Localization of a Domain in the FimH Adhesin of *Escherichia coli* Type 1 Fimbriae Capable of Receptor Recognition and Use of a Domain-specific Antibody to Confer Protection against Experimental Urinary Tract Infection

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Abstract

The FimH subunit of type 1–fimbriated *Escherichia coli* has been implicated as an important determinant of bacterial adherence and colonization of the urinary tract. Here, we sought to localize the functionally important domain(s) within the FimH molecule and to determine if antibodies against this domain would block adherence of type 1–fimbriated *E. coli* to the bladder mucosa in situ and in vivo in an established mouse model of cystitis. We generated translational fusion proteins of disparate regions of the FimH molecule with an affinity tag MalE, and tested each of the fusion products in vitro for functional activity. The minimum region responsible for binding mouse bladder epithelial cells and a soluble mannoprotein, horseradish peroxidase, was contained within residues 1–100 of the FimH molecule. We validated and extended these findings by demonstrating that antibodies directed at the putative binding region of FimH or at synthetic peptides corresponding to epitopes within the binding domain could specifically block type 1 fimbriae-mediated bacterial adherence to bladder epithelial cells in situ and yeast cells in vitro. Next, we compared the ability of mice passively immunized intraperitoneally with antisera raised against residues 1–25 and 253–264 of FimH or 1–13 of FimA to resist bladder colonization in vivo after intravesicular challenge with type 1–fimbriated *E. coli.* Only the antibody directed at the putative binding region of FimH (anti–s-FimH$_{1–25}$) significantly reduced *E. coli* bladder infections in the experimental mouse model of urinary tract infections. Similar results were obtained when the mice were actively immunized with synthetic peptides corresponding to residues 1–25 and 253–264 of FimH or 1–13 of FimA. The mechanism of protection was attributed, at least in part, to inhibition of bacterial adherence to the bladder surface by s-FimH$_{1–25}$–specific antibody molecules that had filtered through the kidneys into the urine. The level of FimH antibodies entering the bladder from the circulatory system of the immunized mice was found to be markedly enhanced upon bacterial challenge. The potential broad spectrum activity of the protective FimH antibody was indicated from its serologic cross-reactivity with various urinary tract bacterial isolates bearing type 1 fimbriae. These findings could be relevant in the design of an efficacious and broadly reactive FimH vaccine against urinary tract infections. (*J. Clin. Invest.* 1997. 100:1123–1136.) Key words: FimH • vaccine • urinary tract infections • type 1 fimbriae • bacterial adhesion

Introduction

Over 80% of all urinary tract infections (UTIs)[1] are caused by the enterobacteria *Escherichia coli*, and an overwhelming majority of these uropathogenic isolates express mannose-binding filamentous organelles called type 1 fimbriae (1–3). Although the importance of type 1 fimbriae in initiating infection by facilitating colonization of the urinary and other mucosal surfaces by *E. coli* and other enterobacteria has been demonstrated by several laboratories (2, 4–6), there was debate in some quarters as to their relative virulence potential (7, 8). Some of the controversy may have subsided, however, in view of a recent report confirming the importance of type 1 fimbriae in the initiation of UTIs in an experimental mouse model (9). Additionally, there is now a growing body of experimental and epidemiological data implicating type 1 fimbriae in the development of inflammatory, and potentially harmful, reactions in the host after bacterial infections (9–14). Perhaps, the best known example is the role of *E. coli* type 1 fimbriae in the development of renal scarring, a frequent sequelae to recurrent UTIs (13–14).

Type 1 fimbriae were first described by Brinton in 1955 and they represent the most widely studied fimbriae of bacteria. Although most of the genetic and structural analyses of type 1 fimbriae have focused on the fimbriae of *E. coli*, studies by Clegg and Gerlach (15), and Gerlach et al. (16) imply that the organization of the gene cluster and the architecture of type 1 fimbriae on enterobacteria are remarkably similar. A cluster of eight to nine closely associated genes located in the bacterial chromosome are responsible for the biogenesis, assembly and function of type 1 fimbriae (17–19). Each type 1 fimbrial filament is ~ 1–2-μm long with a diameter of 7 nm (20). It is a heteropolymer comprising of a major subunit FimA and three mi-

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1. *Abbreviations used in this paper:* KLH, keyhole limpet hemocyanin; MalE, maltose-binding protein; UTIs, urinary tract infections.

nor subunits FimF, FimG, and FimH (17, 18, 21, 22). The FimA subunits constitute > 95% of the total fimbrial proteins and are arranged in a tight right-handed helix forming a central axial hole (20). Immune electron microscopy has revealed FimH to be placed strategically at the distal fimbrial tips and also, longitudinally, at various intervals (23, 24). The FimH molecules that are localized at the fimbrial tips appear to be complexed with FimG, forming a flexible fibrillum structure (25). At this time, it is not known where FimF, the other minor subunit of the fimbriae, is located on this complex organelle.

Several lines of evidence suggest that the determinant on *E. coli* type 1 fimbriae responsible for mediating mannose-specific adherence is its FimH subunit and that the presence of this fimbrial moiety is important for initiating bacterial infections in the urinary tract. First, type 1–fimbriated *E. coli* completely lose their mannose-sensitive binding activity when the *fimH* gene within the type 1 fimbrial gene cluster was inactivated (18, 19, 24, 26). Second, antibodies directed at the amino terminus region of *E. coli* FimH specifically blocked attachment to epithelial cells of various *E. coli* strains and, even more remarkably, the attachment of several other enterobacteria (23, 24). This finding also implied that FimH was antigenically conserved among various species of enterobacteria (23). Third, when a $FimH^-$ mutant was compared with the type 1 fimbriae bearing a wild-type strain for its ability to colonize the mouse bladder in an experimental model of cystitis, the former was markedly less infectious (9, 26). Finally, inert beads coated with recombinant FimH mimicked the same mannose-sensitive binding to host cells as type 1 fimbriated *E. coli*, whereas beads coated with FimA, the major type 1 fimbrial protein, failed to exhibit any binding activity (10, 27). Thus, all of the data to date suggest that FimH, a relatively minor fimbrial component, is responsible for the characteristic mannose-specific binding activity of type 1 fimbriae to various host cells including uroepithelial cells. Complementing these findings is the recent intriguing observation that the cognate FimH receptors on uroepithelial cells were uroplakins, which are plaques of glycoprotein that occupy 70–80% of the apical surface of the uroepithelium (28).

In light of the important role played by FimH in promoting the adherence of *E. coli* and possibly other enterobacteria, we sought to localize the functionally important domain(s) within the FimH molecule. We have used translational fusion proteins comprising of disparate regions of the FimH molecule and antibodies directed at these proteins to map functionally active domains of FimH. We have also tested the efficacy of antibodies directed at functionally relevant regions of FimH in protecting animals against *E. coli*-induced UTIs.

Methods

Bacterial culture, strains, and plasmids. The strains and plasmids used in this work are described in Table I. All strains were cultured in brain heart infusion broth or in Luria broth (BBL Microbiology Systems, Cockeysville, MD). The organisms were tested for type 1 fimbrial expression before they were used for adherence assays or in vivo challenge studies. The presence of type 1 fimbriae on bacteria was determined by immunoelectron microscopy and by mannose-sensitive hemagglutination assays employing guinea pig erythrocytes (23, 24).

Culture of mouse bladder epithelial cells. Immortalized mouse bladder epithelial cells MM45T.BL were obtained from American Type Culture Collection (ATCC, Rockville, MD). The cells were cultured in Dulbecco's modified Eagle's medium (DME) with 10% heat-inactivated FBS at 37°C in a humidified atmosphere of 95% air/5% CO_2.

DNA manipulations. Restriction enzymes and DNA-modifying enzymes were used as recommended by the suppliers (Boehringer Mannheim Biochemicals, Indianapolis, IN; New England Biolabs, Inc., Beverly, MA). The plasmid pSH2 that contains the entire type 1 *fim* gene cluster (18) was used as the template to generate partial *fimH* DNA sequences. PCR products were cloned into plasmid vector pMAL-p2 (New England Biolabs, Inc.) using standard techniques. The vector pMAL-p2 encodes the affinity tag (maltose-binding protein [MalE]) and the (Asp)10 linker, which have a molecular mass of ~ 44 kD. Oligonucleotides were synthesized and purified commercially (Integrated DNA Technologies, Inc., Coralville, IA). The following are the sequences of the forward and reverse oligonucleotide primers used to generate the *fimH* DNA fragments employed in this study: primer Ec1: (5′ - GCC GGA ATT CTG TAA AAC CGC CAA TGG TAC C - 3′), primer Ec2: (5′ - GGG CAA GCT TCT ATT ATT GAT AAA CAA AAG TCA C - 3′), primer Ec3: (5′ - GGG CAA GCT TCT ATT AGC CGC CAG TAG GCA CCA CCA C - 3′), primer Ec4: (5′ - GGG CAA GCT TCT ATT ACG GCT TAT CCG TTC TCG AAT T - 3′), primer Ec5: (5′ - GGG CAA GCT TCT ATT AAA TGG TTT CCG GAT AAT CGT T 3′), primer Ec6: (5′ - GCC GGA ATT CTG CGA TGT TTC TGC TCG TGA T - 3′), primer Ec7: (5′ - GGG CGG ATC CTT ATT GAT AAA CAA AAG TCA C - 3′) , primer Ec8: (5′ - GCC GGA ATT CTG GCC GGT GGC GCT TTA TTT G - 3′), primer Ec9: (5′ - GGG CGG ATC CTT AGC CGC CAG TAG GCA CCA C - 3′), and primer Ec10: (5′ - GCC GGA ATT CCA TAA CGA TTA TCC GGA AAC C - 3′). Primer sets Ec1 and Ec2 , Ec1 and Ec3, Ec1 and Ec4, Ec1 and Ec5, Ec6 and Ec7, Ec8 and Ec9, and Ec9 and Ec10 were used to produce the plasmids pKT100, pKT101, pKT102, pKT103, pKT104, pKT105, and pKT106, respectively. A complete list of plasmids that were generated as well as their gene products are described in Table I. Plasmids were introduced into *E. coli* by a method outlined by Sambrook et al. (29). Factor Xa, expression vector pMAL-p2, amylose resin, antiserum against MalE, and purified MalE were obtained from the New England Biolabs, Inc.

