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Purified P Fimbriae from Two Cloned Gene Clusters of a Single Pyelonephritogenic Strain Adhere to Unique Structures in the Human Kidney

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We have completed immunofluorescence binding studies of purified fimbriae from two clones which express Pap or Pap-2 fimbriae. Although the two fimbrial types exhibited common binding to the uroepithelia of the bladder and renal pelvis and to occasional cells located within the glomeruli which we have termed glomerular elements, only Pap-2 fimbriae adhered to Bowman's capsule. Previous studies have demonstrated that the Galα1→4Gal disaccharide moiety is capable of inhibiting Pap hemagglutination and adherence to uroepithelial cells. Results of our experiments demonstrate that this disaccharide is not sufficient for blocking binding of Pap-2 fimbriae to Bowman's capsule but that GalNAcβ1→3Gal completely blocks Pap-2 adherence to Bowman's capsule. These results indicate that the different hemagglutination capacities of the two clones reflect different receptor specificities and differential tissue tropisms in the urinary tract. These unique receptor specificities may provide uropathogenic strains of *Escherichia coli* carrying multiple chromosomal copies of *pap*-like gene clusters with the advantage of increased numbers of binding sites within the urinary tract. This, in turn, might improve the chances of colonization and the establishment of infection.

Uropathogenic *Escherichia coli* strains have been shown to carry multiple copies of gene clusters which encode P fimbriae (3, 10, 13, 14, 45) and to express more than one P fimbrial type per strain (33–35, 38). P fimbriae, named for their adherence to the human P-blood-group antigens and their association with pyelonephritis, bind to human erythrocytes (11, 15, 20), voided uroepithelial cells (40, 41), and tissues of the urinary tract (44). The minimal receptor required for P-fimbrial adhesion has been determined to be Galα1→4Gal (15, 20). This digalactoside has been shown to inhibit P-fimbrial hemagglutination of erythrocytes (17, 18), binding to uroepithelial cells (16), and adherence to cryostat sections of human tissues (19).

Numerous P fimbriae have been described and classified serologically as F7 to F14 (1, 31, 32). Some of these P fimbriae adhere to structures of the globoseries of glycolipids other than the minimal Galα1→4Gal structure. This was demonstrated by the failure of some P fimbriae to agglutinate Galα1→4Gal-latex beads and erythrocytes which lack globotetraosylceramide, the P antigen (6; J. Karr, R. Hull, and S. Hull, Abstr. Annu. Meet. Am. Soc. Microbiol. 1988, D-162, p. 98). Recently, other globoseries recognizing *E. coli* strains have been described and designated ONAP (O negative, A positive), based on the requirement for both the A terminal structure and the globoseries chain for binding (39). These strains appear to recognize as receptor the globo-A glycolipid, an extended structure which resembles the P-blood-group antigens.

We have previously cloned the second P fimbrial gene cluster from the pyelonephritogenic isolate J96 and designated it *pap*-2 (22; Karr et al., Abstr. Annu. Meet. Am. Soc. Microbiol. 1988). The restriction enzyme maps of the *pap* and *pap*-2 clones reveal homologous sequences (Fig. 1), except in the regions which encode the adhesins. The *pap* gene cluster has been extensively analyzed and mapped (4, 5, 21, 23–25, 27, 28, 43), and it has been demonstrated that the adhesin is encoded within the 3.9-kilobase (kb) *Kpn*I-to-*Eco*RI region of pRHU845. The *pap*-2 gene cluster has been partially mapped by in vitro translation, and the adhesin is encoded within the 3.2-kb *Kpn*I-to-*Bam*HI fragment (unpublished data). Construction of chimeric clones in which the 3.2-kb *Kpn*I-to-*Bam*HI fragment of pJFK102 and the 3.9-kb *Kpn*I-to-*Eco*RI region of pRHU845 were exchanged created chimeric clones with adherence specificities of the parental clones which contained the corresponding regions (unpublished data). Although the *pap*-2 gene cluster is probably identical to the *prs* (*pap*-related sequence) gene cluster (23, 26), these two clones were constructed differently and independently. The *prs* clone has been partially mapped by minicell analysis and restriction enzymes (23, 26), and the

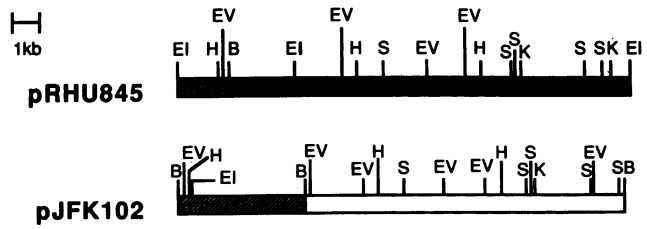

FIG. 1. Restriction endonuclease maps of the two *pap* gene clusters from clones pRHU845 and pJFK102. The two inserts are alike between their common *Eco*RV and *Kpn*I sites but differ to the right of the *Kpn*I sites, where two minor pilins and the adhesins are located. In the region to the right of and including the terminal *Sma*I fragment of pRHU845, the two clones are not comparable since a deletion was introduced in vitro in this region of pRHU845 during subcloning (28). Hatched bars indicate vector DNA: pACYC184 for pRHU845 and pBR322 for pJFK102. The solid bar represents *pap* genes, and the open bar represents *pap*-2 genes. Restriction endonucleases: B, *Bam*HI; EI, *Eco*RI; EV, *Eco*RV; S, *Sma*I; H, *Hin*dIII; K, *Kpn*I.

