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Synthetic peptides corresponding to protective epitopes of *Escherichia coli* digalactoside-binding pilin prevent infection in a murine pyelonephritis model

(antigenic determinants/vaccine)

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ABSTRACT **Synthetic peptides corresponding to five segments of a globoside (Gal-Gal)-binding pilin sequence [residues 5–12 (R5–12), R65–75, R93–104, R103–116, and R131–143], cyanogen bromide fragment II (CNBr-II, R53–163), and purified, intact Gal-Gal pili were prepared as vaccines and tested for their efficacy in a BALB/c murine model of pyelonephritis. Intact Gal-Gal pili, CNBr-II, and synthetic peptides R5–12 and R65–75 engendered antibodies that bound the homologous pilin protein and prevented urine and renal colonization in most vaccine recipients. Protection correlated with serum anti-pilus IgG ELISA titers ≥1:250. The efficacy afforded by synthetic peptides R5–12 and R65–75 in vaccinated mice indicates that linear "antigenic" determinants in separate cyanogen bromide fragments encode "protective" epitopes. Peptides R93–104, R103–116, and R131–143 lacked efficacy, indicating that not all regions of the sequence are serologically equivalent. The crossreactivity of the peptide antisera for different Gal-Gal pilins was also assessed and correlated with the sequence homology of the corresponding regions. Antiserum to peptide R65–75, which corresponds to a region of unconserved sequence in heterologous pilins, bound only the homologous pilin. Thus, it specifies a type-specific protective epitope. Antiserum to synthetic peptide R5–12, which corresponds to a region of conserved sequence, bound Gal-Gal pilins from seven of eight pyelonephritis strains, indicating that it specifies a crossreacting protective epitope.**

Uropathogenic *Escherichia coli* adhere to and colonize urogenital mucosa by means of adhesin proteins that bind epithelial cell surface "receptor" glycoconjugates (1, 2). Two main classes of chromosomally encoded adhesins characteristic of *E. coli* urine isolates have been defined by their receptor specificities. Mannose-binding adhesins are associated with type I or common pili and bind to Tamm–Horsfall glycoprotein in human urine (3), and their agglutination of guinea pig erythrocytes is inhibited by D-mannose (4). Digalactoside (Gal-Gal)-binding adhesins are associated with Gal-Gal pili. They bind globoseries glycolipids on human uroepithelial cells and erythrocytes. α-D-Gal-(1→4)-β-D-Gal, the minimal receptor moiety, causes hemagglutination inhibition (5) and blocks the attachment of Gal-Gal piliated strains to voided uroepithelial cells in urine (6). Type I and Gal-Gal pili are simultaneously expressed by most pyelonephritis strains (7).

The chromosomal DNA of a single urine isolate from a patient with pyelonephritis was used to prepare two recombinant strains that separately express only type I or Gal-Gal pili (8). These and the parent pyelonephritis strain were examined in a murine urinary-tract infection model to determine the pathogenic significance of the two pilus classes (9). Intravesicular administration of the pyelonephritis strain resulted in renal epithelial colonization and parenchymal invasion. The type I piliated recombinant did not readily colonize and never invaded the murine urinary tract. The Gal-Gal piliated recombinant colonized but did not invade, indicating that the Gal-Gal adhesin mediates renal epithelial infectivity, but not invasion. The efficacy of purified type I and Gal-Gal pili as pyelonephritis vaccines was also assessed. The Gal-Gal pilus vaccine alone prevented renal colonization and invasion by the homologous pyelonephritis strain (9), substantiating the uropathogenic role of the Gal-Gal adhesin.

The complete primary structure of Gal-Gal pilin, the major repeating polypeptide of the pilus filament, was obtained for pili from the Gal-Gal binding recombinant strain by automated Edman degradation of overlapping peptide fragments (10) and deduced from the DNA sequence of the structural gene (11) in order to determine the structural basis for vaccine efficacy. Linear "immunogenic" and "antigenic" epitopes‡ were identified with synthetic peptides corresponding to nine regions of the Gal-Gal pilin sequence (12). In the work reported here, five of these peptides were studied in the murine pyelonephritis model to determine their efficacy as vaccines and the location of linear "protective" epitopes.