SDS-PAGE and immunoblotting. Samples of whole bacterial cell extracts or purified fusion proteins were subjected to SDS-PAGE on a 10% slab gel by the method of Laemmli (30). The proteins were subsequently electrophoretically transferred onto nitrocellulose membranes. The immunostaining of specific protein was performed essentially as described by Towbin et al. (31), using antiserum raised against FimH or MalE.

Extraction of periplasmic contents and purification of MalE/FimH fusion proteins. Once the expression of each MalE/FimH fragment chimera was confirmed by immunoblotting, we proceeded to generate large amounts of the fusion protein in the periplasmic fraction of induced cultures (27, 32, 33). In order to induce expression of the fusion protein, 0.3-mM isopropyl-β-D-thiogalactopyranoside (IPTG) was added to the culture medium. Periplasmic fluids from various clones were extracted from 2 liter culture suspensions as described previously (25, 32). Each fusion protein was isolated by passing bacterial periplasmic material through a Sepharose-amylose affinity column. The fusion proteins were bound to the column via their affinity tag (the MalE portion). Bound fusion protein was subsequently eluted by the addition of 10-mM maltose and the purity of the eluate confirmed on an SDS-PAGE gel.

Overlay assays using mouse bladder epithelial cells and horseradish peroxidase. Purified fusion proteins comprising of MalE and full length FimH or MalE and various FimH fragments were subjected to SDS-PAGE and transferred onto nitrocellulose membranes. After blocking in 3% BSA made up in PBS for 1 h, the nitrocellulose blot was overlaid with 5×10^6 biotinylated mouse bladder epithelial cells (33), or 100 mM of horseradish peroxidase made up in PBS (Type VI-A; Sigma Chemical Co., St. Louis, MO) in the presence and absence of 100-mM α-methyl-D-mannoside. Bladder cells were biotinylated as described previously (33, 34). After 1 h of incubation at 22°C, the blot

Table I. Strains and Plasmids Employed in this Study

Strains and plasmids	Properties	Reference/source
Strains		
E. coli ORN103	*thr-1 leu-6 thi-1 Δ(arg*F-*lac)* U*169 xyl-7 ara-13 mtl-2 gal-6 rps*L *tonA2 fhuA2 min*A *min*B *rec*A*13* Δ(*fim*ABCDEFGH)	(18)
E. coli TB1	F$^-$*ara Δ(lac-proAB)rpsL* (Str^r){*ø80 dlacΔ(lacZ)M15Δ*} *hsdR* ($r_k^-m_k+$)	New England Biolabs, Inc.
E. coli CI5	Type 1–fimbriated uropathogenic isolate	(6)
K. pneumoniae CI111	Type 1–fimbriated uropathogenic isolate	(This study)
Citrobacter diversus CI113	Type 1–fimbriated uropathogenic isolate	(This study)
Enterobacter aerogenes CI114	Type 1–fimbriated uropathogenic isolate	(This study)
E. coli CI115	Type 1–fimbriated uropathogenic isolate	(This study)
E. coli CI116	Type 1–fimbriated uropathogenic isolate	(This study)
K. pneumoniae CI117	Type 1–fimbriated uropathogenic isolate	(This study)
E. coli CI118	Type 1–fimbriated uropathogenic isolate	(This study)
Serratia marcescens CI119	Type 1–fimbriated uropathogenic isolate	(This study)
K. pneumoniae CI120	Type 1–fimbriated uropathogenic isolate	(This study)
E. coli CI121	Type 1–fimbriated uropathogenic isolate	(This study)
K. pneumoniae CI122	Type 1–fimbriated uropathogenic isolate	(This study)
E. coli CI123	Type 1–fimbriated uropathogenic isolate	(This study)
E. coli CI124	Type 1–fimbriated uropathogenic isolate	(This study)
Plasmids		
pSH2	*E. coli* type 1 fimbriae encoding plasmid	(18, 19)
pMAL-p2	MalE affinity tag expression vector	New England Biolabs, Inc.
pKT100	MalE/FimH$_{1–277}$ fusion expression plasmid	(This study)
pKT101	MalE/FimH$_{1–158}$ fusion expression plasmid	(This study)
pKT102	MalE/FimH$_{1–100}$ fusion expression plasmid	(This study)
pKT103	MalE/FimH$_{1–50}$ fusion expression plasmid	(This study)
pKT104	MalE/FimH$_{159–277}$ fusion expression plasmid	(This study)
pKT105	MalE/FimH$_{101–158}$ fusion expression plasmid	(This study)
pKT106	MalE/FimH$_{43–158}$ fusion expression plasmid	(This study)

was rinsed several times with PBS and the bound cells were probed with alkaline phosphatase–conjugated avidin followed by the appropriate substrate 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium (BCIP/NBT) (34). Bound horseradish peroxidase was directly detected by the addition of its substrate, 4-chloro-1-naphthol.

Synthesis of peptides. Peptides s-FimH$_{1–25}$, s-FimA$_{1–13}$, and s-FimH$_{253–264}$ were synthesized by solid-phase techniques and purified as described previously (24, 35). The purity of the peptide product was determined by high-performance liquid chromatography on a reverse phase column and by quantitative amino acid analyses (36).

Immunization of animals. Passive immunization was performed by injecting female ICR strains of mice (Harlan Sprague Dawley, Inc., Indianapolis, IN) intraperitoneally with 200 μl of normal rabbit serum or polyclonal rabbit antibody raised against synthetic peptides. 24 h after immunization, serum and urine IgG titers of specific antibody were typically 1:1000 and 1:4, respectively. Active immunization of rabbits or mice was achieved by intramuscular and subcutaneous injection with 200 μg of MalE/FimH fusion protein or 150 μg of keyhole limpet hemocyanin (KLH)-conjugated s-FimH$_{1–25}$, s-FimA$_{1–13}$, s-FimH$_{253–264}$, or PBS emulsified with complete Freund's adjuvant (24, 35). 4 wk later, the animals were boosted with the immunogen emulsified with incomplete Freund's adjuvant. Blood samples were drawn from each of the animals and the serum antibody titer was assayed periodically by ELISA as described previously (37).

Assays of antibody reactivity. Antibodies were detected by an ELISA. Purified MalE/FimH fusion proteins or isolated type 1 fimbriae or synthetic peptides were adsorbed onto ELISA trays as the solid-phase antigen (24, 35).

Assay for antibody-mediated inhibition of the adherence of type 1 fimbriated E. coli to mouse bladders in situ. The association of *E. coli* ORN103(pSH2) expressing recombinant type 1 fimbriae with mouse bladder mucosal surfaces in the presence of various antibodies was examined as described previously by us (6). Briefly, groups of mice (ICR strains) were killed, and their urinary bladders exposed. Urine was removed from the bladders with a needle and syringe, and immediately thereafter 200 μl of bacterial suspension (containing 2×10^8 CFU of bacteria) mixed with 20 μl of antibody was injected through the same needle. The bladders were then incubated for an additional 30 min, after which the mucosa of the bladders were exposed and washed repeatedly with sterile PBS. All bladders were then homogenized, and the number of viable bacteria which remained associated with the bladder mucosa was determined by colony counts of serial dilutions of the homogenates onto plates of MacConkey agar. The results were recorded as the mean number of CFU per bladder.

Assay for antibody-mediated inhibition of type 1 fimbriae-induced yeast cell (Candida albicans) agglutination. The number of type 1–fimbriated bacterial cells to be added to the test system was initially determined by titrating the *E. coli* ORN103(pSH2) bacteria for yeast cell agglutination activity. The lowest concentration of bacteria that produced a strong agglutination reaction was used for the inhibition test. Twofold dilutions (20 μl) of each antibody in PBS was combined with 40 μl of 1.0% yeast cell suspension. The mixtures of bacteria

(40 μl of 10^9 CFU/ml PBS), antibody and yeast cells were incubated at 37°C for 60 min. Yeast cell agglutination inhibition was assessed visually. Antibodies that inhibited *E. coli*–induced yeast agglutination were identified. The antibody titer that completely inhibited yeast cell agglutination was also noted.

In vivo protection assays in mice using an experimental model of bacterial cystitis. The protection studies were carried out in female ICR mice undergoing water diuresis induced by the method of Keane and Freedman (38). Briefly, female mice weighing 25–34 g were maintained on 5% glucose water for 4 d before bacterial challenge and continued until they were killed. Passive or active immunization of the mice was undertaken as described previously. To initiate experimental UTIs, mice were anesthetized with Nembutal sodium solution (Abbott Laboratories, Chicago, IL) and each mouse abdomen was cleansed with Betadine and 70% alcohol. A small abdominal incision was made, the bladder was exposed and the residual urine was removed with a $27_{1/2}$-gauge sterile needle and syringe. Then 50 μl (10^9 CFU/ml PBS bacterial suspension) of type 1–fimbriated *E. coli* CI5 was instilled into the bladder and the abdomen was closed. 5 d after challenge the animals were killed, the bladders were removed, and, after all the urine was drained, homogenized in sterile PBS. Some kidneys were also removed and homogenized in sterile PBS. Various dilutions of these homogenates were cultured on MacConkey agar plates. The mean CFU per bladder and per kidney were determined by counting the number of colonies on the culture plates. Urine was collected from the mice after gentle massage of the abdomen at various intervals during the experiment. The bacterial content of the urine was assayed by plating onto MacConkey agar plates and the antibody content of the urine was monitored by ELISA using MalE/FimH as the solid phase antigen.

