mediate neutrophil-induced renal

* Corresponding author.

parenchymal injury in chronic pyelonephritis. While the expression of type 1 fimbriae is known to enhance bacterial virulence in certain settings and its expression within the lower gastrointestinal tract may be strong, the role of this fimbria in the establishment of peritoneal infection remains unclear (14).

By using two genetically engineered *E. coli* strains, each bearing an antibiotic resistance element inserted at a different site within the type 1 fimbria operon, we examined the role of the type 1 fimbria in intraperitoneal infection in rats. A permanently nonfimbriated strain (designated CAB 69 below) was compared with an analogously constructed strain (designated CAB 70 below) that demonstrates the prototypic phase-variable type 1 fimbria expression. In a series of experiments utilizing these two *E. coli* strains, we examined the influence of type 1 fimbriae on animal mortality and intraperitoneal abscess formation in a rat peritonitis model that mimics clinical infection. In addition, we examined the alterations in *E. coli* recovery from the rat peritoneal cavity, the peritoneal mesothelium, and the blood after the intraperitoneal inoculation of these two strains.

MATERIALS AND METHODS

Animals. Female Sprague-Dawley rats (Hilltop Lab Animals, Inc., Scottdale, Pa.), weighing 180 to 220 g, were housed for at least 5 days and given rat chow and water ad libitum prior to each experiment. Animals were maintained according to National Research Council standards.

Preparation of fecal adjuvant. The peritonitis and abscess model utilized in these experiments employs 1 ml of sterile fecal adjuvant per animal. Cecal contents from adult, female, grain-fed Sprague-Dawley rats were collected, pooled, and combined with prerduced Hanks' balanced salt solution (Sigma Chemical Co., St. Louis, Mo.), glycerol, and brain heart infusion (BHI) broth (BBL Microbiology Systems, Cockeysville, Md.) to a final concentration of 25% (wt/vol). The resulting mixture was strained through four single layers of surgical-mesh gauze to remove large particulate matter, sterilized by autoclave for 40 min at 121°C and 15 lb/in² of pressure and subsequently frozen at -85°C until use.

***E. coli* strains.** Two isogenic *E. coli* O18:K1 strains that differ in their ability to express the type 1 fimbria were used in all experiments. Construction of the two *E. coli* strains was described previously (4). Briefly, the wild-type *pil* DNA sequence in *E. coli* RS218 was replaced with one or the other of two mutant sequences, referred to here as *pilA::tetR* and *pilA⁺::tetR*, without polar effects on *pilE*. These sequences were constructed in vitro from a cloned segment of *E. coli* DNA harboring the *pilA* gene that codes for the major structural subunit of type 1 fimbriae. They differ only in the site of insertion of a restriction fragment bearing the tetracycline resistance determinant from Tn10. Of the two resulting *E. coli* strains, one is stably nonfimbriated and the other demonstrates the prototypic phase-variable type 1 fimbrial expression, designated here as CAB 69 (T1F⁻) and CAB 70 (T1F⁺), respectively (4). CAB 69 has previously been shown to be unable to accomplish type 1 mannose-sensitive binding (4).

Preinoculation bacterial culture conditions. The expression of type 1 fimbriae by *E. coli* can be altered by environmental conditions. Preinoculation culture conditions in the experiments described below were selected to produce inocula containing *E. coli* that are either maximally or minimally fimbriated.

To produce maximal fimbrial expression by CAB 70, both

E. coli strains were grown statically in aerobic conditions in Luria broth (10 g of tryptone [Difco], 5g of yeast extract [Difco], 5g of NaCl [each per liter]) for 48 to 72 h at 37°C (20). To inhibit the expression, both *E. coli* strains were grown in BHI broth overnight with agitation at 37°C. Bacteria produced by both culture methods were collected by centrifugation at 3,500 × g for 10 min, washed twice with normal saline, and quantified by using serial dilutions and plate enumeration on sheep blood agar in an aerobic environment.