MATERIALS AND METHODS

Bacteria and Pilus Purification. *E. coli* strain J 96 (04:K5:H51), a human pyelonephritis isolate, is hemolytic, colicin V-positive, motile, resistant to the bactericidal action of normal human serum, and simultaneously expresses type I and Gal-Gal pili. HU 849 is a recombinant strain prepared from J 96 chromosomal DNA that expresses Gal-Gal binding activity and a J 96 pilus protein that contains the F13 antigen (8). *E. coli* strain AD 110 (06:K2:H1), also termed strain C1212 (13), was provided by I. Orskov and is a human cystitis isolate that expresses $F7_1$ and $F7_2$ Gal-Gal-binding

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Abbreviation: R*x*–*y*, residues *x*–*y*.

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‡Throughout this report, the term "immunogenic epitope" refers to a particular domain in the native protein that is recognized by the immune system and gives rise to antibodies able to bind synthetic peptides corresponding to this domain. "Antigenic epitope" is used to describe domains that are recognized in the native protein by antibodies engendered by synthetic peptides corresponding to that region of the protein. "Protective epitope" designates a linear domain of the native protein that confers protection in the murine pyelonephritis model when administered as a synthetic peptide vaccine.

pili. Gal-Gal-binding recombinant strains carrying plasmids pPIL110-75 (AM 1727) and pPIL110-35 (AM 1727) were provided by I. van Die and express the $F7_1$ and $F7_2$ pilin antigens of strain AD 110, respectively (14). Gal-Gal-binding *E. coli* pyelonephritis strains C1979 (016:K1:H6), which expresses the F12 pilin antigen (13), and 3669 (02:K nontypeable), which expresses the F9 pilin antigen (6), were provided by I. Orskov and C. Svanborg-Eden, respectively. Gal-Gal-binding *E. coli* strains P21, A42, A50, and A8 (7) were isolated from patients with pyelonephritis at Stanford University Hospital by K. Vosti.

Gal-Gal pili from strain HU 849 were prepared from organisms grown on tryptic soy agar (TSA) by a modification of the method of Brinton (15) and their purity was determined by $NaDodSO_4$/PAGE (16) and N-terminal amino acid sequencing.

Cyanogen Bromide Cleavage and Isolation of the CNBr-II Fragment. The Hu 849 Gal-Gal pilus subunit contains a single methionine at position 52, thus yielding two fragments, CNBr-I [residues 1–52 (R1–52)] and CNBr-II (R53–163), upon cleavage with CNBr (17). CNBr-II was isolated by preparative $NaDodSO_4$/PAGE according to the method of Dietzschold *et al.* (18). It was extracted in 10% formic acid and extensively dialyzed against phosphate-buffered saline (0.15 M NaCl/0.01 M sodium phosphate, pH 7.4) and was shown to be homogeneous by analytical $NaDodSO_4$/PAGE followed by silver staining.

Selection and Synthesis of Peptides. The choice of peptides corresponding to segments of the HU 849 Gal-Gal pilus sequence for synthesis has been discussed in detail (12). The peptides were prepared by solid-phase Merrifield synthesis (19) and conjugated to carrier proteins as described (12).

Protein Blotting. Proteins derived from single agar-grown colonies (12) were electrophoretically transferred from $NaDodSO_4$/polyacrylamide gels to nitrocellulose paper as described by Towbin *et al.* (20) and Burnette (21).

Vaccine-Efficacy Studies with Synthetic Pilus Peptides, CNBr-II, and Purified Pili. Groups of 5–15 female BALB/c mice (16 weeks old) were used in these studies (9). They were immunized subcutaneously and intramuscularly with 150 μg of peptide-thyroglobulin conjugate, CNBr-II, or purified HU 849 Gal-Gal pili in complete Freund's adjuvant. Phosphate-buffered saline in complete Freund's adjuvant served as a negative control. Three to four weeks later the mice were given booster injections of the same immunogen in incomplete Freund's adjuvant. Fourteen days later the mice were challenged with 10^8 colony-forming units of J 96 bacteria administered by transurethral intravesicular catheterization as described (9). After 2 days the mice were bled by cardiac puncture and killed for determination of urine and renal colonization according to the method of O'Hanley *et al.* (9).