Results

Construction of chimeric genes comprising malE and various fimH gene segments. Our initial studies were directed at localizing the region(s) in FimH responsible for mediating bacterial adherence. Our strategy was to generate fragments of FimH that could be readily isolated in sufficient amounts to test for functional activity. Recently, it was reported that fimbrial subunits and even subunit fragments could be stabilized and isolated when fused to MalE (39–41). Apparently, MalE stabilized the fimbrial subunit polypeptides in the bacterial periplasm and obviated the need for the coexpression of the chaperone protein (39, 41). Thus, we generated fusion proteins comprising of various domains of FimH by fusing a portion of the *fimH* gene encoding the coding region for the mature polypeptide as a translational extension of *malE*. The 3′ extension fragment was generated by PCR using pSH2 plasmid as the template. This fragment was cloned into the MalE fusion expression vector pMAL-p2, creating a new plasmid which was then used to transform the *E. coli* TB1 strain. The cloning strategy that was used to create the various gene fusion constructs is depicted in Fig. 1. The Asp(10) linkage region between *malE* and the fragment of the *fimH* encoded the recognition sequence of the proteolytic coagulation factor Xa. In this way, the FimH polypeptide could be separated from MalE if necessary.

Because the putative receptor binding domain of the structurally similar PapG fimbrial subunit of P fimbriae of *E. coli* was localized recently to the amino terminus half of the molecule (39), we suspected that the binding domain of FimH would also be at an analogous site. Therefore, most of the fragments we generated were restricted to the amino-terminal half of the FimH molecule.

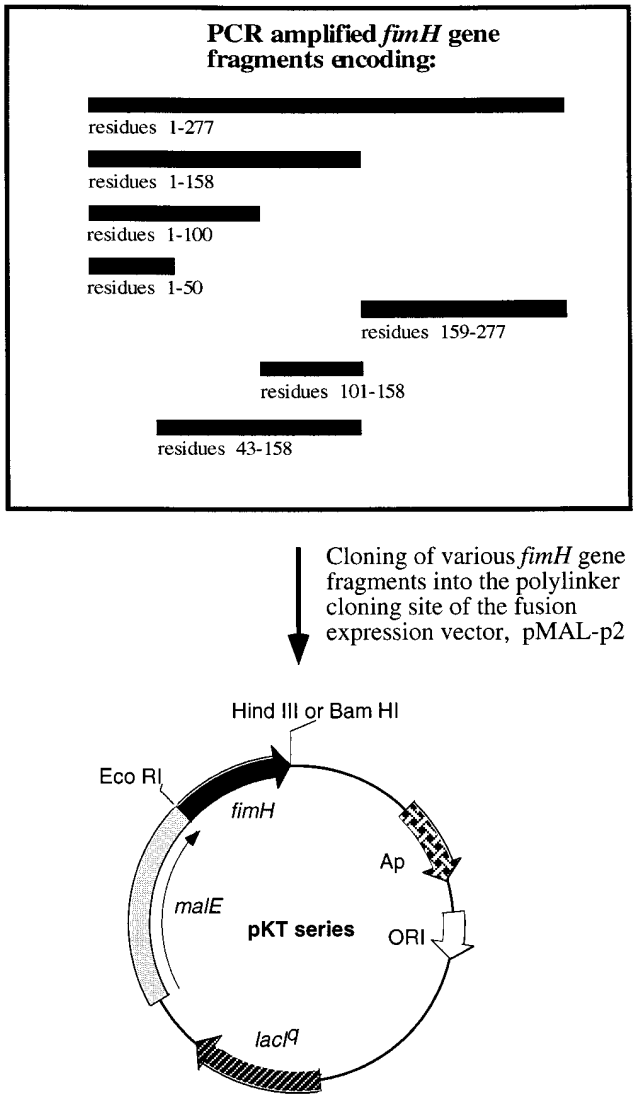

Figure 1. Cloning strategy used to create *malE*/*fimH* gene fusion constructs. Various fragments of the *E. coli fimH* gene were PCR-amplified from the recombinant plasmid pSH2, possessing the type 1 fimbrial operon and cloned into the Eco RI and either Hind III or Bam HI digested sites on the MalE affinity tag expression vector, pMAL-p2. The resulting series of pKT plasmids are described in Table I.

Detection and isolation of translation fusions of MalE with FimH fragments. We confirmed expression of each MalE/FimH fusion protein by subjecting whole cell extracts of the bacterial transformants to SDS-PAGE and Western blotting using antibodies to FimH and MalE as probes. A Western blot of whole cell extracts from bacterial clones expressing the various chimeric proteins is shown in Fig. 2. The blots were reacted with FimH-specific antibodies raised against synthetic peptides s-$FimH_{1–25}$ or s-$FimH_{253–264}$. In every case, the intact fusion protein corresponded to the predominant immunoreactive band in each lane. The other immunoreactive bands of smaller size were presumed to be degraded products of the fusion proteins because they reacted as well with antibodies to MalE (data not shown). Since antibodies raised to β-galactosidase did not react with these bands (data not shown), the im-

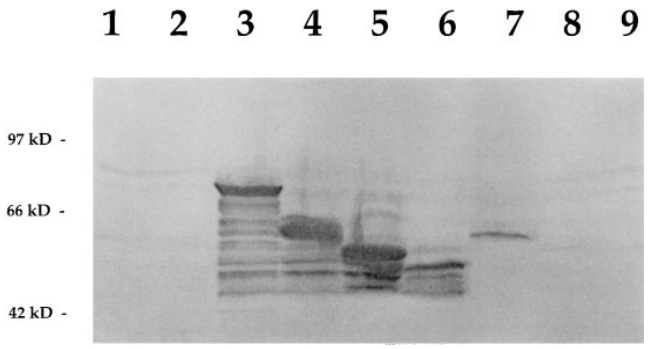

Figure 2. Composite Western blot of whole cell lysates of transformants harboring the various recombinant *malE/fimH* fusion plasmids. Lane *1*: *E. coli* host strain (TB1); lane *2*: TB1 harboring the vector, pMAL-p2; lane *3*: TB1 harboring pKT100 (encoding MalE/$FimH_{1–277}$); lane *4*: TB1 harboring pKT101 (encoding MalE/$FimH_{1–158}$); lane *5*: TB1 harboring pKT102 (encoding MalE/$FimH_{1–100}$); lane *6*: TB1 harboring pKT103 (encoding MalE/$FimH_{1–50}$); lane *7*: TB1 harboring pKT104 (encoding MalE/$FimH_{159–277}$); lane *8*: TB1 harboring pKT105 (encoding MalE/$FimH_{101–158}$); lane *9*: TB1 harboring pKT106 (encoding MalE/$FimH_{43–158}$). Lanes *1–6* were reacted with anti–s-$FimH_{1–25}$ antisera. Lanes *7–9* were reacted with anti–s-$FimH_{253–264}$ antisera. Fusion proteins MalE/$FimH_{43–158}$ (lane *9*) and MalE/$FimH_{101–158}$ (lane *8*) were not detectable on the blot because the FimH antibodies that were used as probes were specific for other regions of the FimH molecule. Note that the bands below the prominent immunoreactive band in each lane are the partially degraded forms of the respective fusion proteins.

munoreactivity appeared specific. As expected, the fusion proteins MalE/$FimH_{43–158}$ and MalE/$FimH_{101–158}$ in lanes *9* and *8*, respectively, of Fig. 2 were not detected because the FimH antibodies that were used as probes were specific for other regions of the FimH molecule.

Once the expression of each MalE/FimH fragment chimera was confirmed by the immunoblot, we proceeded to generate large amounts of each fusion protein in the periplasmic fraction of IPTG-induced cultures and purified the fusion proteins on an amylose affinity column. Fig. 3 shows a Coomassie blue–stained SDS-PAGE preparation of the various MalE/FimH chimeric proteins as well as the MalE protein. The smaller sized bands in each lane are degraded forms of the fusion protein. This was confirmed by amino-terminal sequence analysis of the major bands pooled together. This finding is consistent with previous reports that degraded forms of the MalE fusions copurify with the full length fusion protein during the isolation procedure (39, 41).

Our attempts to purify subfragments of FimH within residues 1 and 100 were largely unsuccessful because of the extreme instability of these fusion proteins in the bacterial periplasm. A preparation of MalE/$FimH_{1–50}$ is shown in Fig. 3, lane *4* where the intact fusion protein is barely perceptible on the gel. However, it is noteworthy that although we were not able to isolate sufficient amounts of MalE/ $FimH_{1–50}$ to test for functional activity, we were able to obtain sufficient amounts of this protein to raise specific antibody.

Identification of functionally active MalE/FimH chimeras. Now that adequate amounts of the chimeric proteins containing various FimH fragments were obtainable, we were in position to directly test them for functional activity. Each chimeric protein was tested for its capacity to mediate mannose-inhibitable binding to mouse bladder epithelial cells and to horseradish peroxidase, a soluble mannoprotein that serves as a receptor for type 1 fimbriae (42). Because MalE does not bind bladder cells or peroxidase (Figs. 4 and 5), it was not necessary to clip off this portion of the fusion protein before testing. To complement these studies, we assessed the ability of polyclonal antibodies that we had raised in rabbits against each of the fusion proteins to block type 1 fimbriae-mediated bacterial attachment to bladder epithelial cells in situ and agglutination of yeast cells in vitro.