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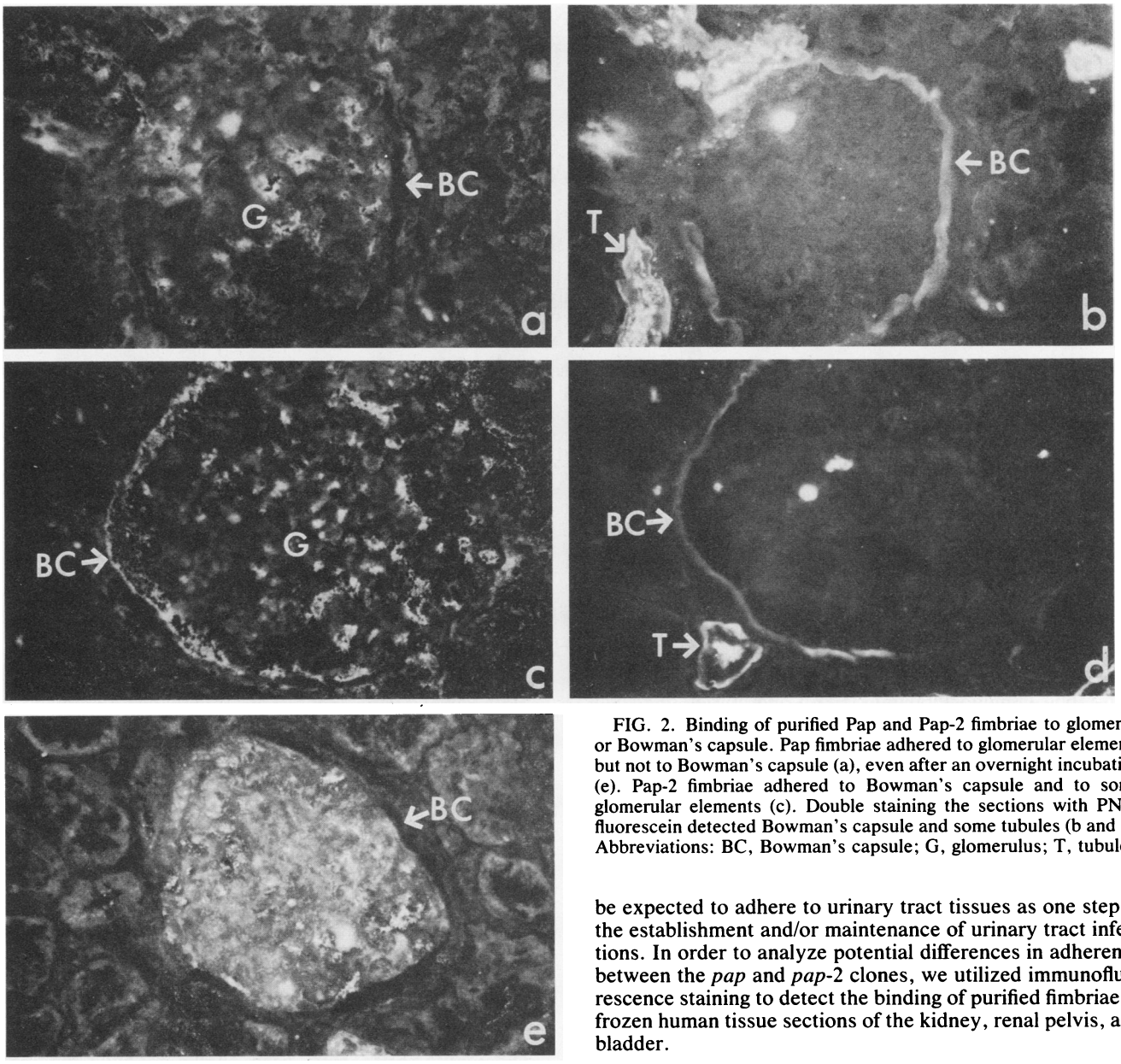

FIG. 2. Binding of purified Pap and Pap-2 fimbriae to glomeruli or Bowman's capsule. Pap fimbriae adhered to glomerular elements but not to Bowman's capsule (a), even after an overnight incubation (e). Pap-2 fimbriae adhered to Bowman's capsule and to some glomerular elements (c). Double staining the sections with PNA-fluorescein detected Bowman's capsule and some tubules (b and d). Abbreviations: BC, Bowman's capsule; G, glomerulus; T, tubule.

results of *pap*-2 analysis suggest that these two clones carry the same gene cluster from strain J96.

We have analyzed the differences in binding between the *pap*-2 and *pap* clones. Both clones are positive for mannose-resistant hemagglutination of human erythrocytes, but only *pap*-2 cells are capable of mannose-resistant hemagglutination of sheep and dog erythrocytes (Karr et al., Abstr. Annu. Meet. Am. Soc. Microbiol. 1988; manuscript in preparation). In contrast, Prs-piliated *E. coli* have been demonstrated to bind to sheep erythrocytes but not to human erythrocytes (23, 26), and the Prs receptor has been identified as the Forssman antigen (26). Results of subsequent studies demonstrate that a Prs-piliated clone agglutinates human AP_1 erythrocytes (22). A comparison of the binding of *prs* and *pap*-2 clones to purified glycolipids bound to thin-layer chromatograms has recently been completed (22).

The adherence of P-fimbriated *E. coli* to human tissues has not been thoroughly studied. Uropathogenic strains would be expected to adhere to urinary tract tissues as one step in the establishment and/or maintenance of urinary tract infections. In order to analyze potential differences in adherence between the *pap* and *pap*-2 clones, we utilized immunofluorescence staining to detect the binding of purified fimbriae to frozen human tissue sections of the kidney, renal pelvis, and bladder.

MATERIALS AND METHODS

Bacterial strains. Strains HU849 (10) and P678-54(pJFK102) (Karr et al., Abstr. Annu. Meet. Am. Soc. Microbiol. 1988) were grown at 37°C on L agar plates with 10 μg of tetracycline per ml or 100 μg of ampicillin per ml, respectively.