Evaluation of Serum Antibodies in Immunized Mice. The sera of immunized mice were tested for specific peptide and HU 849 pilus antibodies by ELISA using horseradish peroxidase-linked goat anti-mouse IgG, IgA, and IgM as second antibody, as described (5).

RESULTS

Selection of Peptides. Five peptides corresponding to linear segments of the 163 amino acid HU 849 pilus subunit (R5–12, R65–75, R93–104, R103–116, and R131–143) were prepared by solid-phase synthesis. The regions selected for peptide synthesis were predicted by the Chou and Fasman (22) and Hopp and Woods (23) algorithms to contain reverse turns and/or hydrophilic stretches, respectively. Distal to the predicted reverse turn, each peptide contained a C- or N-terminal non-natural cysteine through which the peptides were coupled to carrier proteins in one orientation.

Immunogenicity of Peptide-Carrier Conjugates. The linear antigenic structure of HU 849 Gal-Gal pilin was determined with the five peptide-thyroglobulin conjugates in BALB/c mice in order to correlate their serologic properties with the protection they confer in the murine pyelonephritis model (Table 1). Although each peptide-carrier conjugate elicited a strong, homologous peptide antibody response in mice, only antisera to peptides R5–12 and R65–75 bound intact pili (Table 1).

Crossreactivity of Antisera to Peptides R5–12 and R65–75 for Heterologous *E. coli* Gal-Gal Pilins. Gal-Gal pili appear to encode three separate classes of antigenic determinants, according to the specificity of the antibodies they engender. "Type-specific" determinants elicit antibodies that bind only the homologous immunizing pilus protein (12); "F-antigen-specific" determinants elicit antibodies that bind all examples of an F-antigen pilus serotype (13); and "Gal-Gal pilus crossreacting" determinants elicit antibodies that bind heterologous F-pilus antigens exhibiting α-D-Gal-(1-4)-β-D-Gal receptor specificity (12). In order to determine if either or both of the linear "anti-pili" epitopes in the HU 849 Gal-Gal pilin sequence (Table 1) specify type-specific, F-antigen specific, or Gal-Gal crossreacting linear antigenic determinants, the amino acid sequences corresponding to R5–12 and R65–75 were analyzed for homology with the available sequences of other Gal-Gal pili. In addition, the binding specificity of antisera engendered by peptides R5–12

Table 1. Synthetic peptides and their serologic properties

Peptide*	Sequence†	Serologic properties§				
		Reverse-turn probability‡ × 10^4	Anti-peptide	Anti-pili	Cross-reactive	Homologous protection
R5–12	**PQGQ**GKVT(C)	1.86	+	+	+	+
R65–75	AFK**GGNG**AKKG(C)	2.52	+	+	−	+
R93–104	LDT**NGGT**GTAIV(C)	2.05	+	−	−	−
R103–116	(C)IVVQG**AGKN**VVFDG	0.33	+	−	−	−
R131–143	(C)LHYTAVV**KKSS**AV	0.55	+	−	−	−

*Peptide names indicate the corresponding residues in the HU 849 pilin sequence.

†Standard one-letter amino acid symbols; (C), non-natural cysteine residues added for conjugation purposes. The four-residue sequences shown in boldface constitute β-turns as predicted by the Chou and Fasman algorithm (22).

‡Probability of each turn.

§Serologic properties observed (+) or absent (−): anti-peptide, peptide antiserum binds immunizing peptide as determined by ELISA; anti-pili, peptide antiserum binds the homologous (HU 849) Gal-Gal pilin as determined by ELISA and protein blot analysis; crossreactive, peptide antiserum binds heterologous Gal-Gal pilins (Fig. 1) as determined by protein blot analysis; homologous protection, peptide vaccine confers homologous protection to mice challenged with *E. coli* strain J 96 in the murine pyelonephritis model (Table 3).

and R65–75 was determined for Gal-Gal pilins from seven additional *E. coli* pyelonephritis strains.