Binding of MalE/FimH chimeras to mouse bladder epithelial cells. The binding of bladder epithelial cells to the various MalE/FimH chimeras in the overlay assay is shown in Fig. 4 *A*. MalE/$FimH_{1–158}$ (lane *2*), and MalE/$FimH_{1–100}$ (lane *3*) bound bladder cells to a comparable extent as the chimera bearing the full-length adhesin (MalE/$FimH_{1–277}$) (lane *1*). In contrast, MalE/$FimH_{159–277}$ (lane *4*), MalE/$FimH_{101–158}$ (lane *6*) and MalE (lane *7*) failed to bind bladder epithelial cells. Interestingly, the MalE/$FimH_{43–158}$ chimera (lane *5*) is shown to bind bladder epithelial cells, but this was not always a consistent finding. From these observations, we inferred that the bladder epithelial cell–binding domain was localized to a region within residues 1–100 of FimH. It is also noteworthy that all the bladder cell binding reactions were inhibited by α-methyl-D-mannoside confirming that the binding specificity exhibited by the fusion proteins was similar to that of native type 1 fimbriae (Fig. 4 *B*).

Binding of MalE/FimH chimeras to horseradish peroxidase. Because type 1 fimbriae also efficiently bind certain soluble mannoglycoproteins (42), we tested the ability of the various FimH fragments to bind horseradish peroxidase. This assay simultaneously takes advantage of the highly mannosylated nature of peroxidase (reflected in its ability to bind FimH) and its intrinsic enzymatic properties (reflected in its ability to react with the western blot substrate, 4-chloro-1-naphthol). After binding to FimH, bound horseradish peroxi-

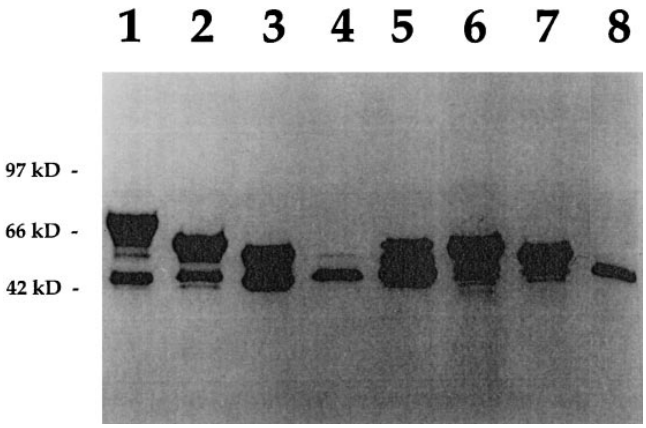

Figure 3. Coomassie blue–stained gel after SDS-PAGE analysis of the amylose affinity column-purified MalE/FimH fusion proteins. Lane *1*: MalE/$FimH_{1–277}$; lane *2*: MalE/$FimH_{1–158}$; lane *3*: MalE/$FimH_{1–100}$; lane *4*: MalE/$FimH_{1–50}$; lane *5*: MalE/$FimH_{159–277}$; lane *6*: MalE/$FimH_{43–158}$; lane *7*: MalE/$FimH_{101–158}$; lane *8*: MalE. The bands of smaller size in each lane correspond to partially degraded forms of the respective fusion proteins.

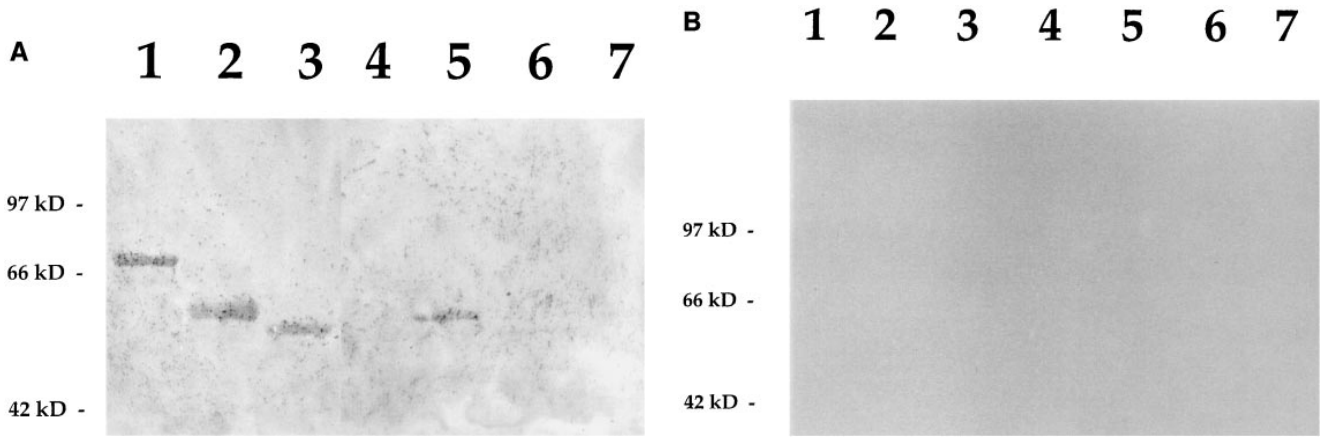

Figure 4. Mannose-sensitive binding of immobilized MalE/FimH fusion proteins to mouse bladder epithelial cells. (*A*) Binding experiment performed in the absence of α-methyl-D-mannoside. (*B*) Binding experiment performed in the presence of 100-mM α-methyl-D-mannoside. Lane *1*: MalE/FimH$_{1–277}$; lane *2*: MalE/FimH$_{1–158}$; lane *3*: MalE/FimH$_{1–100}$; lane *4*: MalE/FimH$_{159–277}$; lane *5*: MalE/FimH$_{43–158}$; lane *6*: MalE/FimH$_{101–158}$; lane *7*: MalE.

dase reacts with the substrate giving rise to precipitin bands shown in Fig. 5 *A*. Thus, the darkly staining band in each lane corresponded to sites of binding of the mannosylated enzyme. MalE/FimH$_{1–158}$ (lane *2*) and MalE/FimH$_{1–100}$ (lane *3*) bound peroxidase in comparable amounts as MalE/FimH$_{1–277}$ (lane *1*). In contrast, MalE/FimH$_{159–277}$ (lane *4*) and MalE/FimH$_{101–158}$ (lane *6*) did not exhibit any binding activity; neither did MalE/FimH$_{43–158}$ (lane *5*), which had exhibited occasional binding to bladder epithelial cells. The peroxidase binding activity of all the immobilized fusion proteins was also completely inhibitable by α-methyl-D-mannoside (Fig. 5 *B*). Taken together, these data implied that the peroxidase binding domain resides within residues 1–100 of FimH. Since MalE/FimH$_{43–158}$ did not exhibit any binding activity, it would appear that residues 1–42 were important for recognition of horseradish peroxidase by FimH.

Inhibition of type 1 fimbriae-mediated E. coli adherence to mouse bladders in situ and yeast cells in vitro by antibodies directed at selected MalE/FimH fusion proteins. To complement the above studies, polyclonal rabbit antisera against selected fusion proteins described above were generated. The ability of these antibodies to block the adherence of type 1–fimbriated *E. coli* ORN103(pSH2) to mouse bladders and to block type 1 fimbriae-mediated agglutination of yeast cells was examined. On the whole, the antibodies that blocked adherence of *E. coli* to mouse bladders in situ also blocked type 1–fimbriated bacteria-induced agglutination of yeast cells (Tables II and III). Antibodies directed at MalE/FimH$_{1–277}$, MalE/FimH$_{1–158}$, and MalE/FimH$_{1–50}$ were inhibitory, whereas the antibody directed at MalE/FimH$_{159–277}$ was not (Table II). Thus, antibodies directed at an epitope within the amino-terminal half of FimH were inhibitors of bacterial adherence to bladder epithelium and yeast cells whereas the antibody directed against the carboxy-terminal half of FimH was not (Tables II and III).

Antibodies directed at a synthetic copy of residues 1–25 of FimH block E. coli adherence to mouse bladders in situ. Based on the adherence and adherence-inhibition assays employing the various MalE/FimH fusion proteins and their re-

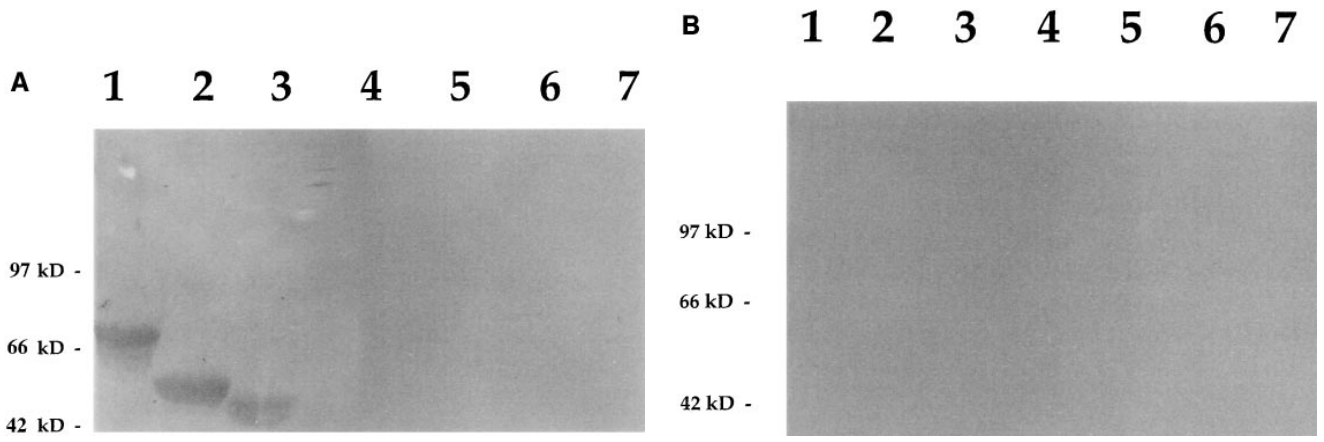

Figure 5. Mannose-sensitive binding of immobilized MalE/FimH fusion proteins to the mannosylated enzyme, horseradish peroxidase. (*A*) Binding experiment performed in the absence of α-methyl-D-mannoside. (*B*) Binding experiment performed in the presence of 100-mM α-methyl-D-mannoside. Lane *1*: MalE/FimH$_{1–277}$; lane *2*: MalE/FimH$_{1–158}$; lane *3*: MalE/FimH$_{1–100}$; lane *4*: MalE/FimH$_{159–277}$; lane *5*: MalE/FimH$_{43–158}$; lane *6*: MalE/FimH$_{101–158}$; lane *7*: MalE.