Fimbrial purification. Bacteria were grown overnight at 37°C on L agar plus the appropriate antibiotic and tested for mannose-resistant hemagglutination of human and sheep erythrocytes. Overnight cultures of L broth with the appropriate antibiotic were inoculated with each strain. Fimbriae were purified from P678-54(pJFK102) and HU849 by heat shock, as described by Wray et al. (47). Fimbriae were stored in phosphate-buffered saline (PBS) (pH 7.1) in aliquots at −70°C.

Human tissue sections. Histopathologically normal surgical specimens of kidney, renal pelvis, and bladder from a 66-year-old male (phenotype B^+ P_1^- P^+) with renal carci-

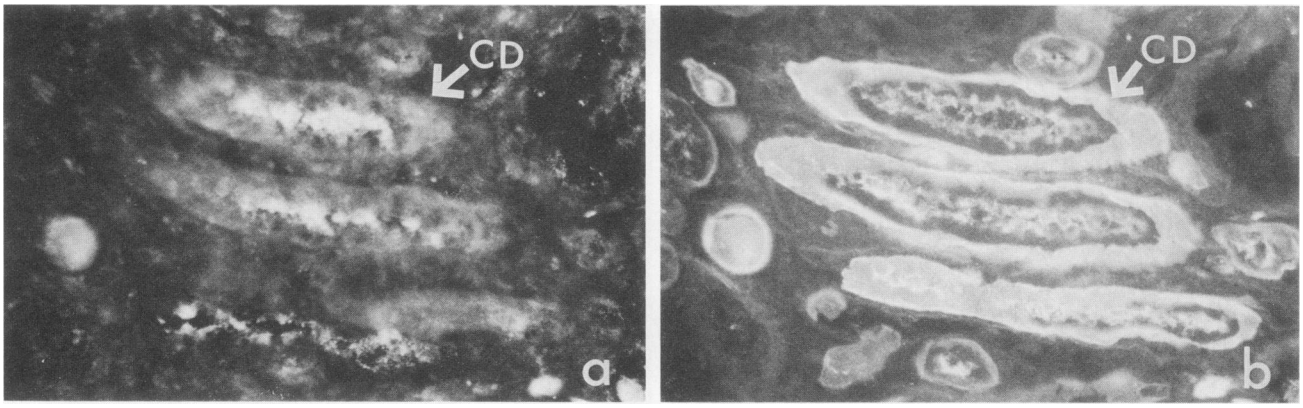

FIG. 3. Pap-2 fimbriae bound to the luminal surface of collecting ducts (CD) (a), as detected by PNA double staining of the same tissue section (b).

noma were snap frozen in liquid nitrogen. Cryostat sections 4 μm thick were cut for immunofluorescence.

Indirect immunofluorescence. Purified fimbriae were suspended at a concentration of 0.5 mg/ml in PBS (pH 7.1) with 0.05% Tween 20, 1% bovine serum albumin (BSA), and 1% methyl-α-D-mannopyranoside. Rabbit anti-HU849 antiserum (12) was extensively adsorbed against P678-54 to remove non-fimbrial-specific binding and then suspended 1:40 in PBS (pH 7.1) with 1% BSA. Normal goat serum diluted 1:10 in 50 mM Tris (pH 7.0) was used to block nonspecific binding. Tetramethylrhodamine isothiocyanate-labeled goat anti-rabbit immunoglobulin G (E-Y Laboratories, Inc., San Mateo, Calif.) was suspended 1:40 in PBS (pH 7.1) with 1% BSA and used to detect bound fimbriae. Fluorescein isothiocyanate-labeled lectin PNA (E-Y Laboratories) was diluted 1:32 in Dulbecco PBS. Immunofluorescence staining of the fimbriae and lectins bound to tissue sections was performed as described by Nowicki et al. (30), with a few modifications. Immediately after the cryostat sections were fixed and washed, 40 μl of normal goat serum was incubated on the tissue sections for 20 min, blotted off, and then replaced by 40 μl of the fimbrial preparation for 45 min, except where noted otherwise. All other incubations of primary and tetramethylrhodamine isothiocyanate-labeled secondary antisera were for 30 min. In order to identify specific structures within the kidney (7–9, 42), fimbria-stained sections were double stained for 30 min with fluorescein isothiocyanate-labeled PNA, the peanut agglutinin from *Arachis hypogaea* which binds to Galβ1→3GalNAc.

Inhibition of fimbrial adhesion. Purified Pap-2 fimbriae (0.5 mg/ml in PBS [pH 7.1] with 0.05% Tween 20, 1% BSA, and 1% methyl-α-D-mannopyranoside) with 0.75% Galα1→4Gal or GalNAcβ1→3Gal were incubated on kidney sections for 45 min before washing and immunofluorescence staining for fimbriae. The negative control contained 1% methyl-α-D-mannopyranoside, as in all the other experiments.

RESULTS

Column-purified Pap and Pap-2 fimbriae were used to determine binding to structures within the kidney, bladder, and renal pelvis. The results of a 45-min incubation of fimbriae with tissue sections (Fig. 2a) show the focal adherence of Pap fimbriae to the glomerulus but not to Bowman's capsule. An overnight incubation of Pap fimbriae on kidney sections revealed that increased incubation time enhanced binding to the glomerulus but not to Bowman's capsule or tubules (Fig. 2e). Figure 2c shows strong binding of Pap-2 fimbriae to Bowman's capsule and focal binding within the glomerulus. Figures 2b and d show the same sections as Fig. 2a and c, respectively, double stained with PNA lectin, which binds specifically to Bowman's capsule and other structures.

