12. S. C. White and G. W. Casarett, *J. Immunol.* **112**, 178 (1974).
13. PBMCs from each donor were plated at 5 × 10^5 cells per well in 96-well microtiter plate in 200 μl of RPMI 1640 medium containing penicillin (100 U/ml), streptomycin (0.1 mg/ml), 2 mM glutamine, 1 mM sodium pyruvate, 10 mM Hepes, and antigen (10 mg/ml) in triplicate. During the last 20 hours of the 72-hour culture period, 1 μCi of [^{3}H]thymidine was added per well. Incorporation was measured by liquid scintillation counting.
14. J. R. Glenney, P. Glenney, K. Weber, *J. Biol. Chem.* **257**, 9781 (1982).
15. D. Perrin and D. Aunis, *Nature* **315**, 589 (1985); D. Perrin, O. K. Langley, D. Aunis, *ibid.* **326**, 498 (1987).
16. D. Perrin, K. Möller, K. Hanke, H.-D. Söling, *J. Cell Biol.* **116**, 127 (1992).
17. J. H. Hartwig, *ibid.* **118**, 1421 (1992).
18. T. L. Leto *et al.*, *J. Biol. Chem.* **264**, 5826 (1989).
19. V. B. Lokeshwar and L. Y. W. Bourguignon, *ibid.* **267**, 21551 (1992).
20. T. C. Saido *et al.*, *ibid.* **268**, 25239 (1993).
21. S. J. Martin *et al.*, *ibid.* **270**, 6425 (1995).
22. J. Van de Water *et al.*, *J. Exp. Med.* **167**, 1791 (1988).
23. L. Portmann, N. Hamada, G. Heinrich, L. J. Degroot, *J. Clin. Endocrinol. Metab.* **6**, 1001 (1985).
24. S. Backkeskov *et al.*, *Nature* **347**, 151 (1990); R. Tisch *et al.*, *ibid.* **366**, 72 (1993).
25. F. A. Karlsson, P. Burman, L. Loof, S. Mardh, *J. Clin. Invest.* **81**, 475 (1988).
26. D. A. Stetler *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 6499 (1982).
27. D. C. Hooper, *Immunol. Today* **8**, 327 (1987).
28. U. K. Laemmli, *Nature* **227**, 680 (1970).
29. E. Macy, M. Kemeny, A. Saxon, *FASEB J.* **2**, 3003 (1988).
30. I. Oadri, H. F. Maguire, A. Siddiqui, *Proc. Natl. Acad. Sci. U.S.A.* **92**, 1003 (1995).
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Prevention of Mucosal *Escherichia coli* Infection by FimH-Adhesin–Based Systemic Vaccination

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Virtually all uropathogenic strains of *Escherichia coli*, the primary cause of cystitis, assemble adhesive surface organelles called type 1 pili that contain the FimH adhesin. Sera from animals vaccinated with candidate FimH vaccines inhibited uropathogenic *E. coli* from binding to human bladder cells in vitro. Immunization with FimH reduced in vivo colonization of the bladder mucosa by more than 99 percent in a murine cystitis model, and immunoglobulin G to FimH was detected in urinary samples from protected mice. Furthermore, passive systemic administration of immune sera to FimH also resulted in reduced bladder colonization by uropathogenic *E. coli*. This approach may represent a means of preventing recurrent and acute infections of the urogenital mucosa.

Adhesive pili mediate the colonization of mucosal surfaces, allowing bacteria to establish infection (*1*, *2*). Over the years much research has been motivated by the goal of using pili as vaccines to prevent bacterial infections (*3*). However, these efforts have often been thwarted by findings that the major immunodominant component of pilus fibers is often antigenically variable and thus would only offer protection against a limited number of bacterial strains. Pilus-associated adhesins, on the other hand, are typically highly conserved among different strains and species of bacteria (*4*, *5*). However, adhesins are often only minor components of pilus fibers (located at the pilus tips), and as a result, intact whole pili do not elicit a strong antibody response to the adhesin (*6*). The use of expression systems to purify large amounts of adhesin has often failed because most adhesins are proteolytically degraded when expressed as an independent moiety (*7*, *8*). However, the discovery that adhesins could be stabilized in active conformations by specific PapD-like chaperones (*2*, *9*, *10*) makes it possible to purify adhesins in large quantity. We used bacterial cystitis as a model infection to test the hypothesis that bacterial adhesins would prevent microbial attachment and colonization when used as a vaccine.

Cystitis is one of the most common disorders prompting medical evaluation in otherwise healthy women. *Escherichia coli* is the most prevalent pathogen associated with lower urinary tract infections, accounting for >85% of asymptomatic bacteriuria and acute cystitis cases (*11*), as well as >60% of recurrent bouts of cystitis. Recent studies have shown that the incidence of *E. coli* cystitis among women 18 to 40 years old ranges from 0.5 to 0.7 infections per person per year (*12*). Such infections result in 7 to 8 million physician and hospital visits per year at a cost of more than $1 billion (*13*).

In contrast to the extensive studies that have implicated P pili as a critical factor in causing upper urinary tract infections (pyelonephritis) (*14*, *15*), very little is known about the molecular basis of bladder infections. A number of studies have suggested that type 1 pili may play a role in bacterial cystitis by mediating colonization to mannose receptors along the bladder mucosa (*16*). In a murine cystitis model it was shown that colonization of the bladder by clinical isolates of *E. coli* was greatly dependent on growth conditions that favored the expression of type 1 pili (*17*, *18*). In both murine and primate models, expression of P pili was unnecessary and insufficient for bladder colonization (*15*, *17*–*19*). Also, in a murine model, type 1–positive isolates survived in higher numbers and induced a greater neutrophil influx into the urine than isogenic type 1–negative mutants (*20*). Reconstitution of type 1 pili in type 1–negative strains restored virulence similar to that of the wild type. In addition, disease severity was greater in children infected with *E. coli* isolates expressing type 1 pili than type 1–negative isolates of the same serotype, same electrophoretic type, and same P piliated and hemolysin phenotypes.