The complete amino acid sequences have been reported for the Gal-Gal pilins of three uropathogenic strains. They are the F13 pilin of J 96 (O4:K5) and the corresponding recombinant strain HU 849 (11), the $F7_2$ variant of AD 110 (O6:K2) (24), and the $F7_1$ variant of KS 71 (O4:K12) (25). In the region corresponding to R65–75 of the HU 849 sequence, the AD 110 and KS 71 sequences have an insertion of three amino acid residues; otherwise, when aligned for maximal homology, seven or eight identical residues are noted. However, antiserum to peptide R65–75 bound only the homologous pilin of strain HU 849 (data not shown), indicating that this region probably encodes a type-specific epitope.

In addition to the HU 849, AD 110, and KS 71 Gal-Gal pilin sequences, the N-terminal amino acid sequences of four other Gal-Gal pilins have been determined (Table 2) through the region corresponding to peptide R5–12. The eight residues comprising this segment were identical in two sequences (HU 849 and the $F7_2$ variant of AD 110); six or seven positions were identical in three additional sequences (the $F7_1$ variants of AD 110 and KS 71 and the F12 antigen of C1979). In contrast, the F9 pilin of strain 3669 lacked significant sequence homology in this region, only two of the eight residues being identical. When eight pyelonephritis strains were analyzed by immunoblotting, seven of the eight Gal-Gal pilins, representing F antigens $F7_1$, $F7_2$, F12, and F13, were bound by antiserum to peptide R5–12 (Fig. 1), thus demonstrating that this region encodes a Gal-Gal pilus crossreacting epitope. Of the tested strains, only the F9 pilin of strain 3669 was not recognized by this serum.

Vaccine Efficacy. The efficacy of subunit pilin vaccines—namely, the five synthetic peptide-thyroglobulin conjugates and the CNBr-II fragment (R53–163) from the HU 849 Gal-Gal pilus, was determined in the BALB/c mouse model of human pyelonephritis (9, 30) and compared with the protection conferred by purified pilus filaments. Immunized mice were subjected to homologous intravesicular challenge with 10^8 colony-forming units of *E. coli* strain J 96, an inoculum that is roughly 10,000-fold the ID_{50} for unvaccinated mice and results in renal and urine colonization and renal invasion in all unvaccinated animals (9). Bacterial colonization of bladder urine and renal tissue was assessed 2 days after challenge as an index of the protection conferred.

Immunization with intact Gal-Gal pili purified from strain HU 849, a recombinant prepared from J 96 DNA, prevented urine and renal colonization by strain J 96 in 16 of 19 (84%) and 18 of 19 (95%) vaccine recipients, respectively (Table 3), corroborating previous reports by O'Hanley *et al.* (9) and Roberts *et al.* (31) that purified Gal-Gal pili confer homologous protection. The CNBr-II fragment of HU 849 pilin (R53–163) prevented urine and renal colonization by strain J 96 in all recipients, indicating that a protective epitope can be encoded by the C-terminal 111 residues of the pilin subunit. Immunization with synthetic peptide R65–75, which corresponds to a region within CNBr-II that contains a type-specific epitope for the F13 pilin of strains J 96 and HU 849 (Table 1), blocked urine and renal colonization in 5 of 8 (65%) and 8 of 8 (100%) mice challenged with strain J 96, respectively (Table 3). Therefore a linear type-specific, protective epitope exists within CNBr-II and can be encoded by a peptide of only 11 amino acids. In contrast, peptides R93–104, R103–116, and R131–143, which correspond to other regions of CNBr-II, did not elicit pilus-specific antisera in the BALB/c mouse (Table 1) or confer protection (Table 3), indicating that not all regions of CNBr-II are serologically equivalent. CNBr-I (R1–52) was not employed as a vaccine in this study. However, synthetic peptide R5–12, which corresponds to a region within CNBr-I that contains homologous sequences (Table 2), elicited antibody that bound $F7_1$, $F7_2$, F12, and F13 pilins (Fig. 1) and prevented urine and renal colonization in each of 11 vaccine recipients (Table 3). Protective epitopes, therefore, reside in separate CNBr fragments of the pilus subunit.