Pap and Pap-2 fimbriae occasionally bound to tubules of the kidney. This staining was weak and not always seen, so tubular staining results were inconclusive. Pap-2 fimbriae are shown adhered to the lumens of some collecting ducts (Fig. 3a) double stained by PNA (Fig. 3b).

Both Pap and Pap-2 fimbriae adhered to the uroepithelia of the renal pelvis and bladder (Fig. 4). The fimbriae from both clones also adhered strongly to smooth muscles from the bladder wall (Fig. 5).

In order to determine whether Pap-2 fimbrial adhesion could be blocked, we added 0.75% Galα1→4Gal or GalNAcβ1→3Gal to the Pap-2 fimbrial preparation prior to incubation of the fimbriae with the kidney sections. Galα1→4Gal did not inhibit binding of Pap-2 fimbriae to Bowman's capsule (Fig. 6a and c). The addition of GalNAcβ1→3Gal eliminated binding to Bowman's capsule but did not inhibit binding of Pap-2 to glomerular elements (6b and d).

DISCUSSION

Different fimbriae have been shown to have different tissue tropisms, for example, P (19) and Dr (30) fimbriae. However, no information was available concerning the tissue tropisms of multiple P fimbriae from the same strain. We have shown that Pap and Pap-2 (F13) have different binding specificities within the human kidney (Table 1). Another type of P fimbria, KS71A ($F7_1$), from the pyelonephritogenic *E. coli* strain KS71, has been analyzed for binding to the human kidney (19, 44). Purified fimbriae from a recombinant clone with KS71A fimbriae showed intense adherence to tubules, including proximal, distal, and collecting ducts, and also to vascular endothelium and parietal epithelia of glomeruli (19). This binding was inhibited by the addition of globotriose (19). KS71A fimbriae do not bind to human kidney sections in the same pattern as Pap or Pap-2 fimbriae. Therefore, different P fimbriae seem to have multiple unique patterns of adherence to human kidney tissue.

In ascending urinary tract infections, bacteria are thought to first colonize the bladder, possibly by adherence to the uroepithelium, and then ascend through the ureter to the

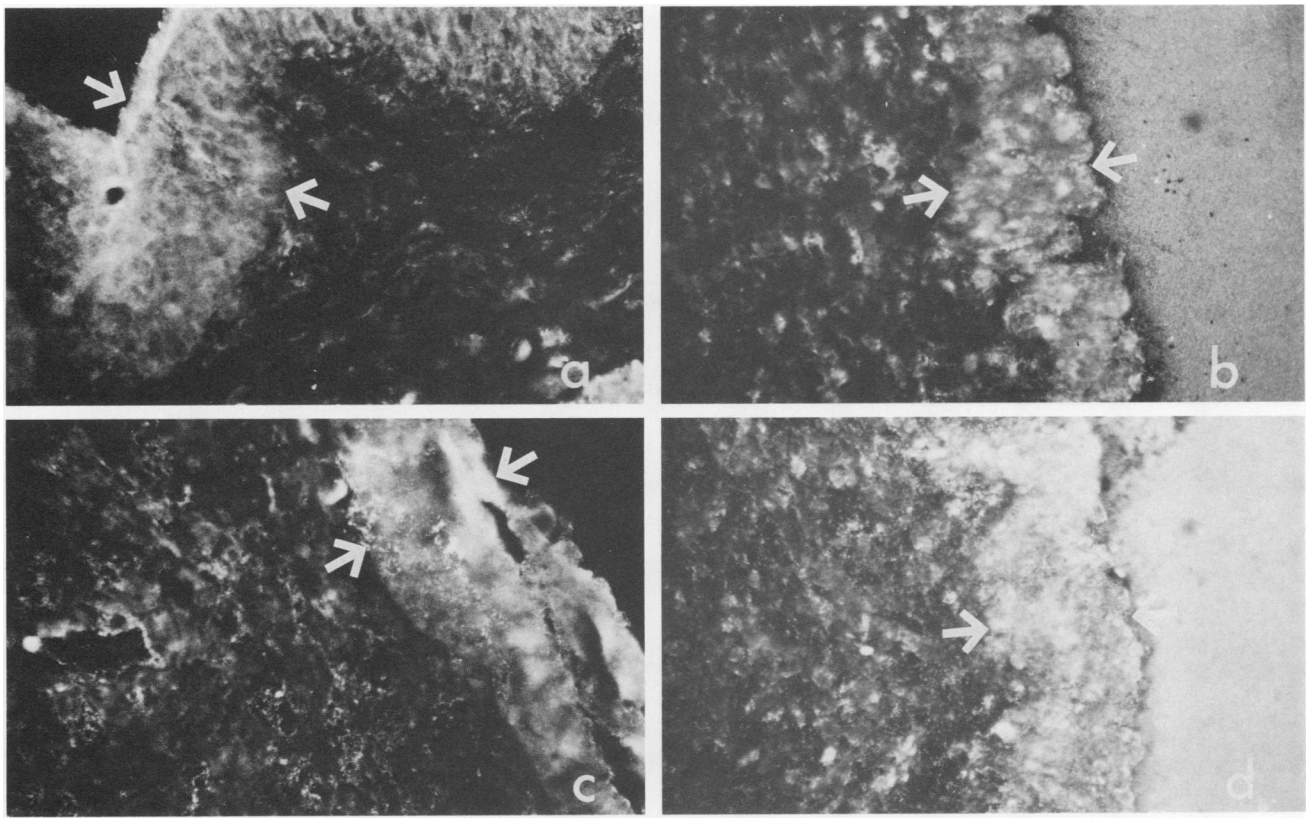

FIG. 4. Both fimbrial preparations adhered to the uroepithelia of the renal pelvis and bladder. The uroepithelia (between the arrows) of the renal pelvis (a and c) and bladder (b and d) are bound by Pap (a and b) and Pap-2 (c and d) purified fimbriae.

kidney, where the renal pelvis is the first renal site to be involved in the acute infectious process. From the renal pelvis, the infection may spread to the interstitial tissues of the kidney, affecting the tubules, glomeruli, and blood vessels. We have shown that there are receptors for two P fimbriae, Pap and Pap-2, located in the uroepithelia of the bladder and renal pelvis and also in the renal parenchyma. The adherence of P fimbriae to these sites may be clinically significant in the establishment of pyelonephritis.