In light of some of these earlier studies, we obtained 57 urinary tract infection isolates of *E. coli* from multiple clinical centers and found that 52 out of 57 (91%) could be induced to express type 1 pili under optimal growth conditions for type 1 pilus expression (*1*, *2*). In contrast, only 30% of the 14 strains that were tested could be induced to express P pili under optimal growth conditions for P pilus expression (*1*, *2*).

The association of type 1 pili with cystitis led us to investigate whether a receptor is present in human bladder mucosa that is recognized by FimH, the adhesin that confers mannose-specific binding activity to type 1 pili (*21*). In situ binding to human tissues has been used to elucidate the roles in virulence of many different adhesins, including pilus-associated adhesins, in a variety of pathogens including *Helicobacter pylori*, *Streptococcus pneumoniae*, *Haemophilus influenzae*, and pyelonephritic *E. coli* (*22*). The NU14 *E. coli* cystitis isolate (*18*) bound avidly to the luminal surface of both mouse (Fig. 1B) and human (Fig. 1D) bladder epithelium. Mannose completely blocked this binding (*23*). A chloramphenicol cassette was recombined into the *fimH* gene in the

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chromosome of NU14, creating the *fimH*$^-$ mutant NU14-1 (*24*). NU14-1 failed to bind to the human and mouse bladder tissues (Fig. 1E). The *fimH*$^-$ mutation on the chromosome of NU14-1 was complemented with the *fimH* gene cloned downstream from the *tac* promoter on plasmid pHJ20 (*8*). Growth in the presence of isopropyl β-D-thiogalactopyranoside (IPTG) to induce expression of *fimH* restored the ability of the bacterial cells to bind to the human (Fig. 1F) and mouse bladder tissue. Complementation with pHJ19, which is the same as pHJ20 except that *fimH* is cloned in the opposite orientation, did not restore binding (Fig. 1E). Finally, the recombinant strain ORN103/pSH2 (type 1$^+$) (*25*) also exhibited mannose-sensitive binding to the mouse and human bladder tissues, and the *fimH*$^-$ isogenic mutant ORN103/pUT2002 (type 1$^+$/*fimH*$^-$) (*26*) did not bind.

We then investigated whether antibody to FimH would bind to type 1–piliated *E. coli*. Antibody was raised in mice to two forms of purified FimH protein: (i) a complex containing the periplasmic chaperone FimC bound to the full-length FimH protein (FimC-H), and (ii) a naturally occurring mannose-binding FimH truncate (FimHt), corresponding to the NH_2-terminal two-thirds of the FimH protein, was purified away from the FimC-H complex (*8*). Antibody was also raised to whole type 1 pili purified from ORN103/pSH2.

Both FimHt and FimC-H induced strong, long-lasting immune responses to isolated FimHt and to FimH associated with whole type 1–pilus organelles (Fig. 2). The responses persisted more than 30 weeks, and booster immunizations with FimHt or FimC-H increased responsiveness. In contrast, type 1 pili elicited poor anti-FimH responses even though mice developed strong responses to whole pilus rods. Immunization studies in rabbits demonstrated similar immunogenicity profiles to those seen in mice (*27*). Antisera to FimHt and to FimC-H bound to recombinant type 1$^+$/FimH$^+$ *E. coli* strains (ORN103/pSH2) but not to the type 1$^+$/FimH$^-$ isogenic mutant (ORN103/pUT2002) as determined by indirect immunofluorescence and flow cytometric analysis. Antibody to the whole pilus bound both ORN103/pSH2 and ORN103/pUT2002, as expected.

The ability of antibodies to the adhesin to prevent bacterial binding to bladder epi-

