INFECTION AND IMMUNITY, Dec. 1990, p. 4055–4062
0019-9567/90/124055-08$02.00/0

Vol. 58, No. 12

pap-2-Encoded Fimbriae Adhere to the P Blood Group-Related Glycosphingolipid Stage-Specific Embryonic Antigen 4 in the Human Kidney

JOAN F. KARR,[1] BOGDAN J. NOWICKI,[1] LUAN D. TRUONG,[2] RICHARD A. HULL,[1] JOHN J. MOULDS,[3] AND SHEILA I. HULL[1]*

Department of Microbiology and Immunology, Baylor College of Medicine,[1] and Department of Pathology, The Methodist Hospital,[2] Houston, Texas 77030, and Gamma Biologicals, Inc., Houston, Texas 77092[3]

Received 9 February 1990/Accepted 27 September 1990

A subtype of P fimbriae, encoded by the *pap-2* gene cluster, has been analyzed for agglutination of erythrocytes and for binding to cryostat sections of the human kidney. We have demonstrated that *pap-2*-encoded fimbriae are capable of binding to erythrocytes from some animal species and to human erythrocytes which express globoside and the LKE (stage-specific embryonic antigen 4 [SSEA-4]) antigen. The *pap-2* fimbriae bind to Bowman's capsule in the human kidney. Monoclonal antibodies directed against glycosphingolipids were used for the detection of specific P blood group-related antigens in the human kidney and on erythrocytes. Preincubation of kidney sections with monoclonal antibody MC813-70, which binds to the SSEA-4 antigen, inhibited adherence of purified *pap-2*-encoded fimbriae to Bowman's capsule. We suggest that one receptor for *pap-2*-encoded fimbriae is the antigen known as LKE (Luke) on human erythrocytes or SSEA-4 in the tissues.

The establishment of ascending urinary tract infections appears to require bacterial adhesion to tissue surfaces of the urinary tract. *Escherichia coli* is the organism which causes the majority of urinary tract infections. *E. coli* from the bowel may colonize the periurethral area (3, 4, 15) and from there establish colonization of the urinary tract through the urethra (12, 14, 35, 37, 38) to the bladder and kidney. Attachment of infecting uropathogenic strains to specific receptors may be mediated by fimbrial adhesins. The presence of different fimbrial types is correlated with urinary tract infections: type 1 is correlated with lower urinary tract infections (18, 22, 34), P fimbriae are correlated with pyelonephritis (21, 23, 24, 28), and Dr hemagglutinins are correlated with cystitis (36).

It was recently reported that 40% of *E. coli* strains isolated from children with pyelonephritis harbor two or three copies of DNA sequences homologous with *pap*, a cloned gene cluster encoding P fimbriae (39). The pyelonephritic isolate J96, from which *pap* was cloned, also carries a second copy of homologous sequences; this second copy of *pap* was cloned, and the binding of its fimbriae to sections of human kidney tissue was studied (27).

Results of indirect immunofluorescence studies revealed that both *pap*- and *pap-2*-encoded purified fimbriae bound to a number of structures in the tissues of the urinary tract but that only *pap-2*-encoded fimbriae adhered to Bowman's capsule in the kidney (27). Preincubation of the fimbriae with GalNacβ1→3Gal blocked the binding of *pap-2*-encoded fimbriae to Bowman's capsule, but Galα1→4Gal, the receptor for P fimbriae, had no inhibitory effect (2, 27).

Lindstedt et al. separated several glycosphingolipids by thin-layer chromatography and showed that the *pap-2* clone, P678-54(pJFK102), bound strongly to Forssman and globo-A antigens but only weakly to globoside and para-Forssman (29). These antigens are a few of the extended globoseries glycosphingolipids which possess an internal Galα1→4Gal disaccharide and could serve as receptors for P-fimbrial binding (2). However, Galα1→4Gal alone was a weak inhibitor of binding by the *pap-2* clone to separated glycolipids (29). Furthermore, Galα1→4Gal-coated latex beads were only weakly agglutinated by the *pap-2* clone (29). Taken together, these results indicate that the receptor for the *pap-2*-encoded fimbrial adhesin is more complex than the defined P-fimbrial Galα1→4Gal receptor (2, 24).