Adherence by both clones to the smooth muscle of the bladder was surprising but has also been observed for some other P fimbriae (46). Two possible explanations exist for this strong binding phenomenon. One is that the receptor is fortuitously present on the muscle fibers and that the adherence to these structures plays no role in the pathogenesis of urinary tract infection. The other possibility is that pyelonephritogenic *E. coli* may ascend the wall of the bladder and ureter to the kidney by intramural spread and by ascending interstitially along the connective tissue and muscle fibers (36). This pathway would require invasion of the bladder wall to reach the subepithelial tissues. According to Anderson (2), the subepithelial tissues of the bladder, ureter, and renal pelvis could provide a direct route of invasion of the kidney.

Results of our previous studies of the *pap*-2 clone indicate that the receptor analog for Pap-2 fimbriae is not

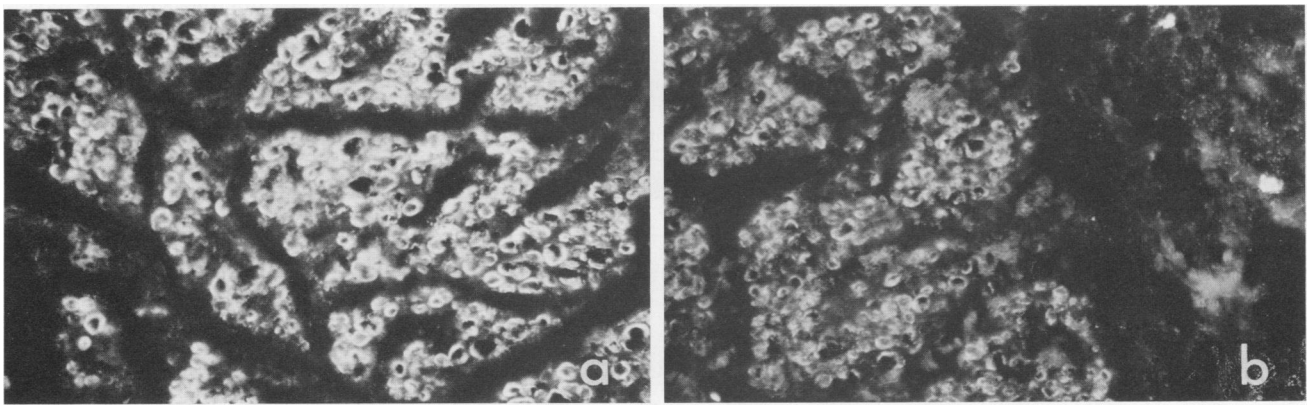

FIG. 5. Pap (a) and Pap-2 (b) bound strongly to the smooth muscle in the bladder wall.