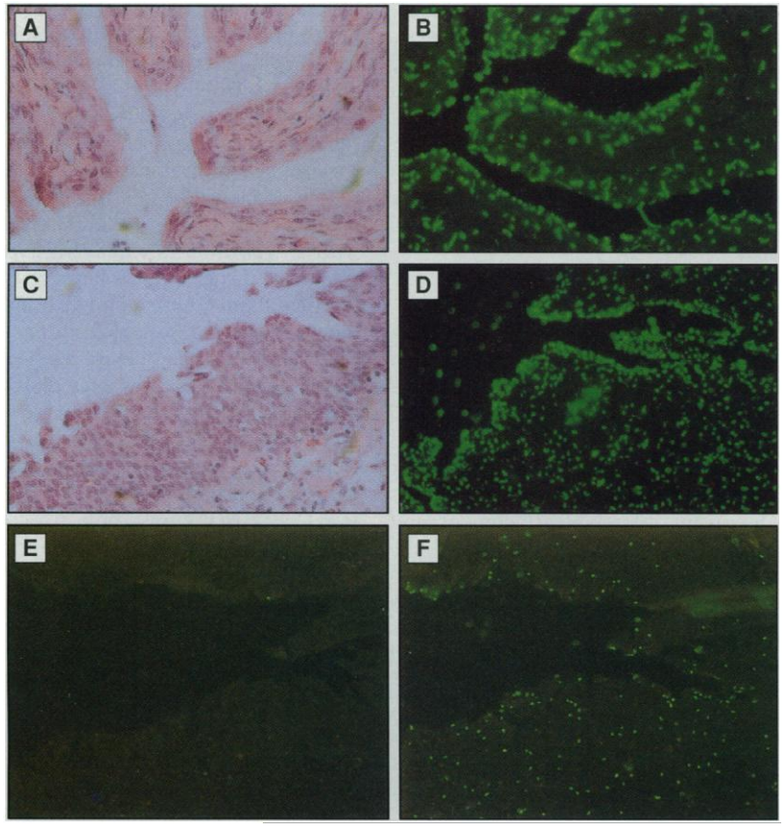

Fig. 1. FimH-mediated binding to human and mouse bladder tissue in situ. Human bladder sections were obtained from the surgical pathology and autopsy files of the Department of Pathology at Washington University. The tissue sections were deparaffinized as described previously and stained with hematoxylin and eosin [(**A**) (mouse) and (**C**) (human)]. The clinical *E. coli* isolate NU14 (type 1$^+$) was labeled with FITC as described (*22*) and tested for its ability to bind to mouse (**B**) and human (**D**) bladder tissue sections in situ. Binding to human bladder tissue was also analyzed for the *fimH*$^-$ variant NU14-1 strain complemented with pHJ19 (phenotypically FimH$^-$, as explained in text) (**E**) or with pHJ20 (*fimH*$^+$) (**F**). C3H bladders were obtained from 9-week-old C3H/HeJ mice. Binding of FITC-labeled bacteria to the tissue sections was essentially as described (*39*). FITC-labeled bacteria were incubated with bladder sections for 5 hours at room temperature, washed with PBS, and observed with a Zeiss Axioskop fluorescent microscope.

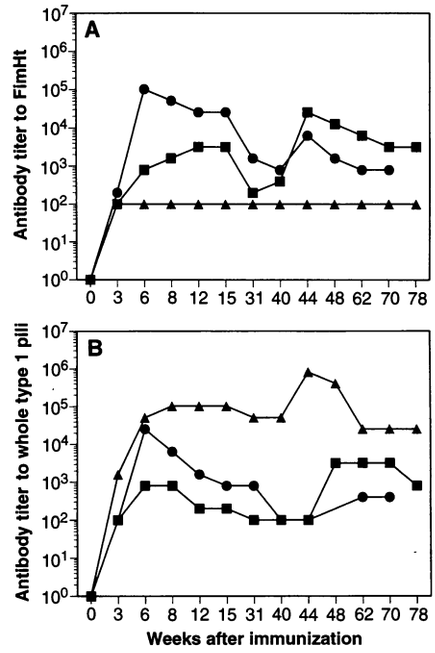

Fig. 2. Murine serum immunoglobulin G (IgG) titers to (**A**) FimHt adhesin and (**B**) whole type 1 pili after immunization with purified adhesin, adhesin-chaperone complex, or whole type 1 pili. The serum immune responses to FimH and whole type 1 pili were evaluated after primary immunization (day 0) [in complete Freund's adjuvant (CFA)] and booster immunization (week 4) [in incomplete Freund's adjuvant (IFA)] with purified FimHt (squares), FimC-H (circles), or whole type 1 pili (triangles) (*8*). The immunogenicity of all proteins was tested in C3H/HeJ mice, five mice per group. Samples from individual mice treated identically were pooled for serologic analysis and diluted 1:100 before serial dilution. Antibody responses were assessed by an ELISA with purified FimHt (A) or whole pili (B) as the capture antigens. Titers reflect the highest dilution of serum reacting twice as strongly as a comparable dilution of preimmune sera obtained from the same mice. The purity of the protein preparations ranged from 95% pure for whole type 1 pili and FimHt to 98 to 99% purity for FimC-H. In all cases the protein preparations were free of any lipopolysaccharide contaminants. Comparable immune response profiles to those shown for the C3H/HeJ mice were seen in BALB/C and C57/BL6 strains of mice.

thelial cells was investigated in vitro by a flow cytometric method originally developed to evaluate Rickettsia-cell attachment (*28*). Antibodies to FimHt (anti-FimHt) and anti–FimC-H blocked the ability of HB101/pSH2 (and strain ORN103/pSH2) and *E. coli* NU14 to bind to bladder cells from the human bladder epithelial cell line J82 (*29*) (Fig. 3). There was a direct relation between the antibody titer to FimH and the ability of the antisera to block microbial attachment (functional inhibitory titer). Furthermore, as titers to FimH dropped or leveled off by 30 to 40 weeks after immunization for FimHt- and FimC-H–immunized mice, the concomitant functional inhibitory titers dropped as well. However, as titers increased after a booster immunization at week 42 (Fig. 2B), the corresponding functional inhibitory titers were elevated. Given these results, we determined the functional inhibitory titers of anti-FimH to a panel of primary clinical isolates from women and children with active urinary tract infections (*30*). Antisera raised to FimHt and FimC-H blocked attachment of 49 out of 52 (~94%)