Normark and colleagues have also independently cloned the second copy of the *pap* homologous sequences from J96 and have named this cloned gene *prs* (30, 31). On the basis of agglutination of sheep erythrocytes and binding to isolated glycolipids by the *prs* clone, this group concluded that the Forssman antigen was the primary receptor for *prs*-encoded fimbriae (31). The Forssman antigen is the major neutral glycosphingolipid on the surface of sheep erythrocytes (47) but is relatively rare in human tissues; for example, it has been found in the kidneys of individuals of blood group A only (5). Perhaps another related antigen is the primary receptor in human kidney tissue.

To further define the exact receptor for *pap-2*-encoded (or *prs*-encoded) fimbriae, we have performed additional studies. After reviewing the published structures of P, Forssman, globo-A, and related antigens, we have chosen erythrocytes of defined P blood group phenotype for hemagglutination assays and various digalactosides as inhibitors of hemagglutination. We have also used monoclonal antibodies (MAbs) both to detect P-related antigens in sections of human kidney tissue and to inhibit the binding of purified fimbriae to the human kidney. The results of these studies suggest that a potential receptor for *pap-2*-encoded fimbriae in the human urinary tract is the antigen known as Luke on human erythrocytes and as stage-specific embryonic antigen 4 (SSEA-4) in tissues.

* Corresponding author.

TABLE 1. MAbs used in these experiments

MAb	Structure(s) recognized	Immunoglobulin class	Immunogen	References
9G7	Globoside (P)	Mouse IgM	Common acute lymphoblastic leukemia antigen	45
5A3	Galactosylgloboside	Mouse IgM	3-Fucosyllactosamine structure 1	32
MC631	SSEA-3 and SSEA-4	Rat IgM	4- to 8-cell-stage mouse embryos	25, 40, 26, 32, 43
MC813-70	SSEA-4 (LKE)	Mouse IgG3	Human teratocarcinoma cell line 2102 Ep	25, 43
A003	Blood group A	Mouse IgM	Human ovarian cyst A_2 substance phenol-insoluble cyst 14	9, 10, 13
M1/87	Forssman antigen	Rat IgM	B10 mouse spleen cells enriched for T cells	41, 42

MATERIALS AND METHODS

Bacteria. P678-54(pRHU845), also called HU849 (21), and P678-54(pJFK102) (27, 29; J. Karr, R. Hull, and S. Hull, Abstr. Annu. Meet. Am. Soc. Microbiol. 1988, D162, p. 98) are P-fimbriated *E. coli* clones which express the *pap*- and *pap-2*-encoded fimbriae, respectively, from the pyelonephritogenic isolate *E. coli* J96 (20).

Blood. Fresh human blood was obtained from normal volunteer donors or from Gamma Biologicals, Houston, Tex. Animal blood was obtained from Pel Freez, Clinical Systems Division, Brown Deer, Wis.

Human cryostat sections. Samples from a histopathologically normal portion of human kidney from a 66-year-old male (phenotype B, P_2) with renal carcinoma were snap frozen in liquid nitrogen immediately following surgical excision. Sections of 4 μm were cut and mounted on glass microscope slides and kept frozen at −70°C until use.

Antibodies. MAbs directed against specific glycolipids are described in Table 1. 9G7 (also called CLB-ery-2) and 5A3 were kindly provided by D. Marcus, Baylor College of Medicine, Houston, Tex. MC631 and MC813-70 were generously donated by D. Solter, the Wistar Institute for Anatomy and Biology, Philadelphia, Pa. Other antibodies were available commercially: M1/87 was from Hybritech Inc., San Diego, Calif., and A003 (40/5G7) was from Bio-Carb, Accurate Chemical and Scientific Corp., Westbury, N.Y. Tissue- or erythrocyte-bound MAbs were detected by the appropriate fluorescence-labeled secondary antibodies: tetramethylrhodamine isothiocyanate (TRITC)-labeled goat anti-rabbit immunoglobulin G (IgG), fluorescein isothiocyanate (FITC)-labeled goat anti-mouse IgG, and FITC-labeled goat anti-mouse IgM from E-Y Laboratories, Inc., San Mateo, Calif., and FITC-labeled goat anti-rat IgM from Cappel Research Reagents, Organon Teknika Corporation, West Chester, Pa.

Hemagglutination. Fresh human or animal erythrocytes were washed three times in phosphate-buffered saline (PBS), pH 7.1, or physiological saline and resuspended at 3% (vol/vol), with or without 1% α-D-methylmannopyranoside, in PBS or saline. Equal volumes of a bacterial suspension in saline (optical density at 600 nm of 1.0) and washed erythrocytes were mixed on a glass slide over ice, and the reactions were read by the unaided eye.