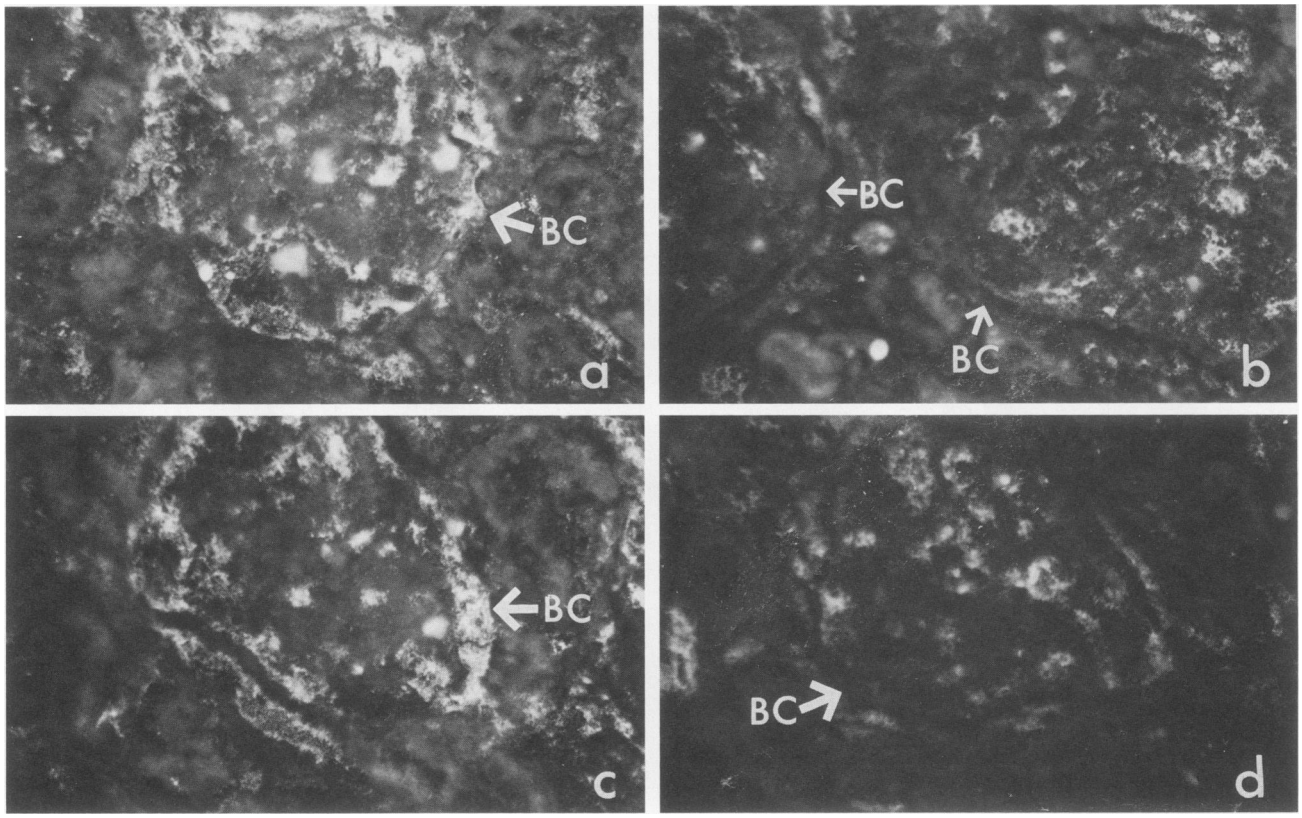

FIG. 6. Inhibition of Pap-2 fimbrial adherence to Bowman's capsule (BC). Preincubation of purified fimbriae with 0.75% Galα1→4Gal had no inhibitory effect on Pap-2 adherence to Bowman's capsule (a and c), but 0.75% GalNAcβ1→3Gal completely blocked this binding (b and d).

Galα1→4Gal (22; Karr et al., Abstr. Annu. Meet. Am. Soc. Microbiol. 1988). In contrast to studies of the *prs* clone (23, 26), our results have demonstrated that the *pap*-2 clone causes hemagglutination of both sheep erythrocytes and those human erythrocytes which express globotetraosylceramide (Karr et al., Abstr. Annu. Meet. Am. Soc. Microbiol. 1988). Since GalNAcβ1→3Gal is found in globotetraosylceramide on P_1 and P_2 erythrocytes and the Forssman antigen on sheep erythrocytes but not on P_1^k, P_2^k, or p erythrocytes, this structure may be required for receptor recognition by Pap-2 fimbriae. However, GalNAcβ1→3Gal does not appear to be the entire receptor structure and may only be part of an extended glycolipid structure that serves as the complete receptor for Pap-2 fimbriae. The data indicate that the receptor epitope recognized by Pap-2 fimbriae on erythrocytes may be found on the Forssman antigen and on a P-related blood group antigen from human erythrocytes.

Our present experiments demonstrate that purified fimbriae from the *pap* and *pap*-2 clones are capable of adhering to structures within the human urinary tract and that GalNAcβ1→3Gal, but not Galα1→4Gal, blocks adherence of Pap-2 fimbriae to Bowman's capsule. PNA recognizes Galβ1→3GalNAc and binds to Bowman's capsule, occasional cells within glomeruli, distal tubules, and collecting ducts of the human kidney (7–9, 42). Pap-2 fimbriae and PNA bind to Bowman's capsule and the luminal surfaces of some collecting ducts, but the binding is not identical.

Several conclusions may be drawn from our experiments. First, there are receptor binding sites for Pap and Pap-2 fimbriae in both the upper and lower urinary tract. Second, Pap and Pap-2 fimbriae express differential tissue tropisms in the kidney. These tropisms are not the same as those of KS71A fimbriae, as described by Korhonen et al. (19). Third, the receptor for Pap-2 fimbriae requires the presence

TABLE 1. Adhesion of purified fimbriae or inhibition of adhesion to frozen human tissue sections

Prepn	Adhesion					
	Kidney			Renal pelvis uroepithelium	Bladder	
	Glomerulus	Bowman's capsule	Tubules		Uroepithelium	Smooth muscle
Pap	++	−	+/−	++	++	++++
Pap-2	++	+++	+/−	++	++	+++
Control, PNA lectin	−	++	+++	−	−	−
Pap-2 + Galα1→4Gal	+	+++	+/−	NT[a]	NT	NT
Pap-2 + GalNAcβ1→3Gal	+	+/−	+/−	NT	NT	NT

[a] NT, Not tested.

of *N*-acetylgalactosamine. The disaccharide Galα1→4Gal is not adequate for inhibition of Pap-2 fimbrial adherence; however, GalNAcβ1→3Gal does block Pap-2 fimbrial binding to Bowman's capsule.

We therefore suggest that although there are many serologically or genetically identified and characterized P fimbriae, the adhesive properties within the P fimbrial family are diverse. P fimbriae cannot simply be categorized as having a Galα1→4Gal minimal receptor specificity. There appear to be subtle differences in receptor recognition in the kidney. Within a single clinical isolate, more than one P fimbrial subtype may be encoded and expressed, as in J96. These multiple fimbrial subtypes, likely carried on separate bacterial subpopulations (29, 37), may provide the bacteria with adherence to multiple receptor sites within the urinary tract, with the advantage of increased opportunities for each bacterial cell to bind to separate tissue elements and establish infection.