*Table II. Inhibition of the Association of Type 1–fimbriated E. coli ORN103(pSH2) with Mouse Bladders In Situ by Antibodies Raised Against MalE/FimH Fusion Proteins or Synthetic Peptide Fragments of FimH or Fim A**

Sera	CFU of *E. coli* per bladder‡ (mean±SEM)
Preimmune serum	$1.9\pm0.3\times10^6$/ml
MalE/FimH$_{1–277}$	$4.6\pm1.2\times10^5$/ml§
MalE/FimH$_{1–50}$	$5.1\pm0.1\times10^5$/ml§
MalE/FimH$_{1–158}$	$1.8\pm0.9\times10^5$/ml§
MalE/FimH$_{159–277}$	$1.8\pm0.3\times10^6$/ml
s-FimH$_{1–25}$	$4.3\pm0.7\times10^5$/ml§
s-FimH$_{253–264}$	$2.7\pm0.8\times10^6$/ml
s-FimA$_{1–13}$	$3.6\pm1.3\times10^6$/ml

*Urine from each bladder was removed with a syringe, and 20 μl of antibody and 200 μl of PBS containing 2×10^8 CFU of the bacteria were instilled into the bladder. After 30 min, the mucosa of the bladders was exposed and washed repeatedly with sterile PBS. The bladders were then homogenized, and the number of viable bacteria which remained associated with the bladder mucosa was determined by colony counts. ‡The data are expressed as mean±SEM (n = at least four mice). §$P <$ 0.005, on comparison of CFU of mice treated with preimmune or MalE/FimH$_{159–277}$ as determined by Student's t test. The protocol used in the care and treatment of the animals was in accordance with institutional guidelines.

spective specific antibodies, we predicted that a major bladder cell binding domain resided within residues 1–100 of FimH. To test this prediction, we synthesized a peptide corresponding to residues 1–25 of FimH and raised polyclonal antibodies in rabbits to this polypeptide. We reasoned that since these antibodies were directed at a region on FimH that was close to or part of the putative binding domain, they would be very effective in blocking the adherence of type 1–fimbriated *E. coli* to bladder cells. To test this notion, we compared the ability of antibodies directed at s-FimH$_{1–25}$ to block *E. coli* adherence to mouse bladders in situ with antibodies directed at s-FimH$_{253–264}$, which corresponds to a region near the carboxy terminus of FimH. For comparative purposes, we also included in this assay, antibodies directed at s-FimA$_{1–13}$, a synthetic copy of the amino terminus region of the major subunit of type 1 fimbriae. As shown in Table II, in contrast to antibodies directed at the other synthetic polypeptides, antibodies directed at s-FimH$_{1–25}$ markedly reduced the adherence of *E. coli* to mouse bladders in situ. Indeed, the level of inhibition was comparable to that seen with antibodies directed at the MalE/FimH$_{1–277}$ fusion protein.

*Table III. Inhibition of E. coli Type 1 Fimbriae-mediated Yeast Agglutination by Antibodies Directed at Various MalE/FimH Fusion Proteins**

Sera	Inhibition of yeast agglutination	Highest dilution of antibody at which inhibition is still detected
Preimmune serum	−	−
MalE/FimH$_{1–277}$	+	40
MalE/FimH$_{1–50}$	+	80
MalE/FimH$_{1–158}$	+	40
MalE/FimH$_{159–277}$	−	−

*Various concentrations of antibody (20 μl volume) were mixed with a fixed amount (40 μl of 1×10^9 CFU/ml PBS) of type 1 fimbriated *E. coli* ORN103(pSH2) suspension. 40 μl of 1% yeast cell suspension was added to this mixture and then incubated at 37°C for 60 min.

Reduction of in vivo E. coli colonization in the bladders of mice passively immunized with s-FimH$_{1–25}$–specific antibody. Because of the effectiveness of antibodies directed at s-FimH$_{1–25}$ in blocking adherence of type 1–fimbriated *E. coli* to mouse bladders in situ, we sought to further test our prediction by examining the efficacy of our antibodies in affording in vivo protection against bacterial UTIs. To undertake this study, we used an established experimental mouse model of urinary tract colonization. The results of the in vivo protection assay is summarized in Table IV. The bladders of mice immunized passively with antibodies against the peptide copy of the FimH amino terminal fragment (s-FimH$_{1–25}$) were colonized by type 1–fimbriated *E. coli* to a significantly lower level compared to mice immunized with either normal rabbit serum or antibody against the peptide copy of the carboxy region of FimH (s-FimH$_{253–264}$) or antibody against the peptide copy of the amino-terminal fragment of FimA (s-FimA$_{1–13}$). It is noteworthy that the differences in bacterial colonization between mice immunized with normal serum, FimH$_{253–264}$ or FimA$_{1–13}$ specific antibodies were not significant.

Since bacteriuria is a good indicator of the course of infection in the urinary tract, we also compared the bacterial numbers in the urine of mice passively immunized with antibodies against s-FimH$_{1–25}$ and s-FimH$_{253–264}$. As shown in Fig. 6, the level of bacteriuria in the mice immunized with s-FimH$_{1–25}$ antibodies was consistently lower than mice immunized with s-FimH$_{253–264}$ antibodies (Table IV). Unlike the latter group, the numbers of bacteria shed into the urine in mice immunized with s-FimH$_{1–25}$ appeared to progressively decrease during the 5 d of the experiment. These findings are consistent with the remarkable differences in the number of bacteria colonizing the bladders in the two groups of mice seen on day 5. The temporary drop in bacterial counts on day 4 in mice immunized

*Table IV. Inhibition of In Vivo Bacterial Colonization of Mouse Bladders by Passive Immunization with s-FimH$_{1–25}$–specific Antibodies**

Sera employed for immunization	CFU of *E. coli* CI5 per bladder‡ (mean±SEM)
Normal rabbit serum	$4.3\pm1.6\times10^6$
Anti–s-FimH$_{1–25}$	$9.2\pm2.2\times10^{2}$§
Anti–s-FimH$_{253–264}$	$6.2\pm2.4\times10^5$
Anti–s-FimA$_{1–13}$	$1.3\pm0.8\times10^6$

*Each mouse was injected intraperitoneally 200 μl of antibody 24 h before challenge with *E. coli* CI5. The reaction of each of the antibodies with isolated type 1 fimbriae was comparable on ELISA. 5 d after intravesicular challenge with *E. coli* CI5 (50 μl of 1×10^9 CFU/ml PBS bacterial suspension), the bladder from each mouse was homogenized and the CFU determined. ‡The data are expressed as mean±SEM (n = at least eight mice). §$P < 0.05$, on comparison of CFU of mice treated with anti–s-FimH$_{1–25}$ serum with CFU of corresponding mice treated with anti–s-FimH$_{253–264}$ or anti–s-FimA$_{1–13}$ as determined by Student's t test. The differences in CFU between mice treated with anti–s-FimH$_{253–264}$, anti–s-FimA$_{1–13}$ or normal serum were not significant.

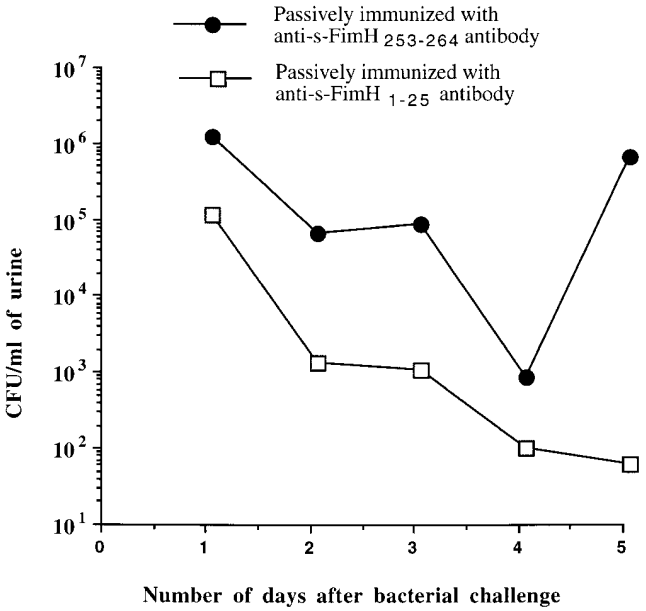

Figure 6. Distinct profiles of bacteriuria in mice after intravesicular challenge with *E. coli* CI5. Mice were passively immunized intraperitoneally with anti–s-FimH$_{1–25}$ and anti–s-FimH$_{253–264}$ antisera. Each group of passively immunized mice was intravesicularly challenged with *E. coli* CI5. Every day after bacterial challenge, urine was collected from at least three mice from each group and pooled. The samples were treated with 0.1% Triton X-100 to release all cell-associated bacteria in the urine and the number of CFU per milliliter of urine in each group was determined.

with antibodies to s-FimH$_{253–264}$ is inexplicable at this time. The amount of FimH-specific antibody in the urine of the two groups of immunized mice was also assessed. In both groups, FimH-specific antibodies were detected in the urines as early as 5 h after intraperitoneal infusion of the FimH antibodies. A dramatic increase in FimH antibodies was seen in both groups of mice when their urine was examined on day 1 after bacterial challenge (Fig. 7). The level of FimH-specific antibodies gradually subsided over the subsequent 4 d. There was no dramatic increase in FimH antibodies in the urines of passively immunized mice that were not challenged with bacteria (Fig. 7). Thus, the sharp spike seen in the FimH antibody levels in the urine appeared to be the direct result of bacterial challenge.

