Role of Phase Variation of Type 1 Fimbriae in a Uropathogenic Escherichia coli Cystitis Isolate during Urinary Tract Infection†

Jennifer A. Snyder,1 Amanda L. Lloyd,2 C. Virginia Lockatell,3 David E. Johnson,3,4 and Harry L. T. Mobley2*

Department of Microbiology and Immunology1 and Division of Infectious Diseases,3 University of Maryland School of Medicine, and Department of Veterans Affairs,4 Baltimore, Maryland 21201, and Department of Microbiology and Immunology, University of Michigan Medical School, Ann Arbor, Michigan 48109

Received 22 August 2005/Returned for modification 4 October 2005/Accepted 4 November 2005

Type 1 fimbrial phase-locked mutants of uropathogenic Escherichia coli cystitis isolate F11 were used to assess the role of the invertible element during urinary tract infection. Compared to the wild type, the phase-locked off mutant was attenuated, and constitutive production of type 1 fimbriae by the phase-locked on mutant did not provide a competitive advantage.

Molecular Koch’s postulates of microbial pathogenesis have been satisfied for the type 1 fimbria of uropathogenic Escherichia coli (UPEC) (2). The expression of this phase-variable adhesin is transcriptionally controlled by a promoter situated on an invertible element, which can switch between two different orientations (1, 15). The phase-on orientation of the invertible element allows transcription of fimA, the main structural subunit, and other accessory genes, resulting in expression of type 1 fimbriae. When the promoter is in the opposite orientation, no type 1 fimbrial transcription occurs, and bacteria are phase-off. The inversion of the invertible element is mediated by two recombinases, FimE, which primarily promotes switching from phase-on to phase-off, and FimB, which can mediate switching in either direction (3, 9).

Type 1 fimbriae are critically important in the establishment of UPEC infections (13, 16). Indeed, we have shown previously that type 1 fimbrial genes are among the most highly expressed genes overall in vivo (17) and that either these genes are upregulated during murine urinary tract infection (UTI) compared to growth inuria broth culture or bacteria expressing type 1 fimbriae are selected in the host. The patterns of type 1 fimbrial expression, however, may differ depending on the clinical source of the UPEC strain (4).

UPEC strain F11 (O6:K15:H31), isolated from the urine of a patient with cystitis (8, 18), has been characterized as a representative cystitis strain (7) and possesses the hly gene encoding hemolysin, the pap gene encoding Fim P fimbriae, the fim gene encoding type 1 fimbriae, and the cnf1 gene encoding cytotoxic necrotizing factor 1 (4). Strain F11 is a member of the B2 clonal group, which is usually more prevalent in cystitis patients than in pyelonephritis patients and is one of the most prevalent cystitis-associated clonal groups.

The clinical syndromes of cystitis and acute pyelonephritis differ significantly in presentation. While undoubtedly the fitness of the host contributes to the clinical outcome, it is likely that there are also substantial differences in strains that cause the distinct outcomes. To study this, we selected a representative cystitis isolate, E. coli F11, and compared the contribution of type 1 fimbrial expression during colonization of the murine urinary tract to the contribution found in perhaps more virulent strains (12). We found substantial genetic differences between this strain and pyelonephritis strain CFT073. Indeed, F11 lacks 661 genes found in strain CFT073, including genes for the aerobactin iron acquisition system, capsular polysaccharide, an autotransporter, and numerous other predicted virulence determinants (Lloyd and Mobley, unpublished data).

Thus, it is straightforward to hypothesize that cystitis strains and pyelonephritis strains may use different strategies for expressing and using their virulence factors, including type 1 fimbriae, in different temporal and spatial manners.

To assess the role of type 1 fimbrial phase variation during infection with a cystitis strain, we locked the invertible element of E. coli cystitis isolate F11 in both the phase-on and phase-off orientations and introduced the resulting mutants into the murine urinary tract either individually or in direct competition with wild-type strain F11.