Protection against *E. coli* urine and renal colonization among the mice immunized with the subunit pilin vaccines correlated with anti-HU 849 Gal-Gal pilus IgG serum titers ≥1:250 (data not shown). Specific anti-pilus IgM or IgA antibodies were not detected in the serum of the immunized mice.

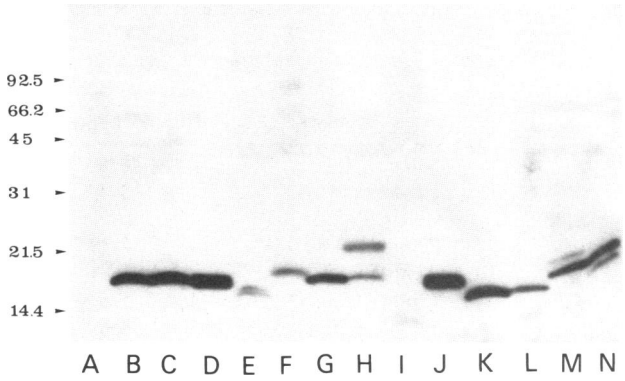

FIG. 1. Protein blot of Gal-Gal-binding *E. coli* strains or purified Gal-Gal pilins probed with R5–12 peptide antiserum diluted 1:200. Bound antibody was detected with ^{125}I-labeled protein A after an 8-hr autoradiographic exposure. Lane A, non-piliated K-12 strain HB101; lane B, strain J 96 (F13); lane C, pap 5 (HB101) (F13); lane D, pilin purified from recombinant strain HU 849 (F13); lane E, C1979 (F12); lane F, AM 1727 carrying plasmid pPIL110-75 (expresses $F7_1$ of AD 110); lane G, AM 1727 carrying pPIL110-35 (expresses $F7_2$ of AD 110); lane H, C1212 (AD 110) ($F7_1$ and $F7_2$); lane I, 3669 (F9); lane J, pilin purified from pap 5 (HB101); lanes K–N, pyelonephritis strains P21, A42, A50, and A8 (see *Materials and Methods*). Positions and sizes (kDa) of standard proteins run in parallel are shown at left.

Table 2. Amino acid sequence homology between synthetic peptide R5–12 from *E. coli* recombinant strain HU 849 Gal-Gal pilin and the Gal-Gal pilins of other uropathogenic strains

Strain	F antigen	Residue											
		1	2	3	4	5	6	7	8	9	10	11	12
HU 849	F13	Ala	Pro	Thr	Ile	**Pro**	**Gln**	**Gly**	**Gln**	**Gly**	**Lys**	**Val**	**Thr**
KS 71	$F7_1$	Ala	Ala	Thr	Ile	**Pro**	**Gln**	**Gly**	**Gln**	**Gly**	Glu	**Val**	**Thr**
AD 110	$F7_1$	Ala	Ala	Thr	Ile	**Pro**	**Gln**	**Gly**	**Gln**	**Gly**	Glu	**Val**	Ala
AD 110	$F7_2$	Ala	Pro	Thr	Ile	**Pro**	**Gln**	**Gly**	**Gln**	**Gly**	**Lys**	**Val**	**Thr**
3669	F9	Thr	Thr	Pro	Thr	Thr	Val	Asn	Gly	**Gly**	Thr	**Val**	His
C1979	F12	Ala	Pro	Thr	Ile	**Pro**	Glu	**Gly**	**Gln**	**Gly**	**Lys**	**Val**	**Thr**

Sequence of the synthetic peptide R5–12 is indicated in boldface, as are conserved residues 5–12 in the other sequences. References pertaining to each sequence and the F-antigen determination are as follows: HU 849 (11); AD 110 (24); KS 71A (26); 3669 (27); and C1979 (28). Note that strain AD 110 expresses two separate Gal-Gal-binding pili, representing the $F7_1$ and $F7_2$ antigens, respectively. Strain AD 110 (24) is identical to strain C1212 (29).