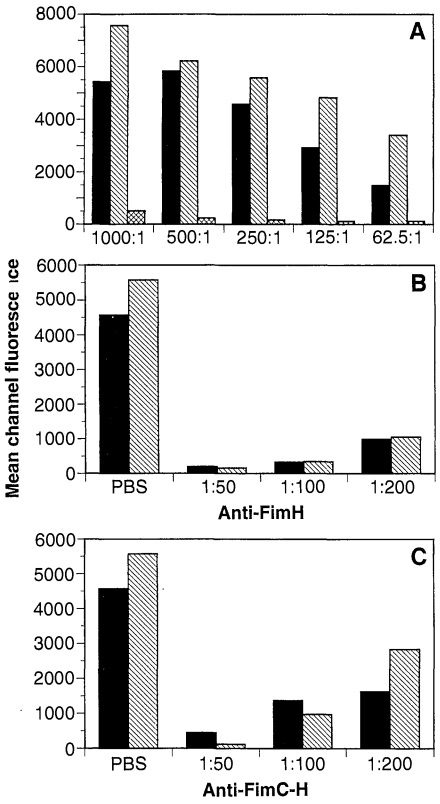

Fig. 3. In vitro binding of type 1–piliated *E. coli* to human bladder epithelial cells and inhibition by anti-FimH. (**A**) Type 1–piliated [HB101/pSH2 (black bars) and NU14 (striped bars)] and nonpiliated [HB101 (cross-hatched)] bacteria were directly labeled with FITC and tested for their ability to bind to bladder cells. Expression of type 1 pili was confirmed by hemagglutination of a 3% solution of guinea pig erythrocytes and inhibition of hemagglutination by a 10 mM solution of α-methyl mannoside (*40*). Labeled bacteria were incubated with 2×10^6 J82 (ATCC HTB1) bladder cells with bacteria:bladder cell ratios of 1000:1 to 62.5:1. Samples were assayed by flow cytometry in a FACStar PLUS (Becton Dickinson). Mean channel fluorescence was used as an indicator of FITC-labeled bacteria bound to J82 cells. The threshold for positivity was set for each experiment by flow cytometric analysis of J82 cells that were incubated with PBS only. For evaluation of FITC labeling of bacteria, gates were set with non–FITC-labeled bacteria. Lysis II software (Becton Dickinson Immunocytometry Systems) was used for analysis of data. (**B** and **C**) The inhibition of bacterial attachment by anti-adhesin was assayed with 2.5×10^8 HB101/pSH2 or NU14 bacteria incubated for 30 min at 37°C with high-titer anti-FimHt or anti-FimC-H (from bleeds at 6 to 9 weeks) at 1:50, 1:100, or 1:200 dilutions in PBS or with PBS alone. After incubation with the antisera, 2×10^6 J82 cells were added and allowed to mix with the bacteria for 30 min at 37°C. The remainder of the adherence assay and evaluation by flow cytometry were carried out as described in (A) (*41*).