Reduction of in vivo E. coli colonization in the bladders of mice actively immunized with synthetic FimH$_{1–25}$. The marked decrease in CFU after passive immunization with specific antibodies against the synthetic FimH$_{1–25}$ peptide in vivo encouraged us to directly assess the synthetic peptide as a vaccine in affording protection against experimental infection. We vaccinated groups of mice with s-FimH$_{1–25}$, s-FimH$_{253–264}$, s-FimA$_{1–13}$, or PBS, and subsequently challenged these hyperimmune mice with *E. coli* as described above. The results obtained from this experiment closely resembled our findings with the passively immunized mice (Table V). Mice actively immunized with s-FimH$_{1–25}$ peptide, exhibited significantly lower levels of bacterial bladder colonization than mice immunized with either s-FimH$_{253–264}$ or s-FimA$_{1–13}$ or PBS, when challenged by type 1–fimbriated *E. coli.* The class of antibody evoked in the serum and urine of mice immunized with FimH peptides is shown in Fig. 8. The predominant antibody class in the serum were of the IgG and IgM class whereas in the urine, the predominant antibodies were of the IgG and IgA class. As described in the previous experiment, there appeared to be a marked increase in the presence of FimH antibody in the urine when bacteria was instilled into the urinary tract (Fig. 8).

Antigenic cross-reactivity of FimH-specific antibodies with urinary tract bacterial isolates bearing type 1 fimbriae. The effectiveness of the FimH vaccine can be greatly enhanced if it exhibits broad activity. We reasoned that if the binding domain of FimH resided in its amino terminus region and since all type 1 fimbriae mediate mannose-sensitive binding activity, this region on FimH must be conserved immunologically among enterobacteria relative to other regions of the FimH molecule or to similar sites of other fimbrial subunits. To test this notion, the polyclonal antibody raised against s-FimH$_{1–25}$ was reacted by ELISA against randomly selected uropathogenic enterobacteria bearing type 1 fimbriae. For comparative purposes, the antibodies against s-FimH $_{253–264}$ and s-FimA$_{1–13}$ were also tested. As shown in Fig. 9, with the exception of *Serratia marcescens* CI119, both FimH-specific antibodies exhibited broad immunoreactivity with all the strains tested, whereas the antibody directed at s-FimA$_{1–13}$ exhibited limited cross-reactivity. This finding suggests that the amino terminus and the carboxy terminus region of FimH are, in most cases, antigenically conserved. In contrast, antibodies directed at s-FimA$_{1–13}$ exhibited either no or low level reactivity with most of the strains tested.

Discussion

In this study, we have made several observations that cumulatively indicate that a receptor binding domain of FimH resides within residues 1–100. These findings include: (*a*) MalE fusion proteins containing residues 1–100 of FimH bind bladder epithelial cells and horseradish peroxidase in a mannose-inhibitable manner; (*b*) a MalE fusion protein containing the entire carboxy half of the FimH molecule (residues 159–277) failed

*Table V. Inhibition of In Vivo Bacterial Colonization of Bladders of Mice that Were Actively Immunized with s-FimH$_{1–25}$ Peptide**

Immunogens	CFU of *E. coli* CI5 per bladder‡ (mean±SEM)
PBS (sham)	$1.5 \pm 0.7 \times 10^6$
s-FimH$_{1–25}$	$8.2 \pm 2.1 \times 10^{3}$§
s-FimH$_{253–264}$	$2.2 \pm 1.7 \times 10^6$
s-FimA$_{1–13}$	$6.1 \pm 2.8 \times 10^6$

*Each mouse was injected intramuscularly and subcutaneously with 150 μg of KLH-conjugated synthetic peptide emulsified in CFA. 4 wk later, each animal was boosted with 150 μg of the same immunogen emulsified in incomplete Freund's Adjuvant. 5 d after intravesicular challenge with *E. coli* CI5 (50 μl of 1×10^9 CFU/ml PBS bacterial suspension), the bladder from each mouse was homogenized and the CFU determined. ‡The data are expressed as mean±SEM (n = at least eight mice). §$P <$ 0.05, on comparison of CFU of mice immunized with s-FimH$_{1–25}$ with CFU of corresponding mice immunized with s-FimH$_{253–264}$, s-FimA$_{1–13}$ or PBS as determined by Student's t test. The differences in CFU between mice treated with anti–s-FimH$_{253–264}$, anti–s-FimA$_{1–13}$ or PBS were not significant.

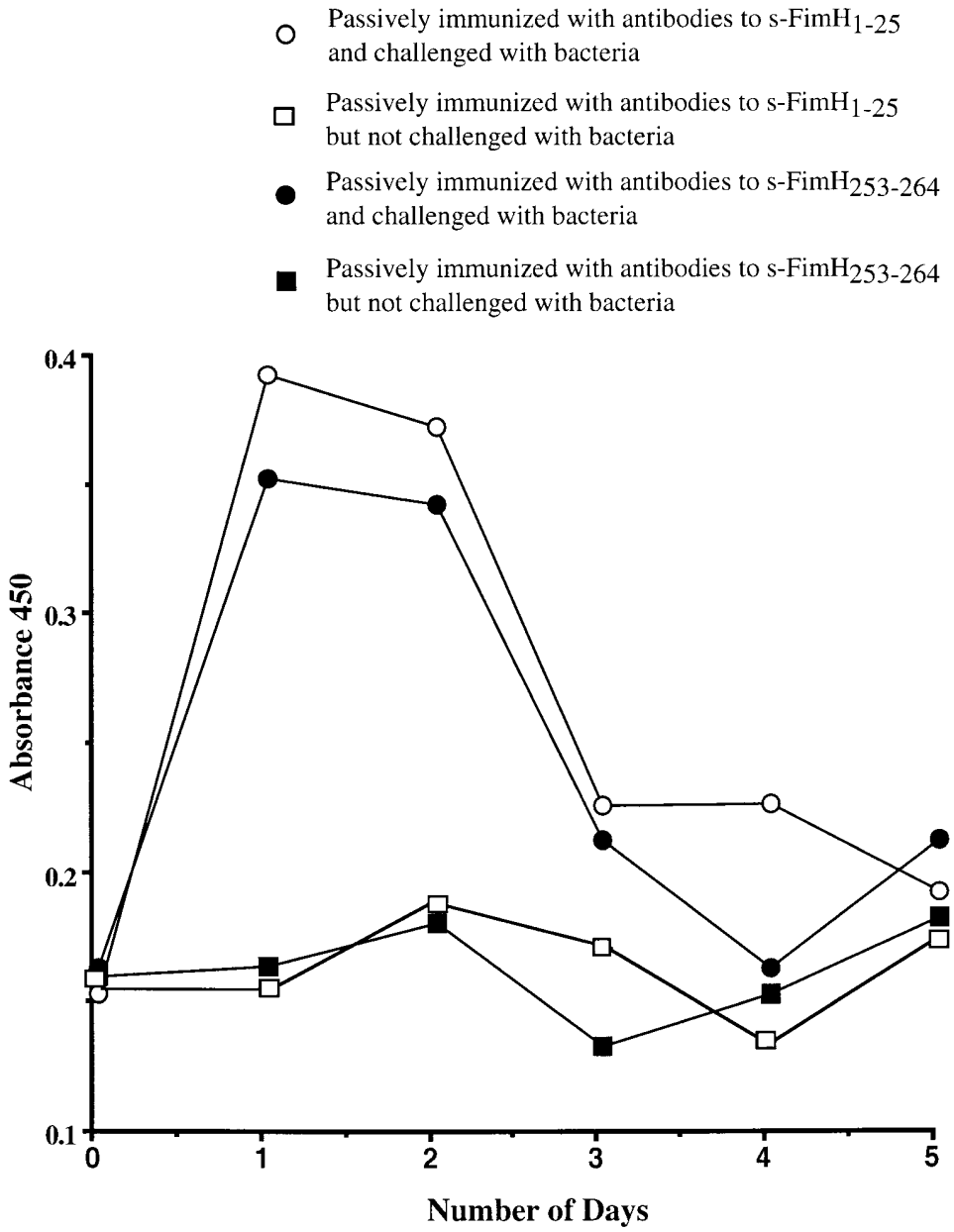

Figure 7. Enhanced urinary secretion of antibodies in passively immunized mice after intravesicular challenge with *E. coli* CI5. Mice were passively immunized intraperitoneally with antibodies to s-$FimH_{1–25}$ or s-$FimH_{253–264}$. 24 h later (*Day 0*), the mice immunized with antibody were segregated into two groups and urine samples were collected and pooled from at least three mice in each group. The first group of mice was intravesicularly challenged with *E. coli* CI5 whereas the second or control group was left unchallenged. Urine was collected every day from at least three mice in each group and pooled. FimH-specific antibody in the urine was detected by an ELISA using MalE/$FimH_{1–277}$ as the solid phase antigen. Urine samples were dialyzed against PBS before they were assayed for antibody.

to exhibit any binding activity; and (*c*) the only FimH antibodies that blocked adherence of type 1–fimbriated *E. coli* to mouse bladders and yeast cells were those directed against epitopes within residues 1–100. Our attempts to further localize the binding region within FimH has been hampered by the instability of the MalE fusion proteins in the bacterial periplasm. Currently, we are in the process of synthesizing overlapping peptides within residues 1–100 to test for functional activity. It is noteworthy that s-$FimH_{1–25}$ failed to exhibit any binding activity despite its capacity to evoke an antiadhesive antibody. Perhaps, s-$FimH_{1–25}$ contains only a portion of the receptor binding region or this peptide is located sufficiently close to the binding domain such that its corresponding antibody sterically blocks FimH-mediated adherence.