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LITERATURE CITED

1. **Abe, C., S. Schmitz, I. Moser, G. Boulnois, N. J. High, I. Ørskov, F. Ørskov, B. Jann, and K. Jann.** 1987. Monoclonal antibodies with fimbrial F1C, F12, F13, and F14 specificities obtained with fimbriae from *E. coli* O4:K12:H⁻. Microb. Pathog. **2**:71–77.
2. **Anderson, W. A. D.** 1966. Kidneys, p. 631–633. *In* W. A. D. Anderson (ed.), Pathology, 5th ed. C. V. Mosby Co., St. Louis.
3. **Arthur, M., C. Campanelli, R. D. Arbeit, C. Kim, S. Steinbach, C. E. Johnson, R. H. Rubin, and R. Goldstein.** 1989. Structure and copy number of gene clusters related to the *pap* P-adhesin operon of uropathogenic *Escherichia coli*. Infect. Immun. **57**: 314–321.
4. **Båga, M., M. Göransson, S. Normark, and B. E. Uhlin.** 1985. Transcriptional activation of a Pap pilus virulence operon from uropathogenic *Escherichia coli*. EMBO J. **4**:3887–3893.
5. **Ekbäck, G., S. Mörner, B. Lund, and S. Normark.** 1986. Correlation of genes in the *pap* gene cluster to expression of globoside-specific adhesin by uropathogenic *Escherichia coli*. FEMS Microbiol. Lett. **34**:355–360.
6. **Enerbäck, S., A.-C. Larsson, H. Leffler, A. Lundell, P. de Man, B. Nilsson, and C. Svanborg-Edén.** 1987. Binding to galactoseα1→4galactoseβ-containing receptors as potential diagnostic tool in urinary tract infection. J. Clin. Microbiol. **25**:407–411.
7. **Hennigar, R. A., B. A. Schulte, and S. S. Spicer.** 1985. Heterogeneous distribution of glycoconjugates in human kidney tubules. Anat. Rec. **211**:376–390.
8. **Holthöfer, H.** 1983. Lectin binding sites in kidney: a comparative study of 14 animal species. J. Histochem. Cytochem. **31**:531–537.
9. **Holthöfer, H., I. Virtanen, E. Pettersson, T. Törnroth, O. Alfthan, E. Linder, and A. Miettinen.** 1981. Lectins as fluorescence microscopic markers for saccharides in the human kidney. Lab. Invest. **45**:391–399.
10. **Hull, R., S. Bieler, S. Falkow, and S. Hull.** 1986. Chromosomal map position of genes encoding P adhesins in uropathogenic *Escherichia coli*. Infect. Immun. **51**:693–695.
11. **Hull, R. A., R. E. Gill, P. Hsu, B. H. Minshew, and S. Falkow.** 1981. Construction and expression of recombinant plasmids encoding type 1 or D-mannose-resistant pili from a urinary tract infection *Escherichia coli* isolate. Infect. Immun. **33**:933–938.
12. **Hull, R. A., S. I. Hull, and S. Falkow.** 1984. Frequency of gene sequences necessary for pyelonephritis-associated pili expression among isolates of *Enterobacteriaceae* from human extraintestinal infections. Infect. Immun. **43**:1064–1067.
13. **Hull, S., S. Clegg, C. Svanborg Eden, and R. Hull.** 1985. Multiple forms of genes in pyelonephritogenic *Escherichia coli* encoding adhesins binding globoseries glycolipid receptors. Infect. Immun. **47**:80–83.
14. **Hull, S. I., S. Bieler, and R. A. Hull.** 1988. Restriction fragment length polymorphism and multiple copies of DNA sequences homologous with probes for P-fimbriae and hemolysin genes among uropathogenic *Escherichia coli*. Can. J. Microbiol. **34**: 307–311.
15. **Källenius, G., R. Möllby, S. B. Svenson, J. Winberg, A. Lundblad, S. Svensson, and B. Cedergren.** 1980. The P^k antigen as receptor for the haemagglutinin of pyelonephritic *Escherichia coli*. FEMS Microbiol. Lett. **7**:297–302.
16. **Källenius, G., S. B. Svenson, R. Möllby, B. Cedergren, H. Hultberg, and J. Winberg.** 1981. Structure of carbohydrate part of receptor on human uroepithelial cells for pyelonephritogenic *Escherichia coli*. Lancet **ii**:604–606.
17. **Källenius, G., S. B. Svenson, R. Möllby, T. Korhonen, J. Winberg, B. Cedergren, I. Helin, and H. Hultberg.** 1982. Carbohydrate receptor structures recognized by uropathogenic *E. coli*. Scand. J. Infect. Dis. Suppl. **33**:52–60.
18. **Korhonen, T. K., V. Väisänen, H. Saxén, H. Hultberg, and S. B. Svenson.** 1982. P-antigen-recognizing fimbriae from human uropathogenic *Escherichia coli* strains. Infect. Immun. **37**:286–291.
19. **Korhonen, T. K., R. Virkola, and H. Holthöfer.** 1986. Localization of binding sites for purified *Escherichia coli* P fimbriae in the human kidney. Infect. Immun. **54**:328–332.
20. **Leffler, H., and C. Svanborg Edén.** 1980. Chemical identification of a glycosphingolipid receptor for *Escherichia coli* attaching to human urinary tract epithelial cells and agglutinating human erythrocytes. FEMS Microbiol. Lett. **8**:127–134.
21. **Lindberg, F. P., B. Lund, and S. Normark.** 1984. Genes of pyelonephritogenic *E. coli* required for digalactoside-specific agglutination of human cells. EMBO J. **3**:1167–1173.
22. **Lindstedt, R., N. Baker, P. Falk, R. Hull, S. Hull, J. Karr, H. Leffler, C. Svanborg Edén, and G. Larson.** 1989. Binding specificities of wild-type and cloned *Escherichia coli* strains that recognize globo-A. Infect. Immun. **57**:3389–3394.
23. **Lund, B., F. Lindberg, B.-I. Marklund, and S. Normark.** 1987. The PapG protein is the α-D-galactopyranosyl-(1→4)-β-D-galactopyranose-binding adhesin of uropathogenic *Escherichia coli*. Proc. Natl. Acad. Sci. USA **84**:5898–5902.
24. **Lund, B., F. Lindberg, and S. Normark.** 1988. Structure and antigenic properties of the tip-located P pilus proteins of uropathogenic *Escherichia coli*. J. Bacteriol. **170**:1887–1894.
25. **Lund, B., F. P. Lindberg, M. Båga, and S. Normark.** 1985. Globoside-specific adhesins of uropathogenic *Escherichia coli* are encoded by similar trans-complementable gene clusters. J. Bacteriol. **162**:1293–1301.
26. **Lund, B., B.-I. Marklund, N. Strömberg, F. Lindberg, K.-A. Karlsson, and S. Normark.** 1988. Uropathogenic *Escherichia coli* can express serologically identical pili of different receptor binding specificities. Mol. Microbiol. **2**:255–263.
27. **Norgren, M., S. Normark, D. Lark, P. O'Hanley, G. Schoolnik, S. Falkow, C. Svanborg-Edén, M. Båga, and B. E. Uhlin.** 1984. Mutations in *E. coli* cistrons affecting adhesion to human cells do not abolish Pap pili fiber formation. EMBO J. **3**:1159–1165.
28. **Normark, S., D. Lark, R. Hull, M. Norgren, M. Båga, P. O'Hanley, G. Schoolnik, and S. Falkow.** 1983. Genetics of digalactoside-binding adhesin from a uropathogenic *Escherichia coli* strain. Infect. Immun. **41**:942–949.
29. **Nowicki, B., M. Rhen, V. Väisänen-Rhen, A. Pere, and T. K. Korhonen.** 1984. Immunofluorescence study of fimbrial phase variation in *Escherichia coli* KS71. J. Bacteriol. **160**:691–695.
30. **Nowicki, B., L. Truong, J. Moulds, and R. Hull.** 1988. Presence of the Dr receptor in normal human tissues and its possible role in the pathogenesis of ascending urinary tract infection. Am. J. Pathol. **133**:1–4.
31. **Ørskov, I., and F. Ørskov.** 1983. Serology of *Escherichia coli* fimbriae. Prog. Allergy **33**:80–105.
32. **Ørskov, I., F. Ørskov, A. Birch-Andersen, P. Klemm, and C. Svanborg-Edén.** 1982. Protein attachment factors: fimbriae in