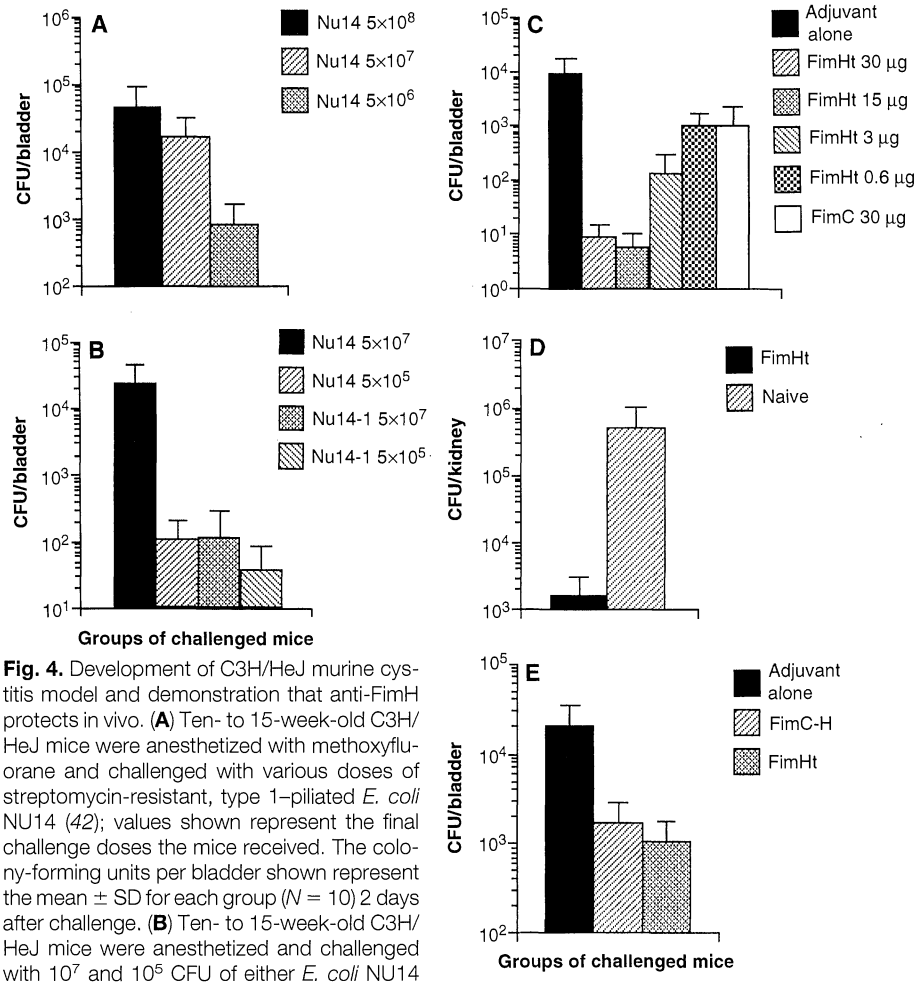

Fig. 4. Development of C3H/HeJ murine cystitis model and demonstration that anti-FimH protects in vivo. (**A**) Ten- to 15-week-old C3H/HeJ mice were anesthetized with methoxyfluorane and challenged with various doses of streptomycin-resistant, type 1–piliated *E. coli* NU14 (*42*); values shown represent the final challenge doses the mice received. The colony-forming units per bladder shown represent the mean ± SD for each group ($N = 10$) 2 days after challenge. (**B**) Ten- to 15-week-old C3H/HeJ mice were anesthetized and challenged with 10^7 and 10^5 CFU of either *E. coli* NU14 (type 1^+/FimH$^+$) or NU14-1 (*fimH*$^-$). The data represent the mean ± SD for each group ($N = 10$) 2 days after challenge. ($P = 0.026$ comparing the NU14 10^7 and NU14-1 10^7 challenge doses; analysis of variance was used in the calculation as well as the Dunnet's t test for multiple comparisons). (**C**) Four groups of C3H/HeJ mice were immunized on day 0 and boosted at week 4 with purified FimHt protein (doses ranging from 0.6 to 30 μg in CFA for the initial immunization and IFA for the booster) and challenged with 5×10^7 CFU of type 1–piliated *E. coli* at week 9 after immunization. The average number of colony-forming units per bladder for each group of 10 mice was evaluated as indicated above. FimC-immunized mice were included as a negative control along with naïve mice. [$P = 0.0001$ comparing the 30-, 15-, and 3-μg doses of FimHt with the naïve data; $P = 0.205$ for the FimC negative control at the highest (30 μg) dose]. (**D**) Two groups of C3H/HeJ mice were inoculated on day 0 and boosted at week 4 with purified FimHt protein (15 μg in CFA then in IFA, respectively) or with CFA/IFA adjuvant alone and challenged with 5×10^7 CFU of type 1–piliated *E. coli* strain at week 9 after immunization. Average number of colony-forming units per kidney for each group of 10 mice was evaluated as indicated above 7 days after the intraurethral challenge ($P = 0.295$). (**E**) Undiluted, high-titer antisera to FimH collected from mice vaccinated with 15 μg of either FimHt or FimC-H was administered passively in 0.1-ml volumes to C3H/HeJ mice by the intraperitoneal route on day 0, 4 hours before intraurethral challenge with 5×10^7 CFU of *E. coli* NU14. Recoverability of NU14 from the bladder was assessed 2 days after challenge as described above ($P = 0.008$, FimH versus naïve; $P = 0.004$, FimC-H versus naïve).

primary clinical *E. coli* UTI isolates induced to express type 1 pili (*31*). A subset of strains induced to express P pili, S pili, or both in addition to type 1 pili were also inhibited from attachment to the bladder epithelial cells by anti-FimH (*32*).

Antibodies to whole type 1 pili, purified from ORN103/pSH2, were poor inhibitors of bacterial binding to bladder epithelial cells; antisera to whole type 1 pili blocked <50% of the clinical isolates even at a 1:50 dilution of antiserum. This is presumably due to the variation in antigenicity of FimA among clinical isolates and may in part be due to the inability of whole type 1 pili to elicit significant anti-FimH responses (Fig. 2B). Preimmune sera and antisera from mice given adjuvant alone or antisera from mice immunized with a control FimC chaperone protein did not inhibit binding.

J82 bladder cells that were sorted from the flow cytometric adherence assay were also analyzed by fluorescent microscopy. The number of fluorescent bacteria attached to 40 bladder cells was visually quantitated. Adherence values (mean number of bacteria ± SD per cell) for two representative cystitis isolates, NU14 and EC72, were 35.2 ± 9.5 and 33.9 ± 4.9 bacteria/cell, respectively, in the absence of FimH-specific antibody; incubation with a 1:50 dilution of anti-FimH completely blocked attachment. Furthermore, the same examination of samples taken from bacteria incubated with different dilutions of anti-FimH confirmed the dose-dependency of this phenomenon.

Intraurethral inoculation of C3H mice with 5×10^7 type 1–piliated *E. coli* (strain NU14) resulted in a highly reproducible colonization of the mouse bladder (Fig. 4A) (*33*). Piliated bacteria persisted in the bladder for at least 7 days [10^4 colony-forming units (CFU)/bladder] and produced ascending infection into the kidney.

The role of mannose-binding type 1 pili in bladder colonization was investigated by testing the effect of the *fimH*$^-$ mutation in the murine model. Inoculation with the *fimH*$^-$ mutant, NU14-1, resulted in little or no colonization of the mouse bladder (Fig. 4B), supporting the hypothesis that FimH plays a critical role in colonization of the bladder.

C3H mice were immunized with the various FimH vaccines and challenged with the NU14 clinical isolate 9 weeks after the primary immunization. Vaccinated animals exhibited a 100- to 1000-fold reduction in the number of organisms recovered from the bladders as compared with adjuvant- or FimC-immunized controls (Fig. 4C). Similar results were seen with the FimC-H vaccine. Protection with the FimHt vaccine was seen as late as 29 weeks after immunization, the latest time point tested (*34*). In addition, the anti-FimH also blocked an ascending infection into the kidney over a 7-day period (Fig. 4D). These protective effects were correlated with the presence of anti-FimH in the urine. Mice that received the FimH vaccine had significant amounts of IgG to FimH in their urine, whereas unimmunized mice, or mice vaccinated with FimC alone, did not have any measurable amounts of anti-FimH in urinary secretions.

To confirm that the observed protection was mediated by anti-FimH, we challenged naïve C3H mice with 5×10^7 type 1–piliated *E. coli* strain NU14 after passive, intraperitoneal administration of hyperimmune mouse FimH antisera raised to either the FimC-H or FimHt proteins. In both cases sera containing anti-FimH resulted in a 100- to 150-fold reduction in the number of organisms recovered from the bladder 2 days after challenge (Fig. 4E). Sera from mice that received only adjuvant did not protect at all.