Verification of fimbrial expression. The presence or absence of type 1 fimbrial expression by each of the strains was confirmed by *Candida albicans* agglutination. Briefly, 0.05 ml of a 10¹⁰-CFU/ml concentration of bacteria suspended in normal saline was mixed with an equal volume of suspension containing 10⁸ to 10⁹ CFU of *C. albicans* (clinical isolate) per ml in normal saline on glass slides, and the amount of agglutination was graded as 0 to 3+ (17). Positive agglutination reactions were repeated in the presence of 5% D-mannose (Sigma) to confirm that agglutination was due to the type 1 adhesin (27).

***Bacteroides fragilis*.** *B. fragilis* (ATCC 23745) was grown anaerobically at 37°C for 24 h in a mixture containing 37 g of BHI broth, 5 g of yeast extract, 1 mg of vitamin K₁, 5 mg of hemin, 1 mg of resazurin, and 0.5 g of cysteine (Carr-Scarborough Microbiologicals, Decatur, Ga.), collected by centrifugation at 3,500 × g for 10 min, washed twice in normal saline, and quantified by using serial dilutions and plate enumeration on brucella agar in an anaerobic chamber.

Mortality and abscess formation—animal inoculation and analysis. To examine the influence of the type 1 fimbria on animal mortality and abscess formation in peritonitis, mixed intraperitoneal infections were produced in cohort groups of rats by percutaneous intraperitoneal injection of 1 ml of an inoculum containing sterile fecal adjuvant, 10³ *E. coli* organisms (either CAB 69 or CAB 70), and 10⁵ *B. fragilis* organisms into animals anesthetized via inhalation of halothane. After inoculation, animals were immediately given 10 ml of 0.9% saline by subcutaneous injection and observed for mortality for 10 days. Animals surviving at 10 days were sacrificed and analyzed for intraperitoneal abscess formation.

In the first set of experiments, the nonfimbriated strain CAB 69 was compared with the heavily fimbriated strain CAB 70. Both strains of *E. coli* were grown in Luria broth to enhance expression of the type 1 fimbria and injected as described above. Two cohort groups of five animals (total animals per group, 10) received inocula containing 10² CAB 69 or 10² CAB 70 and 10⁵ *B. fragilis* organisms. In addition, another cohort of animals (10 per group) received inocula with the number of *E. coli* increased to 10⁴ organisms.

In the second group of experiments, to determine whether suppression of fimbrial growth by CAB 70 within the inocula would alter the outcome of peritonitis, cohort groups were inoculated as described above with inocula containing 10³ organisms of either of the two strains grown in BHI broth with agitation. Three cohorts were inoculated on separate occasions for a total of 18 animals in each group.

After inoculation, the animals were observed for mortality for 10 days. The time of death was recorded for animals in each cohort in each of the experiments. The surviving animals underwent laparotomy at 10 days, and abscesses were collected, weighed, and quantitatively cultured aerobically and anaerobically.

***E. coli* recovery from peritoneum, blood, and mesothelium.** The influence of the type 1 fimbria on the recovery of *E. coli* from within the peritoneum and the bloodstream during early

FIG. 1. Yeast agglutination test demonstrating the degree of type 1 fimbrial expression by the two *E. coli* strains when grown under different conditions as described in the text. CAB 70 produces marked agglutination of yeast cells when grown aerobically without aeration in Luria broth for 48 to 72 h. In contrast, CAB 70 produces only weak agglutination when grown overnight, aerobically, with aeration in BHI broth. CAB 69 fails to produce any agglutination under either of the growth conditions.

peritonitis was also examined. The two *E. coli* strains were grown to favor fimbrial expression as described above. Animals were inoculated by intraperitoneal injection with 10^3 *E. coli* organisms in sterile stool adjuvant or 10^7 *E. coli* organisms in normal saline and analyzed at 4 h (*E. coli* in normal saline) and 1 and 6 h (*E. coli* in sterile stool adjuvant). At the time of analysis, blood was collected by cardiac puncture with heparinized syringes, peritoneal lavage was performed with 10 ml of normal saline, and mesothelial biopsies were performed from the lateral abdominal wall after gently rinsing the surface with 2 ml of phosphate-buffered saline. Quantitative cultures were performed on all samples, and results were expressed in $\log_{10}$ CFU per peritoneum, per milliliter of blood, or per biopsy (total surface area, 78.5 mm^2).