Construction of E. coli F11 type 1 fimbrial phase-locked mutants. The mutant of E. coli F11 in which the type 1 fimbrial invertible element is permanently locked in the phase-off orientation was designated F11-Off; likewise, the phase-locked on mutant was designated F11-On. To construct these phase-locked mutants, PCR primers were designed to amplify the invertible element in either the off or on phase. A kanamycin resistance cassette (Kan) was inserted into an engineered BglII site in the left inverted repeat of the invertible element, which prevented the inverted element from switching phases. The disrupted invertible element carrying the Kan cassette was cloned into pGP704 and then transformed into E. coli strain S17-1A-pir. The plasmid was conjugated into E. coli F11, and exconjugants that resulted in homologous recombination were selected based on their antibiotic resistance phenotypes (see Fig. S1 in the supplemental material).

The genotypes of the phase-locked mutants were confirmed...
by the previously described PCR invertible element assay (11) after both in vitro and in vivo passage. PCR was used to amplify the region spanning the type 1 fimbrial invertible element. Asymmetric digestion of the PCR product with SnaBI then allowed visualization of the invertible element phase (Fig. 1A). Growth of *E. coli* F11-ON in Luria broth yielded fragments that indicated that the invertible element was phase-locked on. Likewise, this assay indicated that the invertible element of F11-OFF was phase-locked off. F11 contained a mixture of invertible elements in the phase-off and phase-on orientations (Fig. 1B). Urine that was collected 48 h after transurethral inoculation of mice with the F11-ON mutant similarly demonstrated that the invertible element was phase-locked on in vivo. In vivo-grown wild-type strain F11 contained only phase-on invertible elements, indicating the preference of the wild-type strain for this orientation at this time during infection (Fig. 1C). No PCR products were obtained from the urine of mice infected with F11-OFF, most likely due to the low level of colonization observed for this strain (Fig. 2D; see below). Thus, the type 1 fimbrial invertible elements of the mutants were not capable of phase variation.

*E. coli* F11 and the phase-locked mutants were phenotypically characterized during in vitro growth in broth, which favors type 1 fimbrial expression (14), and on an agar surface, where the bacteria are predominately phase-off (11). F11-ON was agglutinated by guinea pig erythrocytes (inhibitable by 50 mM mannose) whether it was obtained from broth (titer, 1:64) or from agar (titer, 1:8) and by anti-FimH antiserum under both culture conditions, demonstrating that there was constitutive expression of type 1 fimbriae. F11-OFF was not agglutinated by anti-FimH antiserum, nor was it hemagglutinated by guinea pig erythrocytes under any culture conditions, which is consistent with a lack of type 1 fimbrial expression. Wild-type strain F11 exhibited the ability to phase vary, as agglutination by anti-FimH antiserum and by guinea erythrocytes (titer, 1:32; inhibitable by 50 mM mannose) was observed when the organism was cultured in Luria broth with aeration but not when it was cultured on Luria agar plates. Additionally, Western blot analysis confirmed the presence of the type 1 fimbrial tip adhesin FimH in F11 and F11-ON (data not shown) (in this assay, whole-cell bacterial samples in sodium dodecyl sulfate sample buffer [50 mM] were acid dissociated by adding 1 µl 1 N HCl and boiling for 5 min; the solution was neutralized with 1 µl 1 N NaOH, and the protein was subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis).

The growth rates of F11 and the phase-locked mutants, cultured independently in Luria broth at 37°C with aeration, were not statistically significantly different. The generation time for F11 was 36.5 min, the generation time for F11-ON was 34.3 min, and the generation time for F11-OFF was 33.5 min.

**Comparison of *E. coli* F11 and F11-ON in the experimental murine model of UTI.** *E. coli* F11 (1.34 × 10^9 CFU) and F11-ON (1.23 × 10^9 CFU) were used individually in the experimental mouse model of UTI to assess the contribution of phase variation to virulence during UTI. Forty female CBA/J mice were transurethrally inoculated for each strain, as previously described (6, 7). The inoculum for each strain was prepared on Luria agar slants containing kanamycin (50 µg/ml) as appropriate and was resuspended in phosphate-buffered saline. Fifty microliters of the bacterial suspension was delivered