To determine if protection was neutrophil dependent, we rendered nonimmunized and FimH-immunized mice neutropenic before intraurethral challenge with type 1–piliated *E. coli* (*35*). Nonimmunized, neutropenic mice showed a 100-fold increase in the number of organisms in the bladder relative to immunocompetent, nonimmunized mice (10^6 CFU/bladder compared with 10^4 CFU/bladder). Immunization with FimH vaccine reduced colonization in both neutropenic and non-neutropenic mice to equivalent levels of 10^2 CFU/bladder. Thus the absence of neutrophils did not impede the antibacterial activities of the FimH vaccine in vivo.

We have demonstrated that the binding of FimH to a receptor that is exposed on the luminal surface of both the human and mouse bladder epithelium is critical for uropathogenic *E. coli* strains to colonize the bladder and cause cystitis; previous studies have suggested that the bladder receptor for FimH may be uroplakin (*36*). We have found that IgG to FimH blocks colonization in vivo and protects mice against a mucosal infection of the bladder and subsequent ascending urinary tract infections. Although the exact mechanism of protection by anti-FimH is not known, the in vitro binding data along with the neutropenic mouse experiments would suggest that the ability of anti-FimH to reduce attachment to host epithelium is a significant factor in preventing the establishment of infection.

Although type 1 pili clearly play a role in urinary tract infections, it is not known whether they contribute to colonization of the gastrointestinal tract. It remains to be determined what effect, if any, anti-FimH may have on the normal flora of the gastrointestinal tract. However, earlier studies in humans demonstrated that whole type 1 pilus vaccines derived from an enterotoxigenic *E. coli* strain had no adverse effects on gastrointestinal physiology or motility even when administered at doses as high as 1800 μg (*37*).

Adhesin-based vaccines may be an effective strategy to ward off other diseases such as otitis media, pneumonia, meningitis, pyelonephritis, and gonorrhea. Organisms responsible for these diseases, *H. influenzae*, *Klebsiella pneumoniae*, *Neisseria meningitidis*, pyelonephritic *E. coli*, and *Neisseria gonorrhoeae*, respectively, express pilus-associated adhesins (*38*). Vaccination with these bacterial adhesins may also elicit antibodies that will effectively block microbial colonization of mucosal surfaces and be an effective means to treat or prevent these infections as well.

REFERENCES AND NOTES

1. S. J. Hultgren, S. Normark, S. N. Abraham, *Annu. Rev. Microbiol.* **45**, 383 (1991); F. Jacob-Dubuisson, M. Kuehn, S. J. Hultgren, *Trends Microbiol.* **1**, 50 (1993); S. J. Hultgren and C. H. Jones, *ASM News* **61**, 457 (1995).
2. S. J. Hultgren *et al.*, *Cell* **73**, 887 (1993).
3. E. H. Beachey, *J. Infect. Dis.* **143**, 325 (1981); S. D. Acre, R. E. Isaacson, L. A. Babiuk, R. A. Kapitany, *Infect. Immun.* **25**, 121 (1979); C. Brinton *et al.*, in *Current Chemotherapy and Infectious Diseases*, J. D. Nelson and C. Grassi, Eds. (American Society for Microbiology, Washington, DC, 1980), pp. 1242–1245; R. E. Isaacson, E. A. Dean, R. I. Morgan, H. W. Moon, *Infect. Immun.* **29**, 824 (1980); R. I. Morgan, R. E. Isaacson, H. W. Moon, C. C. Brinton, C. C. To, *ibid.* **22**, 771 (1978); B. Nagy, H. W. Moon, R. E. Isaacson, C. C. To, C. C. Brinton, *ibid.* **21**, 269 (1978); J. M. Rutter and G. W. Jones, *Nature* **242**, 531 (1973); C. C. Brinton *et al.*, in *Immunobiology of* Neisseria gonorrhoeae, C. F. Brooks and E. C. Gotschlich, Eds. (American Society for Microbiology, Washington, DC, 1978), pp. 155–178 ; F. J. Silverblatt and L. S. Cohen, *J. Clin. Invest.* **64**, 333 (1979); F. J. Silverblatt and I. Ofek, *J. Infect. Dis.* **138**, 664 (1978); E. Tramont *et al.*, in *Current Chemotherapy and Infectious Diseases*, J. D. Nelson and C. Grassi, Eds. (American Society for Microbiology, Washington, DC, 1980), pp. 1240–1242.
4. S. N. Abraham, D. Sun, J. B. Dale, E. H. Beachey, *Nature* **336**, 682 (1988); B. I. Marklund *et al.*, *Mol. Microbiol.* **6**, 2225 (1992).
5. G. F. Gerlach, S. Clegg, B. L. Allen, *J. Bacteriol.* **171**, 1262 (1989).
6. F. Lindberg, B. Lund, L. Johansson, S. Normark, *Nature* **328**, 84 (1987); M. S. Hanson and C. C. Brinton, *ibid.* **332**, 265 (1988).
7. S. J. Hultgren *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 4357 (1989); M. J. Kuehn, S. Normark, S. J. Hultgren, *ibid.* **88**, 10586 (1991).
8. C. H. Jones *et al.*, *ibid.* **92**, 2081 (1995).
9. D. L. Hung, S. D. Knight, R. M. Woods, J. S. Pinkner, S. J. Hultgren, *EMBO J.* **15**, 3792 (1996).
10. A. Holmgren, M. Kuehn, C. I. Branden, S. J. Hultgren, *ibid.* **11**, 1617 (1992); L. N. Slonim, J. S. Pinkner, C. I. Branden, S. J. Hultgren, *ibid.*, p. 4747; M. J. Kuehn *et al.*, *Science* **262**, 1234 (1993); C. H. Jones *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **90**, 8397 (1993).
11. J. D. Sobel, *Med. Clin. North Am.* **75**, 253 (1991).
12. T. M. Hooton *et al.*, *N. Engl. J. Med.* **335**, 468 (1996).
13. W. E. Stamm and T. M. Hooton, *ibid.* **329**, 1328 (1993); C. M. Kunin, *Clin. Infect. Dis.* **18**, 1 (1994).
14. A. Lomberg and C. Svanborg-Eden, *Monogr. Allergy* **24**, 35 (1988).
15. J. A. Roberts *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **91**,