Our findings are consistent with previous studies that have attempted to localize the binding epitopes on the PapG adhesin of P fimbriae of uropathogenic *E. coli* (39). In those investigations, the amino and carboxy halves of the PapG molecule were fused to the MalE affinity tag and tested for binding activity. The amino-terminal half but not the carboxy-terminal half of PapG was found to mimic binding reactions that were characteristic of native P fimbriae (39, 43, 44). Our studies are also consistent with earlier transposon insertion analyses of *E. coli fimH* that indicated mutations localized to the 5′ region of the *fimH* gene were more likely to abrogate adhesive functions of the fimbriae than mutations at other sites in the gene (18). However, it is noteworthy that several other fimbrial adhesins appear to mediate binding via their carboxy terminus region (45–49).

An analysis of the covalent structure of several FimH moieties of *E. coli* and *Klebsiella pneumoniae* suggests that the putative receptor binding domain is conserved among at least two enterobacterial species (16, and our unpublished data). On the other hand, the linear sequence of amino acids com-

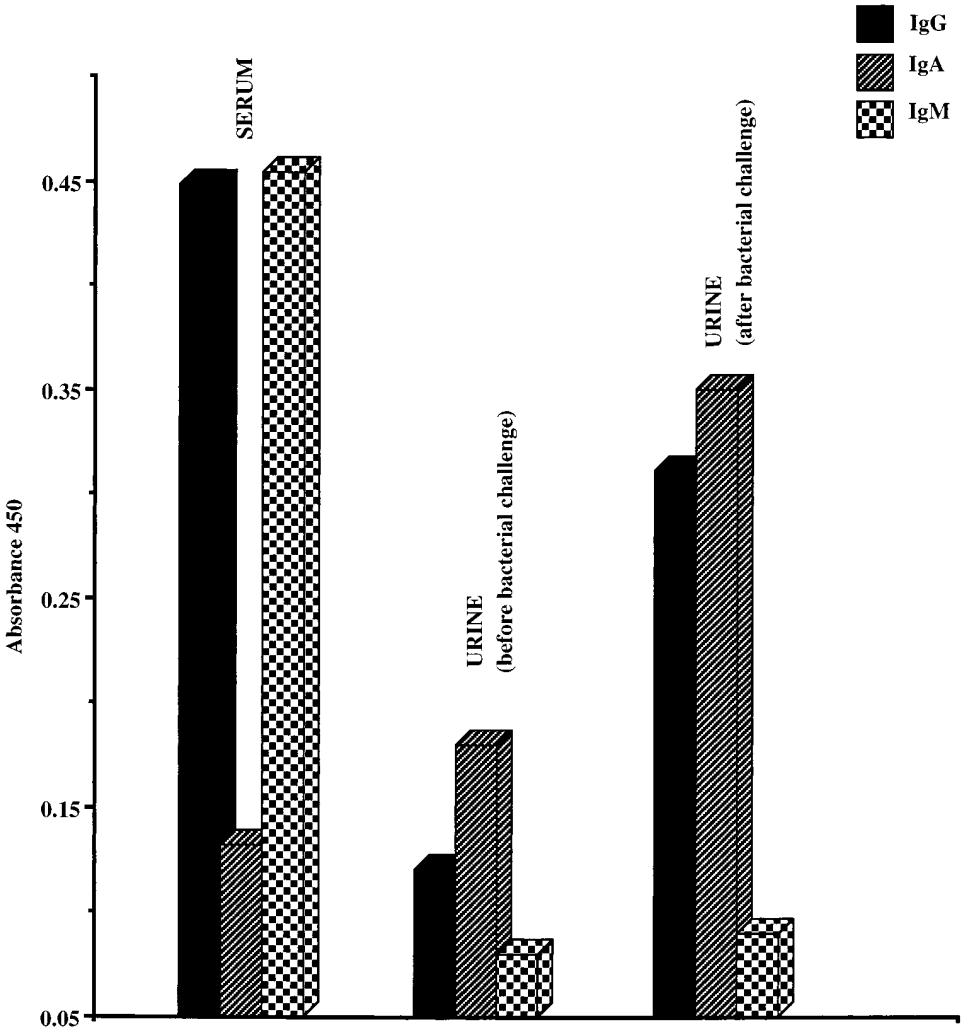

Figure 8. Classes of FimH-specific antibody in serum and urine of mice actively immunized with s-$FimH_{1–25}$. Serum and urine samples were collected on day 0, just before bacterial challenge. To determine the effect of bacterial infection on secretion of antibodies in the urinary tract, urine was also collected from the mice on day 1 after bacterial challenge. Serum and urine from at least three mice were pooled and assayed for each class of antibody. Urine samples were dialyzed against PBS before they were assayed for antibody. The ELISA employed MalE/$FimH_{1–277}$ as the solid phase antigen and antibody specific for each immunoglobulin class was used as the secondary antibody.

prising residues 1–100 of FimH is dissimilar from sequences found on corresponding regions on other fimbrial subunits, implying that it may contain distinct protein motifs that confer mannose-binding property. This 100 amino acid stretch, however, bears little sequence homology to other mannose-binding plant lectins, such as Concanavalin A, and human lectins, such as the mannose-binding proteins of human serum (50, 51). This may not be surprising, as sugar-binding domains on eukaryotic lectins typically involve discontinuous regions of the molecule and are highly conformation dependent. Thus, the structural features of mannose-binding proteins of prokaryotic origin may be quite distinct from eukaryotic lectins with similar binding specificity and may reflect a lack of evolutionary kinship.

Although immobilized and presumably denatured polypeptides of FimH mediate mannose-sensitive binding interactions with bladder epithelial cells and with a mannosylated glycoprotein, it cannot be readily inferred that the receptor-binding activity of FimH is mediated by a linear sequence of amino acids. At this time it is not possible to rule out limited renaturation or oligomerization of the MalE/FimH fragments during the electroblotting process that precedes exposure to the mannosylated substrates (Figs. 4 and 5). The slight differences in the recognition of the bladder cells and horseradish peroxidase exhibited by the various MalE/FimH fragments highlights the complexity of the binding interaction mediated by FimH. It suggests that the nature of the receptor or the substrate on which the receptor is presented are additional factors that can influence the interactive sites within FimH. It is noteworthy that just because the regions on FimH downstream of residues 1–100 do not exhibit any detectable receptor binding activity, one cannot conclude that these regions are not involved in receptor recognition. Clearly, alternate approaches such as crystallographic analysis of FimH-receptor complexes will be invaluable for providing more definitive data on the precise location of the receptor binding site(s) within FimH. However, it is also conceivable that the ultimate determinant of the binding region may be the architecturally complex fimbrial filament on which the FimH moiety is critically poised. Indeed, a recent report has suggested that the distinct fine sugar-binding specificity exhibited by type 1 fimbriae of *E. coli* and *K. pneumoniae* is markedly affected by the fimbrial shaft on which the FimH is borne (52).

Our studies provide the first definitive evidence that antibodies directed specifically at a functionally relevant component of bacterial fimbriae can afford protection against bacterial colonization in vivo. When type 1 fimbriae were initially tested as vaccines, they were found to be of limited value in

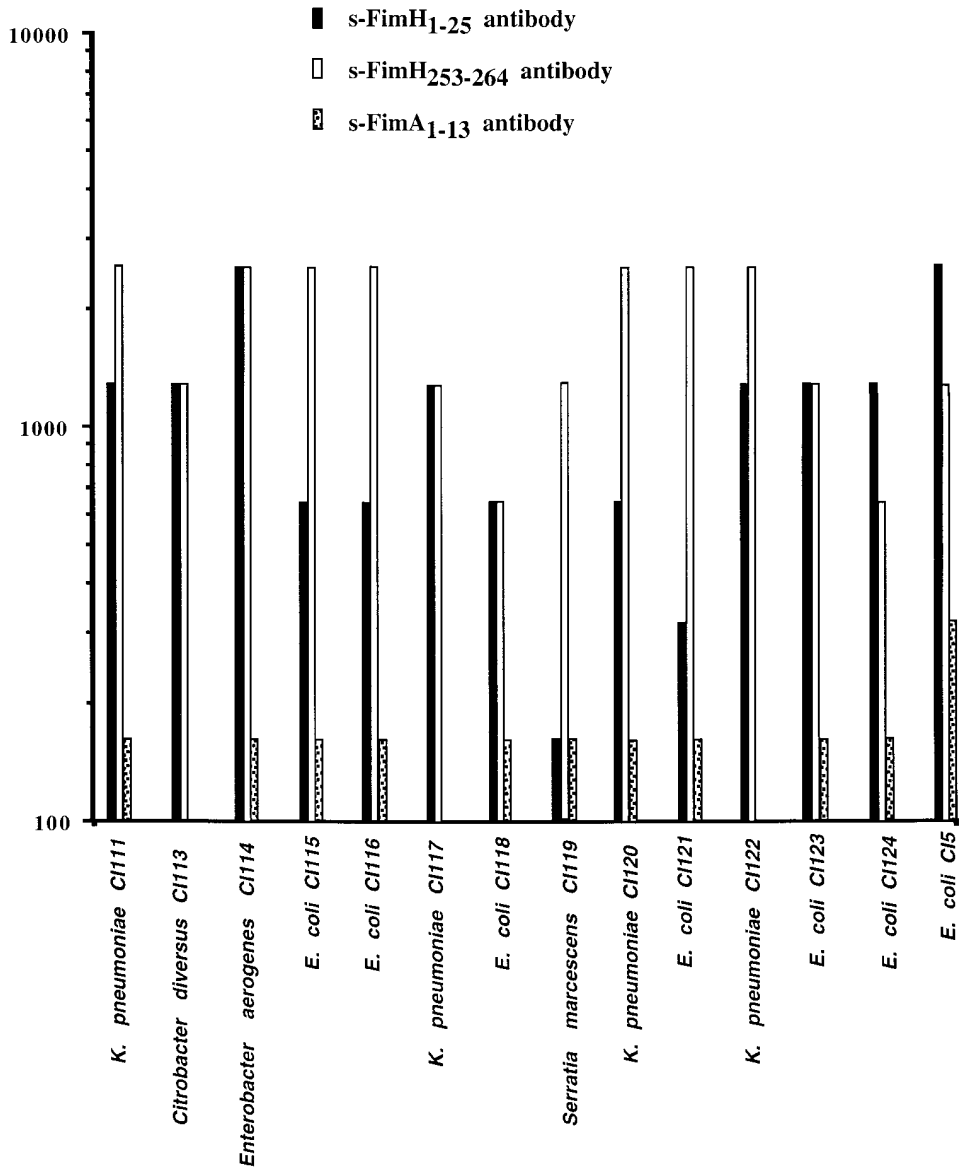

Figure 9. Broad crossreactivity of FimH-specific antibody among urinary tract bacterial isolates. Various type 1–fimbriated enterobacterial urinary tract isolates were immobilized on microtiter trays and then reacted in an ELISA with antibodies raised against s-$FimH_{1–25}$, s-$FimH_{253–264}$ and s-$FimA_{1–13}$.