Table 3. Vaccine efficacy for prevention of urinary tract infection by *E. coli* strain J 96*

	J 96 colonization‡			
	Urine		Kidney	
Immunogen†	No. +/total	RCD	No. +/total	RCD
Saline	8/8	4.5 ± 1.1	8/8	3.5 ± 1.3
HU 849 pili	3/19	0.3 ± 0.7	1/19	0.1 ± 0.2
CNBr-II (R53–163)§	0/7	0.0 ± 0.0	0/7	0.0 ± 0.0
Peptide R5–12	0/11	0.0 ± 0.0	0/11	0.0 ± 0.0
Peptide R65–75	3/8	0.4 ± 0.5	0/8	0.0 ± 0.0
Peptide R93–104§	5/5	4.0 ± 1.0	5/5	4.6 ± 0.6
Peptide R103–116	8/8	5.0 ± 0.0	8/8	4.5 ± 1.1
Peptide R131–143	15/15	4.8 ± 0.6	15/15	3.7 ± 1.0

*Challenge dose: 10^8 colony-forming units by urethral intravesicular catheterization.
†Administered to 16-week-old female BALB/c mice in Freund's adjuvant by subcutaneous and intramuscular injections as described in *Materials and Methods*.
‡Assessed by culture 2 days after challenge dose; relative colonization density (RCD) is expressed as the mean (± SD) of J 96 colonizing the urine or the right kidney per total number of inoculated mice or the total number of kidneys examined, respectively (see *Materials and Methods*).
§Mice received only one immunization.

DISCUSSION

E. coli is the most common bacterial species isolated from endogenous infections of the female urinary tract (32). Pyelonephritis strains exhibit a constellation of phenotypic properties that act in concert to colonize, invade, and injure renal epithelium. The first discernible event in this pathogenic sequence is the specific adherence of the bacteria to uroepithelial cell surface glycoconjugates that serve as receptors for lectin-like adhesin proteins. In contrast to most *E. coli* isolates from the feces of healthy women, pyelonephritis strains express Gal-Gal pili-associated adhesins that bind to the α-D-Gal-(1-4)-β-D-Gal moiety of globoseries glycolipids (1, 2, 7). The pathogenic significance of Gal-Gal pili is also supported by (*i*) the presence of Gal-Gal adhesin receptors in urogenital mucosa (9), (*ii*) the colonizing capacity of Gal-Gal piliated recombinant strains for the renal epithelia of susceptible mammalian species (9, 30), and (*iii*) the efficacy of purified Gal-Gal pilus vaccines in the BALB/c mouse and primate pyelonephritis models (9, 31).

The linear immunogenic and antigenic structure of the HU 849 Gal-Gal pilus subunit was defined in a previous study (12) with synthetic peptides corresponding to nine regions of the pilus sequence and rabbit anti-pilus and anti-peptide sera. Five peptides corresponding to R25–38, R38–50, R48–61, R65–75, and R103–116 were bound by antibody to the intact HU 849 pilus filament and were thus designated linear immunogenic epitopes (12). Four peptide-thyroglobulin conjugates corresponding to R5–12, R65–75, R93–104, and R119–131 elicited antibodies that bound intact HU 849 pilus filaments. These were designated linear antigenic epitopes (12). We now report that two synthetic peptide vaccines corresponding to R5–12 and R65–75 of the HU 849 Gal-Gal pilus sequence prevent renal epithelial and urine colonization in the BALB/c mouse pyelonephritis model and therefore specify linear protective epitopes.