11889 (1994).
16. J. P. Duguid and D. C. Old, in *Bacterial Adherence, Receptors and Recognition*, E. H. Beachey, Ed. (Chapman & Hall, London, 1977), pp. 184–217; J. E. Salit and E. Gotschlich, *J. Exp. Med.* **146**, 1182 (1977); I. Ofek, A. Mosek, N. Sharon, *Infect. Immun.* **34**, 708 (1981); A. J. Schaeffer, S. K. Amundsen, L. N. Schnidt, *ibid.* **24**, 753 (1979).
17. S. J. Hultgren, T. N. Porter, A. J. Schaeffer, J. L. Duncan, *Infect. Immun.* **50**, 370 (1985).
18. S. J. Hultgren, W. R. Schwann, A. J. Schaeffer, J. L. Duncan, *ibid.* **54**, 613 (1986).
19. H. L. T. Mobley *et al.*, *Mol. Microbiol.* **10**, 143 (1993).
20. H. Connell *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **93**, 9827 (1996).
21. L. Maurer and P. E. Orndorff, *J. Bacteriol.* **169**, 640 (1987); M. S. Hanson and C. C. Brinton, *ibid.* **170**, 3350 (1988); S. N. Abraham, J. D. Goguen, D. Sun, P. Klemm, E. H. Beachey, *ibid.* **169**, 5530 (1987).
22. P. Falk *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **90**, 2035 (1993); D. R. Cundell, N. P. Gerard, C. Gerard, I. Idanpaan-Heikkila, E. I. Tuomanen, *Nature* **377**, 435 (1995); J. W. St. Geme III, *Infect. Immun.* **62**, 3881 (1994); R. Lindstedt *et al.*, *ibid.* **57**, 3389 (1989).
23. To assess mannose inhibition of binding activity by type 1–piliated NU14, we suspended fluorescein isothiocyanate (FITC)–labeled NU14 in a 15% mannose solution in phosphate-buffered saline (PBS) before the in situ tissue hybridization assay described in Fig. 1.
24. A suicide vector, pJEB521, was constructed by insertion of a fragment containing the type 1 operon region (with a chloramphenicol resistance marker inserted into the *fimH* gene at the Kpn I site) into the pCVD442 suicide plasmid {provided by M. Lombardo, M. S. Donenberg, and J. B. Kaper [*Infect. Immun.* **59**, 4310 (1991)]}. PCR analysis of the pJEB521 plasmid confirmed correct insertion of the chloramphenicol resistance marker in the *fimH* gene. The *fimH*$^-$ strain, NU14-1, was constructed by introducing pJEB521 from SM10λpir into the *E. coli* clinical isolate NU14 by filter mating. Selection for incorporation of the *fimH* knockout sequences into the NU14 strain was by chloramphenicol and ampicillin resistance, with subsequent analysis for the loss of the ability to hemagglutinate guinea pig red blood cells. Colonies that had lost hemagglutination activity were repeatedly passed in liquid media lacking ampicillin. Serial dilutions were plated on chloramphenicol media and screened for the loss of ampicillin resistance, indicative of the loss of suicide vector sequences. Chloramphenicol-resistant colonies that were ampicillin sensitive were again screened for the inability to hemagglutinate guinea pig red blood cells. One such colony (ampicillin sensitive, chloramphenicol resistant, hemagglutination negative) was designated NU14-1 and used for the binding studies as well as for further comparative studies; DNA blots of chromosomal DNA from NU14-1 confirmed that the mutation was specifically at the *fim*H locus. Electron microscopy revealed that the mutation also greatly reduced piliation.
25. P. E. Orndorff and S. Falkow, *J. Bacteriol.* **159**, 736 (1984); F. Jacob-Dubuisson, F. Heuser, K. Dodson, S. Normark, S. J. Hultgren, *EMBO J.* **12**, 837 (1993).
26. F. C. Minion, S. N. Abraham, E. H. Beachey, J. D. Goguen, *J. Bacteriol.* **165**, 1033 (1989).
27. New Zealand White rabbits (female, 2.5 kg) were injected subcutaneously with 200 μg of FimC-H, FimHt, or whole type 1 pili (day 0) and boosted with 50 μg of each of the corresponding proteins (week 4). Bleeds were obtained every 3 to 4 weeks, and endpoint titers to FimH and whole type 1 pili were determined by enzyme-linked immunosorbent assay (ELISA) as described in Fig. 2.
28. H. Li and D. H. Walker, *Infect. Immun.* **60**, 2030 (1992).
29. W. W. Agace *et al.*, *ibid.* **61**, 602 (1993).
30. *Escherichia coli* was isolated from urine specimens obtained from adult female or pediatric patients with urinary tract infections at Columbia Presbyterian Medical Center in New York or Children's Hospital in Boston, MA. Bacteria were grown in static broth at 37°C for 48 hours to induce type 1 pilus expression (*24*). Expression of type 1 pili was confirmed by hemagglutination of a 3% solution of guinea pig erythrocytes and inhibition of hemagglutination by a 10 mM solution of α-methyl mannoside (*39*).
31. D. C. Dodd and B. I. Eisenstein, *Infect. Immun.* **38**, 764 (1982).
32. *Escherichia coli* strains were induced for P pilus expression by overnight growth on tryptic soy agar (TSA) at 37°C. Organisms were tested for mannose-resistant hemagglutination (MRHA) of a 3% suspension of human red blood cells (HuRBCs) to screen for P pilus expression. Strains that were positive for MRHA of 3% HuRBCs were tested for inhibition by antisera to PapGII to confirm P pilus expression. *Escherichia coli* strains were also tested for MRHA of a 3% suspension of swine red blood cells (SwRBCs) to screen for S pilus expression.
33. L. Hagberg *et al.*, *Infect. Immun.* **40**, 273 (1983); P. O'Hanley, D. Lark, S. Falkow, G. Schoolnik, *J. Clin. Invest.* **75**, 347 (1985); H. L. T. Mobley *et al.*, *Infect. Immun.* **58**, 1281 (1990).
34. Two groups of C3H/HeJ mice were inoculated on day 0 and boosted at week 4 with purified FimHt protein (15 μg in CFA, then in IFA, respectively) or with CFA or IFA adjuvant alone and challenged with 5×10^7 CFU of type 1–piliated *E. coli* strain NU14 at week 29 after immunization. The average number of colony-forming units per bladder for each group of 10 mice was evaluated as described for Fig. 4C 2 days after the intraurethral challenge. A 100-fold reduction was seen in the number of recoverable organisms for FimHt-immunized mice as compared with CFA or IFA naïve controls ($P = 0.0001$).
35. H. H. Collins *et al.*, *J. Infect. Dis.* **159**, 1073 (1989); S. M. Opal *et al.*, *ibid.* **161**, 1148 (1990).
36. X. R. Wu, T. T. Sun, J. J. Medina, *Proc. Natl. Acad. Sci. U.S.A.* **93**, 9630 (1996).
37. M. M. Levine, R. E. Black, C. C. Brinton, *Scand. J. Infect. Dis. Suppl.* **33**, 83 (1982).
38. S. Marieke van Ham, L. van Alphen, F. R. Mooi, J. P. M. van Putten, *Infect. Immun.* **63**, 4883 (1995); K. W. McCrea, W. J. Watson, J. R. Gilsdorf, C. F. Marrs, *ibid.* **62**, 4992 (1994); B. Lund, F. P. Lindberg, M. Baga, S. Normark, *J. Bacteriol.* **162**, 1293 (1985); M. J. Kuehn, J. Heuser, S. Normark, S. J. Hultgren, *Nature* **356**, 252 (1992); X. Nassif *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **91**, 3769 (1994); T. Rudel, I. Scheuerpflug, T. F. Meyer, *Nature* **373**, 357 (1995).
39. R. Striker, U. Nilsson, A. Stonecipher, G. Magnusson, S. J. Hultgren, *Mol. Microbiol.* **16**, 1021 (1995).
40. J. P. Duguid, S. Clegg, M. I. Wilson, *J. Med. Microbiol.* **12**, 213 (1979); N. Firon, I. Ofek, N. Sharon, *Carbohydr. Res.* **120**, 235 (1983); C. S. Giampapa, S. N. Abraham, T. M. Chiang, E. H. Beachey, *J. Biol. Chem.* **263**, 5362 (1988).
41. The bacteria–epithelial cell mixtures in the binding assay were incubated while being mixed at 37°C to achieve maximal binding, then gently washed two times with PBS to remove nonadherent bacteria; after the second wash the epithelial cell pellet was resuspended in PBS, and the samples were assayed by flow cytometry. Background fluorescence was determined by analysis of J82 cells alone.
42. Mice were inoculated with bacteria by inserting a polyethylene catheter adapted to a flat-end 30-gauge needle on a 1-ml tuberculin syringe into the bladder transurethrally and infusing 0.05 ml of a bacterial suspension in PBS. Two days after challenge, the mice were killed and the bladders were removed aseptically, homogenized, and cultured on TSA plates supplemented with streptomycin. To assess ascending infection into the kidney, we killed the mice and removed the kidneys aseptically 7 days after challenge, homogenized them, and cultured them on TSA plates supplemented with streptomycin.
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Partitioning of Large and Minichromosomes in *Trypanosoma brucei*