protecting animals and humans from bacteria expressing heterologous fimbriae (53–56). Most of the antibodies evoked with the purified fimbrial preparation were directed primarily at the structural FimA subunit (which constitutes over 95% of the total fimbrial proteins), but none appeared to be directed at the minor FimH adhesive component. Furthermore, the FimA subunit was antigenically heterogeneous even among strains of the same species (57, 58). Thus, the protection afforded by employing purified type 1 fimbriae as a vaccine was limited to relatively a few bacterial strains expressing homologous type 1 fimbriae. Once FimH was identified as the adhesin (17–19), we demonstrated that purified FimH was in fact immunogenic in animals (23, 24). We also showed that relatively small synthetic peptides copying the amino terminus region of *E. coli* FimH were highly immunogenic when conjugated with KLH and that the resulting antibodies were broadly cross-reactive not only with type 1 fimbriae from other *E. coli* strains, but also with fimbriae from a variety of genera and species in the family *Enterobacteriaceae* (23, 24). In this study, we demonstrate that the amino-terminal region of FimH contains a major determinant of receptor binding and that antibodies directed specifically at this region of FimH block binding of type 1–fimbriated *E. coli* to bladder epithelial cells in situ and confer protection against bacterial infection in an experimental mouse model of UTI. The magnitude of the protection can be ascertained by the fact that bladders of mice immunized passively with antibodies to s-$FimH_{1–25}$ exhibited at least 100-fold lower levels of bladder colonization than mice infused with antibodies to s-$FimA_{1–13}$ or s-$FimH_{253–264}$. The in vivo effectiveness of the s-$FimH_{1–25}$-specific antibody in mice was demonstrated after both passive immunization with hyperimmune serum and active immunization with the s-$FimH_{1–25}$ peptide. Since the value of a vaccine candidate can be greatly enhanced if it is shown to possess broad spectrum activity, we examined the reactivity of our protective s-$FimH_{1–25}$-specific antiserum with several randomly selected type 1–fimbriated urinary tract bacterial isolates. In contrast to the antibody directed at s-$FimA_{1–13}$ that exhibited limited cross-reactivity, s-$FimH_{1–25}$-specific anti-

serum reacted well with almost all the type 1–fimbriated bacteria that were tested. This finding is consistent with the notion that residues 1–25 are within the mannose-binding domain on FimH and is likely to be structurally conserved among all type 1 fimbriae because of their common mannose-binding property. Interestingly, the nonprotective $FimH_{253–264}$-specific serum was also found to be highly cross-reactive. The region spanned by residues 253–264 forms part of the putative chaperone binding region of FimH (43) and has been shown by Zeheb and Orr (33) and Hultgren and coworkers (41, 43) to be structurally conserved among many fimbrial subunits. Hence it is not surprising that this region of FimH was found to be antigenically conserved.

Most studies that have reported protection against experimental bacterial UTIs after vaccination with fimbriae or passive infusion of fimbriae-specific antibodies have inferred that the mechanism involves inhibition of bacterial adherence to mucosal epithelial cells (6, 59–61). Our findings are entirely consistent with that notion. We found that the only protective antibody in vivo was that directed at s-$FimH_{1–25}$. This antibody, unlike the antibody against s-$FimH_{253–264}$ and s-$FimA_{1–13}$, blocked adherence of type 1 fimbriated *E. coli* to bladder walls in situ and to yeast cells in vitro. There was no appreciable difference in the levels of $FimH_{1–25}$-specific and $FimH_{253–264}$-specific antibodies in the urines of mice during the course of the entire experiment (Fig. 7). Thus, the lack of protection seen with the latter antibody was not because of reduced levels of urinary antibodies. This finding also implies that other antibody-mediated antimicrobial functions such as bacterial aggregation and opsonization were not consequential in the urinary tract, because the s-$FimH_{253–264}$-specific antibody readily binds the type 1 fimbriae of the challenge bacteria and can potentially perform these functions just as well as the antibody against s-$FimH_{1–25}$. Thus, we speculate that the mode of protection effected by the $FimH_{1–25}$-specific antibody was through blocking and possibly reversing specific adherence of the challenge strain to the walls of the bladder, effectively preventing bacteria from establishing an early foothold.

To confirm that the protective antibodies could reach the bladder, we screened the urine of passively immunized mice for the presence of FimH-specific antibodies. To ensure that we were assaying only functionally competent antibodies, we used isolated MalE/$FimH_{1–277}$ as the solid-phase antigen in the ELISA assay. Thus, only antibodies that recognized and bound FimH were detected. We determined that both s-$FimH_{1–25}$ and s-$FimH_{253–264}$-specific antibodies were detected in the urine of mice as early as 5 h after intraperitoneal immunization with antibody. This is consistent with previous studies that have reported infiltration of intact serum antibodies into the urine of normal individuals (62). Serum-derived antibodies may also account for a portion of the antibodies in the urine of the actively immunized mice. Interestingly, FimH-specific IgA antibodies were detected in the serum as well as in the urine of the mice. Because the amounts of IgA relative to IgG levels in the urines appeared to be higher than that found in the serum, the IgA antibodies detected in the urine were likely to be locally generated, which is consistent with previous studies showing that subcutaneous or parenteral injection of antigens can elicit specific IgA (and IgG) responses in distant mucosal surfaces including the urinary tract (63–65). Remarkably, there was a dramatic increase in the urinary FimH-antibody levels immediately after instillation of bacteria into the bladders of immunized mice. Conceivably, a portion of the intravesicularly instilled bacteria had migrated into the kidneys, where they induced inflammation resulting in proteinuria. Acute inflammatory reactions in the kidneys can cause malfunctioning of the glomerular filtration system, precipitating leakage of blood proteins including antibody molecules into the urine (66, 67). The degree of leakage is variable and often dependent on the extent of inflammation in the kidneys (66, 67). Evidence that appreciable numbers of bacteria reached the kidneys after intravesicular instillation and caused renal inflammation comes from positive bacterial cultures of kidney homogenates (data not shown) as well as from histological examination of kidney sections of selected mice from each challenge group that revealed definite signs of inflammation (data not shown). Although the spontaneous migration of bacteria into the kidneys could be attributable, at least in part, to the experimental procedure that we used, it is conceivable that in a natural infection, hypersecretion of serum antibodies into the urine will occur when cystitis progresses to pyelonephritis. In the actively immunized mice, FimH antibodies of the IgM and IgG class were detectable in the serum but only IgG antibodies were detected in the urine indicating that IgM antibodies, which are considerably larger molecules than IgG, were excluded from entry into the urine. Thus, in spite of renal inflammation, there still existed some selectivity in the leakage of serum proteins into the urine.

While many bacterial components could potentially contribute to renal inflammation it is noteworthy that uropathogenic bacteria expressing type 1 fimbriae are an important cause of renal inflammation leading to scarring (13, 14). Several recent reports have shown that the FimH moiety of type 1 fimbriae is a potent inflammogen and have provided valuable clues to the underlying molecular and cellular mechanisms involved (10, 11, 68). Mast cells, strategically located underneath the mucosal epithelial cells and around blood vessels, appear to be central to the inflammatory process. These cells, upon contact with the enterobacterial FimH, spontaneously release a large variety of inflammatory mediators (10, 11). One such mast cell product elicited by FimH is histamine (10), a potent vasoactive amine, which markedly increases local blood flow and vascular permeability. These vascular events then facilitate the arrival of inflammatory cells and serum antibodies to the site of bacterial infection. Thus, a consequence of bacterial entry into the kidney could be hypersecretion of antibodies and other antimicrobial blood components into the urine. This phenomenon is especially intriguing because the host appears to be using the virulence properties of the bacteria to facilitate clearance of the pathogen. This bacteria-initiated trafficking of antibodies and other blood components into the urinary tract could represent a novel mechanism by which the systemic immune system can effect protection against microbes invading distal mucosal surfaces.

Finally, it must be emphasized that UTIs are a major complication among pregnant and elderly women with some estimates of the rate of bacteriuria among pregnant women ranging as high as 25% (69, 70). Treatment of these cases has become increasingly difficult as a result of the emergence of multiply resistant pathogens. Indeed, several recent reports have described urinary isolates of *E. coli* and *K. pneumoniae* from hospitalized patients, that are resistant to all available antibiotics including β-lactams, amino glycosides, and glycopeptides (71). Because of this alarming situation, new approaches

for the prevention and management of UTIs are clearly warranted. A potentially viable option is the use of vaccines comprising of one or more well-defined bacterial virulence determinants that would evoke broad protective immunity in individuals predisposed to UTIs. Our studies indicate that a vaccine comprising of a functionally relevant region of bacterial FimH exhibits considerable potential in evoking broadly protective immunity against UTIs. Furthermore, they contribute to the growing body of evidence that have implicated type 1 fimbriae as a critical determinant of bacterial virulence in the urinary tract.

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