Statistics. Dichotomous variables were analyzed by using a chi-square test, and means ($\pm$ standard errors of the means) were compared by using unpaired Student's *t* test with the aid of an Apple Macintosh computer (Cupertino, Calif.) and statistical packages (StatWorks, CricketSoftware, Philadelphia, Pa.; StatView 512+, Abacus Concepts, Calabasas, Calif.).

RESULTS

Influence of the type 1 fimbria on mortality and abscess formation in peritonitis. (i) **Enhanced fimbrial expression within inocula by CAB 70.** To examine the influence of the type 1 fimbria on the outcome of mixed intraperitoneal infection, conditions were chosen that maximized differences in type 1 fimbrial expression within the inocula. Peritonitis was induced with inocula that contained either the nonfimbriated strain CAB 69 or the fimbriated strain CAB 70, both grown in Luria broth to favor fimbrial produc-

tion as described above. *E. coli* suspensions were assayed for the expression of type 1 fimbriae by using the yeast agglutination test immediately prior to their addition to the inoculum solutions and subsequent injection. The results are illustrated on the right in Fig. 1. CAB 69 failed to produce any agglutination, verifying the absence of the type 1 fimbria, and CAB 70 consistently produced 3+ agglutination that could be eluted by the addition of D-mannose, verifying strong type 1 fimbrial expression.

After inoculation, the animals were monitored for mortality for 10 days, and the animals surviving at 10 days were analyzed for intraperitoneal abscesses. Data are shown in Table 1. Mortality resulting from inoculation of the two strains grown under these conditions differed significantly. CAB 69, lacking the type 1 adhesin, resulted in a 60% (6 of 10) mortality, while the heavily fimbriated strain CAB 70 produced no mortality among 10 animals ($P < 0.01$). Thus, the nonfimbriated mutant demonstrated an enhanced virulence relative to the strain with prototypic type 1 fimbrial expression.

Although mortality differed significantly, analysis of abscesses at 10 days revealed no significant differences between the number of abscesses formed ($P = 0.76$), the total abscess weight per animal ($P = 0.38$), or the total number of *E. coli* and *B. fragilis* organisms cultured from the abscesses.

The results described above were obtained with inocula that contained 10^2 organisms of either CAB 69 or CAB 70, and no mortality was obtained in the CAB 70 group. The number of *E. coli* per inoculum was therefore increased to 10^4 so that lethality might be observed in the CAB 70 group. With the increased inoculum size, both the mortality and the time required for mortality were significantly different. The nonfimbriated strain CAB 69 produced 100% mortality (10 of 10) within 48 h. In contrast, the fimbriated strain CAB 70

TABLE 1. Mortality and abscess data 10 days postinduction of peritonitis

Culture conditions	<i>E. coli</i> strain inoculated	Size of inoculum ^a	No. of rats inoculated	Mortality (%)	No. of rats analyzed	Abscess		No. of <i>E. coli</i> (log ₁₀)/rat ^a	No. of <i>B. fragilis</i> (log ₁₀)/rat ^a
						No./rat ^a	Wt (mg)/rat ^a		
Luria broth without aeration ^b	CAB 69 (T1F ⁻)	$(6.7 \pm 0.04) \times 10^2$	10	60 ^c	4	4.0 $\pm$ 0.4	857 $\pm$ 264	7.76 $\pm$ 0.32	8.26 $\pm$ 0.30
	CAB 70 (T1F ⁺)	$(7.2 \pm 0.03) \times 10^2$	10	0 ^c	10	4.2 $\pm$ 0.4	692 $\pm$ 56	7.94 $\pm$ 0.17	8.27 $\pm$ 0.21
BHI broth with aeration ^d	CAB 69 (T1F ⁻)	$(7.5 \pm 4.3) \times 10^3$	19	84	3	3.7 $\pm$ 2.1	273 $\pm$ 110	7.21 $\pm$ 0.81	7.11 $\pm$ 1.18
	CAB 70 (T1F ⁺)	$(8.0 \pm 5.7) \times 10^3$	18	67	6	2.5 $\pm$ 0.8	193 $\pm$ 75	6.78 $\pm$ 0.45	6.76 $\pm$ 0.58