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The *Trypanosoma brucei* nuclear genome contains about 100 minichromosomes of between 50 to 150 kilobases and about 20 chromosomes of 0.2 to 6 megabase pairs. Minichromosomes contain nontranscribed copies of variant surface glycoprotein (VSG) genes and are thought to expand the VSG gene pool. Varying VSG expression allows the parasite to avoid elimination by the host immune system. The mechanism of inheritance of *T. brucei* chromosomes was investigated by in situ hybridization in combination with immunofluorescence. The minichromosome population segregated with precision, by association with the central intranuclear mitotic spindle. However, their positional dynamics differed from that of the large chromosomes, which were partitioned by kinetochore microtubules.

Trypanosoma brucei is a flagellated protozoan parasite that separated from the eukaryotic lineage very early in the evolution of eukaryotes (*1*, *2*). The extracellular parasite survives in the bloodstream of the host by periodically changing its VSG coat, a process known as antigenic variation (*3*). VSG genes, which are the only known open reading frames on the 100 or so minichromosomes of *T. brucei*, are transcriptionally inactive (*4*–*8*). Minichromosomes are thought to increase the repertoire of VSG genes, which can be transposed to expression sites on the larger chromosomes (*9*).

In eukaryotic cells, chromosomes are typically segregated by association with a bipolar mitotic spindle. This ensures an almost perfect mechanism to faithfully segregate chromosomes during cell division. Although there is an intranuclear mitotic spindle in *T. brucei*, indirect evidence argues against a microtubule-dependent segregation mechanism for at least part of the genome. Elec-

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