---

**FIG. 1.** Genotypic characterization of the invertible element. The invertible element was amplified by PCR and digested with SnaBI. Asymmetric cutting demonstrated the phase of the invertible element in wild-type strain F11 and mutants that are phase-locked on or off (A). Strains were cultured in vitro in Luria broth (B) or transurethrally inoculated into mice, whose urine was collected at 48 h postinoculation and used for PCR (C).
to each mouse. At each time examined (4, 24, 48, 72, and 168 h postinoculation) urine samples were collected before eight mice were sacrificed. The bladders and kidneys were removed, weighed, and homogenized in phosphate-buffered saline. Samples were quantitatively cultured at various times postinoculation. Each symbol represents one sample from a mouse. Colonization data were compared for F11 and F11-ON (A to C), and for F11 and F11-OFF (D to F). Values at the lower limit of detection (1 x 10^2 CFU) are spread out for ease of viewing. Median values for the times are connected by a solid line (F11) or a dashed line (phase-locked mutant), but the lines do not imply that there is a continuous function. Data for F11 colonization are shown in relation to each phase-locked mutant for ease of comparison.

FIG. 2. Independent inoculations with F11 and phase-locked mutants in the murine model of UTI. Mice were transurethrally inoculated individually with wild-type *E. coli* strain F11, F11-ON, or F11-OFF. Urine (A and D), bladder (B and E), and kidney (C and F) samples were quantitatively cultured at various times postinoculation. Each symbol represents one sample from a mouse. Colonization data were compared for F11 and F11-ON (A to C), and for F11 and F11-OFF (D to F). Values at the lower limit of detection (1 x 10^2 CFU) are spread out for ease of viewing. Median values for the times are connected by a solid line (F11) or a dashed line (phase-locked mutant), but the lines do not imply that there is a continuous function. Data for F11 colonization are shown in relation to each phase-locked mutant for ease of comparison.

The comparison of the *E. coli* F11 and F11-ON data from these individual inoculation studies demonstrated that there was little difference in colonization in the urine, bladder, or kidney samples taken at any time during infection (Fig. 2A to C). The urine collected from infected mice showed that wild-type strain F11 had a slight (<1-log), but statistically significant, advantage over F11-ON at 4 h postinoculation (*P* = 0.0207). After this, the colonization data for these strains were not statistically different (Fig. 2A). The numbers of CFU/g of bladder for these strains demonstrated that there was no statistically significant difference at any time monitored. The median colonization levels in the bladder remained high through-
out infection, between approximately 1 × 10^5 and 1 × 10^7 CFU/g of bladder (Fig. 2B). Examination of the colonization in the kidneys also revealed that there was no statistically significant difference between F11 and F11-ON. The median colonization levels were initially 1 × 10^5 CFU/g of kidney at 4 h postinoculation and then decreased during infection to approximately 1 × 10^3 CFU/g by 168 h postinoculation (Fig. 2C).

A more sensitive method to assay for the effects of type 1 fimbriae on virulence is coinoculation of mice with wild-type and mutant strains, rather than inoculation with individual strains (5, 10). Suspensions of E. coli F11 and E. coli F11-ON were mixed at a 1:1 ratio. Mice (eight mice at each time) were transurethrally inoculated with the mixed suspension, which delivered 2.83 × 10^9 total CFU per mouse. Median values were determined, and the Wilcoxon matched-pairs test of non-parametric data was used to compare the colonization levels for F11 and F11-ON in each sample (Fig. 3A to C). Neither F11 nor F11-ON exhibited a competitive advantage over the other strain in the urine, bladder, or kidney samples collected during infection. These coinoculations, together with the individual inoculations, indicate that locking the invertible element in the phase-on orientation provides no competitive advantage to F11-ON, at least with the inocula tested.