^a Mean $\pm$ standard error of the mean.^b Favors fimbrial expression.^c $P < 0.01$ for CAB 69 versus CAB 70 by chi-square test.^d Inhibits fimbrial expression.

produced only a 50% mortality (5 of 10), and the mortality that occurred was delayed, with only two of the five deaths occurring prior to 48 h ($P < 0.01$ by chi-square test).

(ii) **Suppressed fimbrial expression within inocula by CAB 70.** Because the expression of type 1 fimbriae by *E. coli* is phase variable and influenced by environmental conditions, we postulated that the ability to down-regulate fimbrial expression could enhance the virulence of the bacterium within the peritoneum. We therefore determined whether inhibition of fimbrial expression within the inoculum would abrogate the differences in mortality demonstrated above. Animals were inoculated as described above, but the inocula were prepared by using *E. coli* strains grown overnight in BHI broth with agitation at 37°C, conditions that inhibit fimbrial expression. The bacteria were collected and quantified as described above, and the suppression of fimbrial expression was verified by yeast agglutination as illustrated on the left in Fig. 1. The yeast agglutination assay revealed that CAB 70 (T1F⁺) produced very weak (1+) agglutination that could be eluted with D-mannose. Thus, fimbrial expression was inhibited but not totally eliminated under these

conditions. Again, as expected, CAB 69 (T1F⁻) produced no agglutination.

Mortality and abscess data are shown in Table 1. In the experiment that produced these data, when both strains were grown in BHI broth with aeration, the overall mortality at 10 days did not differ significantly, being 84% for CAB 69 (T1F⁻) and 67% for CAB 70 (T1F⁺) ($P = 0.79$). However, a difference in the time course of mortality was demonstrated, with the nonfimbriated strain producing mortality more rapidly. Ninety-four percent (15 of 16) of the deaths occurred within 48 h of inoculation of the nonfimbriated strain, while 50% of the mortality (6 of 12) occurred within 48 h of inoculation with the fimbriated strain ($P < 0.01$) (Table 1 and Fig. 2). Therefore, when the expression of fimbriae within the inoculum by CAB 70 was minimized, the two strains produced similar overall mortalities. However, the nonfimbriated mutant still maintained some degree of enhanced virulence, producing mortality more rapidly than the type-1-fimbria-bearing strain.

Analysis of animals surviving at 10 days revealed that no significant differences existed between CAB 69 and CAB 70

FIG. 2. Time course of mortality after the induction of peritonitis with either *E. coli* CAB 69 (type 1 fimbria negative) or *E. coli* CAB 70 (type 1 fimbria positive) when the two strains were grown to inhibit expression of the type 1 fimbriae. The percentages of mortality occurring before and after 48 h are shown for each strain. P was < 0.01 by chi-square test for CAB 69 versus CAB 70.

TABLE 2. *E. coli* recovered from the peritoneum, blood, and mesothelium when inoculated in normal saline or sterile stool adjuvant

Inoculated in:	Strain	<i>E. coli</i> recovered ($\log_{10} \pm$ SEM) from:		
		Peritoneum ($n = 8$)	Blood ($n = 5$)	Mesothelium ($n = 5$)
Normal saline	CAB69	7.59 $\pm$ 0.06 ^a	5.76 $\pm$ 0.14	2.85 $\pm$ 0.17
	CAB70	7.22 $\pm$ 0.12 ^a	5.34 $\pm$ 0.27	2.54 $\pm$ 0.15
Sterile stool adjuvant	CAB69	6.58 $\pm$ 0.28	3.32 $\pm$ 0.36 ^b	3.61 $\pm$ 0.20
	CAB70	5.44 $\pm$ 0.34	2.26 $\pm$ 0.35 ^b	2.44 $\pm$ 0.24

^a $P \leq 0.02$, CAB 69 versus CAB 70.^b $P \leq 0.05$, CAB 69 versus CAB 70.

with respect to the number of intraperitoneal abscesses per animal ($P = 0.25$), the total abscess weight per animal ($P = 0.23$), or the total number of *E. coli* and *B. fragilis* organisms cultured from the abscesses per animal (Table 1), although the number of animals surviving makes conclusions difficult.