adhering *Escherichia coli* strains. Semin. Infect. Dis. **4**:97–103.
33. **Pere, A.** 1986. P fimbriae on uropathogenic *Escherichia coli* O16:K1 and O18 strains. FEMS Microbiol. Lett. **37**:19–26.
34. **Pere, A., R. K. Selander, and T. K. Korhonen.** 1988. Characterization of P fimbriae on O1, O7, O75, rough, and nontypable strains of *Escherichia coli*. Infect. Immun. **56**:1288–1294.
35. **Pere, A., V. Väisänen-Rhen, M. Rhen, J. Tenhunen, and T. K. Korhonen.** 1986. Analysis of P fimbriae on *Escherichia coli* O2, O4, and O6 strains by immunoprecipitation. Infect. Immun. **51**:618–625.
36. **Rényi-Vámos, F., and F. Balogh.** 1979. Pyelonephritis, p. 46–54. Akadémiai Kiadó, Budapest.
37. **Rhen, M., P. H. Mäkelä, and T. K. Korhonen.** 1983. P-fimbriae of *Escherichia coli* are subject to phase variation. FEMS Microbiol. Lett. **19**:267–271.
38. **Rhen, M., E. Wahlström, and T. K. Korhonen.** 1983. P-fimbriae of *Escherichia coli*: fractionation by immune precipitation. FEMS Microbiol. Lett. **18**:227–232.
39. **Senior, D., N. Baker, B. Cedergren, P. Falk, G. Larson, R. Lindstedt, and C. Svanborg Edén.** 1988. Globo-A: a new receptor specificity for attaching *Escherichia coli*. FEBS Lett. **237**: 123–127.
40. **Svanborg Eden, C., B. Eriksson, and L. A. Hanson.** 1977. Adhesion of *Escherichia coli* to human uroepithelial cells in vitro. Infect. Immun. **18**:767–774.
41. **Svanborg Edén, C., B. Eriksson, L. A. Hanson, U. Jodal, B. Kaijser, G. Lidin Janson, U. Lindberg, and S. Olling.** 1978. Adhesion to normal human uroepithelial cells of *Escherichia coli* from children with various forms of urinary tract infection. J. Pediatr. **93**:398–403.
42. **Truong, L. D., V. T. Phung, Y. Yoshikawa, and C. A. Mattioli.** 1988. Glycoconjugates in normal human kidney—a histochemical study using 13 biotinylated lectins. Histochemistry **90**: 51–60.
43. **Uhlin, B. E., M. Norgren, M. Båga, and S. Normark.** 1985. Adhesion to human cells by *Escherichia coli* lacking the major subunit of a digalactoside-specific pilus-adhesin. Proc. Natl. Acad. Sci. USA **83**:1800–1804.
44. **Väisänen-Rhen, V., M. Rhen, E. Linder, and T. K. Korhonen.** 1985. Adhesion of *Escherichia coli* to human kidney cryostat sections. FEMS Microbiol. Lett. **27**:179–182.
45. **Van Die, I., G. Spierings, I. van Megen, E. Zuidweg, W. Hoekstra, and H. Bergmans.** 1985. Cloning and genetic organization of the gene cluster encoding $F7_1$ fimbriae of a uropathogenic *Escherichia coli* and comparison with the $F7_2$ gene cluster. FEMS Microbiol. Lett. **28**:329–334.
46. **Virkola, R., B. Westerlund, H. Holthöfer, J. Parkkinen, M. Kekomäki, and T. K. Korhonen.** 1988. Binding characteristics of *Escherichia coli* adhesins in human urinary bladder. Infect. Immun. **56**:2615–2622.
47. **Wray, S. K., S. A. Hull, R. G. Cook, J. Barrish, and R. A. Hull.** 1986. Identification and characterization of a uroepithelial cell adhesin from a uropathogenic isolate of *Proteus mirabilis*. Infect. Immun. **54**:43–49.