**Comparison of E. coli F11 and F11-OFF in the experimental murine model of UTI.** A suspension of 1.39 × 10^9 CFU of E. coli F11-OFF was transurethrally inoculated into the bladders of mice to assess virulence during UTI. The colonization levels of F11-OFF were compared to those of wild-type F11 in a set of individual inoculation studies (Fig. 2D to F) identical to the experiments performed with F11-ON. In contrast to the similar colonization patterns observed for E. coli F11 and F11-ON during murine UTI, F11-OFF exhibited an overall disadvantage in colonization compared to wild-type strain F11. In the urine collected from infected mice, the median number of CFU/ml of urine of F11-OFF was about 1 log lower than the urine collected from infected mice, the median number of CFU/ml of urine of F11-OFF was about 1 log lower than the urine collected from infected mice, the median colonization levels were similar for F11-OFF and F11 at 4 and 24 h postinoculation. After this the median levels for F11-OFF were at the lower limit of detection in samples collected at 48, 72, and 168 h postinoculation, while wild-type strain F11 continued to colonize at levels that were about 2 logs higher. Interestingly, there was not a statistically significant difference between these strains in urine collected during the coinoculation studies.

E. coli F11-OFF was clearly outcompeted by wild-type strain F11 in the bladder. Statistically significant differences in colonization were observed in bladder samples collected at 24, 48, 72, and 168 h postinoculation (P = 0.0078). The numbers of CFU/g of bladder for F11-OFF were at the lower limit of detection at 72 and 168 h postinoculation, whereas the levels of F11 colonization were about 3 logs higher at these times. This pattern of colonization for F11-OFF and F11 again mirrors the pattern observed with individual inoculations, in which the greatest disparity between strains was observed in bladder samples collected at the latest times monitored during UTI (Fig. 3E).

F11-OFF and F11 competed equally well in the kidneys during the beginning of coinoculation. However, the colonization level of F11-OFF decreased at 168 h postinoculation, and this level was about 3 logs lower than the level of F11, which was statistically significant (P = 0.0313) (Fig. 3F).

Together with the individual inoculation studies, the coinoculation studies demonstrated that locking the type 1 fimbrial invertible element in the phase-off orientation attenuated E. coli F11-OFF during experimental murine UTI. The critical contribution of the type 1 fimbria, a proven virulence factor of UPEC (2), was thus confirmed for a representative cystitis isolate. We specifically found that the lack of type 1 fimbriae had profound effects on bladder colonization at all times monitored during the infections. Overall, the greatest difference in colonization between F11-OFF and F11 was always observed at times near the end of the infection (72 or 168 h). This may indicate that type 1 fimbriae are necessary for both the establishment and the persistence of this cystitis isolate in the urinary tract, a trend which has been observed previously for other UPEC strains (2, 16).

**Coinoculation with E. coli F11 and F11-rif.** One could argue that the patterns of colonization observed are random and that one strain outcompeting an isogenic mutant or vice versa is a chance event. To address this possibility, wild-type strain F11 was coinoculated with a rifampin-resistant clone of the same strain (F11-rif) selected by repeated passage of F11 on rifampin-containing medium and is stably resistant to this antibiotic). The number of CFU of F11 was determined by subtracting the number of CFU of the rifampin-resistant mutant from the total number of CFU on medium lacking rifampin. Overall, both the wild type and the rifampin-resistant strain displayed similar patterns of colonization, and neither strain outcompeted the other by the last time monitored (Fig. 4A to C). Indeed, there were no significant differences after the first day, and there was never a statistical difference for any time in the kidney. At only 3 of the 15 times compared were the numbers of CFU/ml of urine or CFU/g of tissue statistically different, and the medians differed by 1 log or less. These data were in stark contrast to the data obtained for coinoculation of
FIG. 3. Coinoculations with F11 and each phase-locked mutant in the murine model of UTI. Mice were transurethrally coinoculated with a bacterial suspension containing *E. coli* wild-type strain F11 and F11-ON at a 1:1 ratio (A to C) or *E. coli* F11 and F11-OFF at a 1:1 ratio (D to F). Urine (A and D), bladder (B and E), and kidney (C and F) samples were quantitatively cultured at various times postinoculation. Each sample from individual mice yielded a value for the wild-type strain and the phase-locked mutant. Values at or below the lower limit of detection (1 × 10^2 CFU) are spread out for ease of viewing. Note that a urine sample could not be obtained from four mice at 4 h postinoculation; thus *n = 4* at this time (D). Median values for the times are connected by a solid line (F11) or a dashed line (phase-locked mutant), but the lines do not imply that there is a continuous function.
F11 and F11-OFF, which showed that at 6 of the 15 times examined the values were statistically different and that there were 1- to 4-log differences in the median colonization levels (Fig. 3D to F). This experiment demonstrated that the patterns of colonization observed for F11 and F11-OFF were not due to random chance enrichment of one strain.