Of interest are the differences in the abscess data when the Luria broth without aeration culture conditions are compared with the BHI broth with aeration culture conditions. Although the groups were not treated as cohorts and the number of surviving animals in the CAB 69 groups was small, the data illustrate the importance of bacterial culture conditions when examining the virulence properties of organisms. Comparing the results obtained when the *E. coli* organisms were grown in Luria broth, without agitation, for 48 to 72 h with those obtained when they were grown in BHI broth, with agitation, overnight suggests that growth under the former conditions may increase the number of abscesses produced, the total abscess weight per animal, and the number of organisms recovered per animal. These differences occur even though the inocula are smaller when grown in Luria broth, and the differences are particularly pronounced with CAB 70 (P values all <0.02).

***E. coli* recovery from early peritoneal infection and peritonitis.** The mechanism of the enhanced virulence of the fimbria-deficient strain CAB 69 is unknown at present. The type 1 fimbria is known to enhance binding to phagocytic cells in vitro; however, the significance of this enhanced binding in vivo remains controversial. In addition, whether type 1 fimbriae mediate binding to the peritoneal mesothelium, altering clearance through diaphragmatic lacunae, is also unclear. We investigated whether the type 1 fimbria alters *E. coli* recovery from the blood, the peritoneal cavity, and the peritoneal mesothelial surface. When 10^7 organisms of either CAB 69 or CAB 70 suspended in normal saline was inoculated, the nonfimbriated strain CAB 69 was recovered in significantly larger numbers from the peritoneum but not from the blood or mesothelium (Table 2). When the two strains injected with sterile stool adjuvant were compared, the numbers of both strains recovered at 1 h were too low to allow analysis. However, at 6 h, the number of CAB 69 organisms recovered was more than 1 order of magnitude greater than that of CAB 70 from the blood, peritoneum, and mesothelium (Table 2). In addition, no significant adherence to the mesothelium by either strain could be demonstrated in vitro (data not shown).

DISCUSSION

Peritonitis and intraabdominal abscesses continue to threaten the lives of individuals, with overall mortality, excluding periappendiceal abscesses, in the range of 25 to 40% (6, 12, 29). Although great strides have recently been

made in the delineation of the host response to these infections, very little is known about the role of the pathogen in the bacterium-host relationship within the peritoneum. A number of *E. coli* virulence factors have been identified in extraintestinal infections. For example, certain O and K serotypes appear with marked frequency in septicemia, neonatal meningitis, and urinary infections (19, 24, 26). P and S fimbria enhance colonization in pyelonephritis, and alpha-hemolysin secretion promotes cell injury and scarring (28). Whether these or other factors play a significant role in peritoneal infections remains unknown.

Type 1 fimbriae are distributed widely among bacteria, including *E. coli* (2, 23). Their significance in the pathogenesis of infection varies with the organ system involved and has yet to be fully elucidated in most systems. In most localized infections, type 1 fimbrial expression seems to enhance virulence. For example, type 1 fimbriae promote the colonization of the oropharynx of rats with *E. coli* and are thought to mediate attachment to the bladder mucosa in cystitis (4, 9, 11). In chronic pyelonephritis, they cause activation and degranulation of neutrophils, a process that leads to renal scarring (17). On the other hand, when bacteria are free within the bloodstream, type 1 fimbrial expression improves clearance by the host and thus decreases virulence in that setting (2). In vitro studies demonstrate that type 1 fimbriae mediate adherence to a wide variety of cells including erythrocytes, certain mucosal cells, neutrophils, macrophages, and peritoneal mesothelial cells (1-5, 13, 15, 21). The presence of type 1 fimbriae appears to enhance phagocytosis by macrophages but may actually interfere with intracellular killing by these cells (3, 5, 13, 20). Fimbrial attachment to neutrophils induces an oxidative burst, primary and secondary granule release, as well as leukotriene B₄ generation (15, 17). Thus, type 1 fimbrial expression could alter the outcome of an intraabdominal infection by a number of mechanisms.