The structural basis for the efficacy of peptides R5–12 and R65–75 was not directly addressed in this study. Both peptides correspond to regions that are predicted to constitute hydrophilic β-turns in the native protein and which might therefore comprise immunogenic surface projections of the molecule (12). Both were found to encode antigenic epitopes in the mouse and rabbit. Accordingly, the protection conferred by peptides R5–12 and R65–75 might simply reflect the avidity of the respective peptide antibodies for pili and the concomitant loss of pilus function *in situ* as a result of steric hindrance, allosteric effects, or the agglutination of pilus filaments. Alternatively, since the specificity of peptide antibodies is presumed to be "predetermined" (33) by the sequence of the immunizing peptide, the protection conferred by peptides R5–12 and R65–75 might have implications for the structure–function analysis of the pilus subunit. If so, these data imply the existence of two linear domains, separated by 53 intervening residues, that are critical for pilus function and which we have termed protective epitopes. In the native molecule both regions could be spatially close as a result of protein folding. Mutational analysis of the Gal-Gal pilus operon indicates that separate cistrons encode the pilus subunit and the lectin-like adhesin protein *per se* (34). Specific adherence appears to be mediated by a binding organelle composed of the pilus subunit, the adhesin protein, and possibly other gene products that function together as a ternary complex. Therefore, the protective epitopes as defined above could be structural domains within the pilus subunit that govern the correct configuration of the Gal-Gal binding complex. Another interpretation of the data is the possibility that the bound antibodies perturb these domains indirectly.

Similar features of secondary structure and hydrophilicity were predicted for peptides R93–104, R103–116, and R131–143 (Table 1), which lacked efficacy. However, the antisera they elicited bound the immunizing peptide but not the homologous pilus filament, suggesting that the conformations of these peptides in solution and of the corresponding regions in the folded protein were sufficiently different to preclude the production of anti-pilus antibodies.

The entire amino acid sequences have been reported for Gal-Gal pilus proteins from three *E. coli* urine isolates: HU 849, a recombinant strain prepared from strain J 96 (11), and strains AD 110 (24) and KS 71 (25). Their hydrophilicity profiles are similar (24) and the N- and C-terminal regions are highly conserved. However, the intervening regions contain substantial segments of sequence heterogeneity, which probably accounts for the observed antigenic diversity of Gal-Gal pili. Indeed, crossed immunoelectrophoresis of Gal-Gal pili has led to the identification of at least eight F-antigen types: $F7_1$, $F7_2$, F8, F9, F10, F11, F12, and F13 (13). The significance of this for the immunoprophylaxis of pyelonephritis has not been evaluated, since only homologous strains have been used in efficacy studies of whole Gal-Gal pilus vaccines (9, 31). However, the efficacy of other pilus vaccines has been confined to the homologous immunogen (35), stimulating a search for protective epitopes that also evoke crossreacting antibodies. Peptide R65–75 encodes an immunogenic, antigenic, and protective epitope (Table 1); corresponds to a hydrophilic maximum of pilin (12); is predicted to have a strong β-turn potential; contains

regions of unconserved sequence (including a three amino acid deletion in the J 96 protein); and elicits antibodies that bind only the homologous pilus proteins of strains J 96 and HU 849. Thus, this region in HU 849 Gal-Gal pilin appears to constitute an immunogenic, strain-specific determinant in which amino acid substitutions, deletions, and additions in the corresponding regions of other pilus proteins from separate strains could create antigenic diversity. Since peptide R65–75 bound only one of eight tested Gal-Gal pilins, it is likely to confer only homologous protection. Peptide R5–12 also encodes a protective epitope but corresponds to a conserved region near the N terminus that is not normally immunogenic (Table 1) (12). Thus, it is likely that this segment has not evolved under the selective pressure of the host immune response and that it may be structurally or functionally critical. The region corresponding to R5–12 in the F13 Gal-Gal pilin of strain HU 849 has eight of eight identical residues with the $F7_2$ Gal-Gal pilin of strain AD 110, seven of eight identical residues with the $F7_1$ pilin of strain KS 71 and the F12 pilin of strain C1979, and six of eight identical residues with the $F7_1$ pilin of strain AD 110 (Table 2). Accordingly, peptide R5–12 antisera bound the pilins of seven of eight pyelonephritis isolates, indicating that it might also confer heterologous protection.

The findings in this report suggest that efficacious immunizing peptides from antigenically diverse members of a microbial protein family may correspond to linear domains that are highly conserved, are not normally immunogenic within the folded molecule, are functionally or structurally essential, and specify protective epitopes. This study indicates that an eight-residue peptide with these properties can confer protection on a mucosal surface equal to that obtained with the intact protein.

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