Comparative analysis of *E. coli* F11 and *E. coli* CFT073. Analysis of our findings in light of previous results provided a novel and different perspective on the studies of phase-locked mutants. We previously demonstrated that the dynamic temporal regulation of the type 1 fimbrial invertible element during experimental UTI correlated with the clinical manifestation of UTI in the patient from which the strain was isolated (4). The invertible elements of the representative pyelonephritis isolate *E. coli* CFT073 were only about 34% phase-on at 24 h postinoculation and were found to be phase-off thereafter. In contrast, the invertible elements of cystitis isolates, including wild-type *E. coli* F11, were about 85% phase-on at 24 h and remained mostly phase-on throughout infection. The assertion that wild-type strain F11 is mostly phase-on in vivo and the F11-OFF mutant never expresses type 1 fimbriae helps explain the disparate patterns of colonization observed for these strains. Likewise, the data described above also explain the similar patterns of colonization for *E. coli* F11-ON and F11. The invertible element of F11-ON is phase-on in 100% of the population, while the invertible element of wild-type strain F11 is phase-on in most of the population during infection. It would be interesting to perform additional experiments using smaller inocula to detect more subtle differences in colonization potential.

We have previously explored the role of invertible element phase switching in the pyelonephritis strain *E. coli* CFT073 (5). In both this isolate and cystitis isolate F11, locking the invertible element phase-off attenuated the strain. The key differences are in the timing, magnitude, and location of attenuation compared to the parental strain. Whereas CFT073-OFF (phase-locked off for type 1 fimbriae) exhibited decreased colonization only at 24 h postinoculation throughout the urinary tract, F11-OFF was most severely attenuated in the bladder at all times from 4 to 168 h postinoculation. Additionally, the greatest difference between the phase-locked off strain and the corresponding wild-type strain was at 24 h for *E. coli* CFT073, in contrast to 168 h postinoculation for *E. coli* F11. Whereas CFT073-ON (phase-locked on for type 1 fimbriae) colonized at a significantly higher level than wild-type strain CFT073 at 24 h postinoculation, there was no difference between the levels of colonization of F11-ON and wild-type strain F11. The dissimilarities in colonization between phase-locked mutants of CFT073 and F11 may be due to the distinct temporal patterns of invertible element phase switching. In this discussion, we are emphasizing the role of type 1 fimbrial expression in colonization of the urinary tract. However, we recognize that the genomes of the two strains differ by hundreds of genes (Lloyd and Mobley, unpublished data), which could have multiple effects on colonization that have not been characterized yet.

Coordinated regulation among adhesins may be a phenomenon that also influences the clinical outcome of infection. For example, *E. coli* strain CFT073 may possess up to 12 adhesins (19). In contrast, *E. coli* F11-OFF may not possess an adhesin to facilitate adherence in the absence of type 1 fimbriae. These
scenarios may explain the rebound in colonization by CFT073-OFF after 24 h postinoculation, compared to the continued decrease in the level of F11-OFF throughout infection.

If we had hypothesized that the invertible element itself (that is, the ability to phase vary type 1 fimbria production) is a virulence factor, the hypothesis would have been refuted. Upholding the hypothesis would have required that the wild-type strain independently outcompeted both phase-on and phase-off mutants. Indeed, only the phase-off mutant was outcompeted by the wild type. Nevertheless, the ability to phase vary at specific times during infection may indicate that the invertible element has an important role in determining colonization in the urinary tract.

This work was supported by National Institutes of Health Public Health grant AI043363 (H.L.T.M.). We thank Michael S. Donnenberg for kindly providing F11-rif for use in this study.

**REFERENCES**