Complicating the study of this fimbria is its ability to undergo phase variation. This property allows an individual bacterium to turn fimbrial production on and off, a process that occurs spontaneously approximately every 10^3 cellular divisions (23). In addition, in vitro expression of type 1 fimbriae is also influenced by environmental conditions. Expression is favored by growth in an anaerobic environment, in minimal nutrient broth without agitation (20, 83). In vivo, the degree of fimbrial expression has also been shown to vary. Within the large bowel, approximately 70% of the *E. coli* organisms possess the ability to express the type 1 fimbria, and expression within the bowel is thought by some authors to be high (14, 22). Therefore, an inoculum into the peritoneal cavity resulting from a perforation of the large bowel could result in the introduction of predominantly fimbriated strains. However, the role of phase variation in

clinical infections is poorly understood and controversial, and the role of adherence, either in general or that mediated by type 1 fimbriae, in the pathogenesis of peritoneal infections remains speculative at this point.

Data presented herein demonstrate that type 1 fimbrial expression by *E. coli* decreases virulence in this model. Engineered strains of *E. coli*, having no known phenotypic differences other than their ability to produce the type 1 fimbria, were used to study the role of this fimbria in peritonitis. The enhancement of fimbrial expression within the inoculum by the fimbria-bearing strain produced a markedly reduced mortality relative to that of the non-type-1-fimbriated strain (0 versus 60%). Fimbrial expression at the time of inoculation appears to have been an important component in this phenomenon, since suppression of fimbrial expression by the type 1 fimbriated strain within the inoculum resulted in an overall mortality that was not significantly different from that of the nonfimbriated strain under these conditions. However, the time course to mortality from the nonfimbriated strain was more rapid. The delay in mortality produced by the fimbriated *E. coli* strain relative to its nonfimbriated mutant is likely the result of residual fimbrial expression in the inoculum even under these culture conditions. However, the possibility of an intraperitoneal shift to the fimbriated form by CAB 70 was not investigated in this study.

An analysis of organism recovery from the peritoneum, blood, and mesothelium reveals that inoculation of the nonfimbriated strain results in a greater number of bacteria within the peritoneum at 4 and 6 h regardless of whether or not adjuvant was added. When stool adjuvant was used, the nonfimbriated strain also resulted in greater recovery from both the blood and the mesothelium. Analysis of the animals at 10 days suggests that the ability to produce type 1 fimbria may not play a significant role in the abscess stage of infection, but data are too limited to draw definite conclusions.

We chose a rat peritonitis model that utilizes sterile fecal adjuvant and *B. fragilis* to mimic clinical intraabdominal infections. Since type 1 fimbrial expression undergoes phase variation and environmental conditions alter the amount of expression by *E. coli*, substances that alter the intraperitoneal environment may profoundly affect fimbrial growth. Peritonitis generally results from the loss of the integrity of the gastrointestinal tract, with the subsequent introduction into the peritoneum of aerobic and anaerobic organisms in addition to fecal matter and blood. This results in a polymicrobial infection, with *E. coli* and *B. fragilis* being isolated most frequently (16). Experimental models have demonstrated a synergistic relationship between these organisms in peritonitis (22). In addition, the introduction of sterile fecal material not only lowers the 50% lethal dose (LD_{50}) of a simultaneous bacterial inoculation but by itself influences the peritoneal environment (25). Models that fail to employ some type of adjuvant fail to produce persistent intraabdominal infection and probably more closely represent an intravenous inoculation since vast numbers of organisms are rapidly cleared through the diaphragmatic lacunae into the bloodstream (7).

Two studies have previously examined the influence of type 1 fimbriae within the peritoneum of mice, each obtaining different results and reaching different conclusions. Alkan et al. (1) used a single clinical *E. coli* isolate (O25:K⁻:H1) and varied the growth conditions to produce inocula that contained a majority of either fimbriated or nonfimbriated bacteria. Type II porcine stomach mucin was used as an

adjuvant, and the LD_{50} was determined for each inoculum. In addition, bacterial recovery from the peritoneum and degree of piliation after inoculation were determined for each of the inocula. Results demonstrated a decreased LD_{50} with fimbriated bacteria, a more rapid proliferation within the peritoneum with this inoculum, and a rapid shift to a nonfimbriated state. Interpretation of these results requires some caution, however. First, alteration of the growth conditions to produce the inocula undoubtedly influences numerous bacterial characteristics other than type 1 fimbrial expression. In our studies, when growth conditions were varied, growth of CAB 69 and CAB 70 in Luria broth produced greater total abscess weight per animal at 10 days in addition to an increased recovery of *E. coli* and *B. fragilis* organisms per animal. Second, the use of stomach mucin as an adjuvant cannot necessarily be generalized to the clinical setting. Various adjuvant substances may influence the host response quite differently, and the presence of other organisms, particularly anaerobes, may alter both the peritoneal environment and the characteristics of other organisms in clinical intraperitoneal infections.

In another study, Nowicki et al. (20) again used a single strain to examine the influence of type 1 fimbrial expression within the peritoneum of mice. A clinical isolate (O18:K1:H7) was utilized, and a fractionation technique was employed to separate fimbriated from nonfimbriated strains. *E. coli* organisms were introduced in normal saline without adjuvant substances. Contrary to the study of Alkan et al., expression of type 1 fimbria increased the LD_{50} and resulted in reduced recovery from the peritoneum and the blood. Again, a nonfimbriated state seemed to be favored within the peritoneum. As in the study of Alkan et al., separation of bacteria may alter a number of factors that are important to *E. coli* virulence. Additionally, inoculation of bacteria into the peritoneum in normal saline generally results in a rapid clearance of organisms through the diaphragmatic lacunae into the bloodstream. This model, therefore, may differ very significantly from clinical events that cause secondary peritonitis. The conflicting results between the studies may be due not only to differences in the models but also to differences in the *E. coli* strains. Possibly, the expression of type 1 fimbriae could enhance the virulence of one strain while decreasing that of another even if all other factors were constant.

The apparent decrease in virulence in our model that resulted from the expression of type 1 fimbria may occur via a number of mechanisms. Enhanced phagocytosis could occur secondarily to mesothelial adherence. However, we have been unable to demonstrate significant mesothelial adherence in vitro or in vivo at 1 h (data not shown). In addition, greater numbers of the non-type-1-fimbriated mutant were cultured from the mesothelium at 6 h. Type 1 fimbriae may cause adherence to fiber contained in the fecal adjuvant, possibly preventing a bolus of bacteria from being swept through the diaphragmatic lacunae into the bloodstream. We speculate that the differences in mortality and organism recovery are the result of improved clearance by phagocytes secondary to type 1 binding to these cells. These fimbriae are known to cause binding to both macrophages and neutrophils and to enhance phagocytosis and neutrophil activation in vitro. Activation of inflammatory cells could also alter the peritoneal environment, influencing the growth of both *E. coli* and *B. fragilis*. Whatever the mechanism, this early benefit to the host from fimbrial expression may be lost in the latter stages of infection, although the small number of

animals surviving to analysis makes such conclusions premature.

The role of phase-variable expression of type 1 fimbriae in the pathogenesis of infection remains controversial. Investigation of the role of this phenomenon in the establishment of colonization and infection remains difficult. These data suggest that down-regulation of fimbrial expression may be of benefit to *E. coli* within the peritoneum. If fimbrial expression in the anaerobic environment of the large intestine is high, as suggested by some investigators, then the ability to rapidly shift to a nonfimbriated state may be of great pathogenic significance.

Certainly not all *E. coli* strains have equivalent abilities to induce infection within the abdomen. These results suggest that, unlike other infections where fimbriae have generally been shown to enhance the infectious potential of bacteria, adherence within the peritoneum may prove to be beneficial to the host.

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