Inhibition of hemagglutination. Bacteria were resuspended at an optical density at 600 nm of 1.0 and diluted twofold to determine the endpoint which still completely agglutinated. Suspensions at a titer twofold higher than the endpoint were used in the hemagglutination inhibition assay. The digalactosides Galβ1→3GalNAc, GalNAcβ1→3Gal, and Galα1→4Gal were purchased from Sigma Chemical Co., St. Louis, Mo. These disaccharides were dissolved in PBS. Twenty microliters of 3% erythrocytes in PBS was mixed with 10 μl of dilutions of disaccharides in PBS on a glass slide over ice. Bacterial suspension (10 μl) was added to this mixture. Agglutination reactions were read with the unaided eye.

Purification of fimbriae. Fimbriae were purified as described previously (27, 46).

Fimbrial binding to tissues. Indirect immunofluorescence of fimbrial adherence to human kidney tissue sections was described previously (27, 37). Briefly, purified fimbriae bound to cryostat sections were detected by rabbit anti-fimbriae antibody followed by TRITC-labeled goat anti-rabbit antibody.

Binding of MAbs to human kidney tissues. MAbs bound to tissue sections were detected as follows. MAbs diluted 1:100 in Dulbecco's PBS (40 μl) were incubated for 45 min on tissues which had been fixed in 3.5% paraformaldehyde in PBS, washed, and incubated with normal goat sera to block nonspecific binding. The MAbs were blotted off the tissue sections, and the slides were then washed three times in PBS, followed by a 30-min incubation with 40 μl of FITC-labeled goat anti-rat or anti-mouse secondary antibody (1:40 in PBS, pH 7.1, with 1% bovine serum albumin). The goat antisera was removed by blotting, and the slides were washed three times. The tissue sections were covered by 70% glycerol in PBS (pH 7.0) and a glass coverslip and examined under a Leitz Dialux fluorescence microscope.

Inhibition of fimbrial adherence by MAbs. Tissue sections fixed, washed, and blocked by normal goat sera were incubated with 40 μl of diluted MAbs for 45 min, blotted, washed three times in PBS (pH 7.1), and then incubated for 45 min with 40 μl of 0.5 mg of purified fimbriae per ml with 1% bovine serum albumin–1% α-methyl-mannose–0.05% Tween-20 in PBS, pH 7.1. The sections were then blotted, washed, and incubated with 40 μl of 1:40 rabbit anti-fimbriae antisera and 1% bovine serum albumin in Dulbecco's PBS in combination with one of the following: 1:20 goat anti-mouse IgM–FITC, 1:20 goat anti-mouse IgG–FITC, or 1:20 goat anti-rat IgM–FITC. After 45 min, the tissues were blotted

TABLE 2. Bacterial agglutination of human type O blood with known P phenotypes

P blood phenotype	P antigens on erythrocytes	Agglutination of erythrocytes[a]		
		P678-54 (pRHU845) *pap*	P678-54 (pJFK102) *pap-2*	J96 *pap* *pap-2*
p	None[b]	−	−	−
P_2^k	P^K	+	−	+
P_1^k	P^K, P_1[b]	+	−	+
P_2	P^K, P^b	+	+	+
P_1	P^K, P, P_1	+	+	+
Luke negative	P^K, P, P_1[b]	+	−	+

[a] +, Positive reaction; −, no agglutination.
[b] Erythrocytes from two different donors were tested.

TABLE 3. Titers of hemagglutination by *pap* and *pap-2* fimbriated clones

Erythrocyte	Titer of hemagglutination by[a]:	
	P678-54(pRHU845) *pap*	P678-54(pJFK102) *pap-2*
Human A_2, P_1	64	8
Sheep	4	32
Dog	<2	8

[a] Titers are given as the reciprocal of the dilution of a bacterial suspension (optical density at 600 nm of 1.0) that caused complete hemagglutination of a 3% suspension of washed erythrocytes.

and washed three times, followed by an incubation of 45 min with goat anti-rabbit–TRITC, diluted 1:40 in PBS, pH 7.1. The slides were then washed three times, and the tissues were covered by glycerol–PBS.

Agglutination of erythrocytes by MAbs. Thirty microliters of washed erythrocytes suspended at 3% in saline were incubated with 5 μl of each MAb in a 1.5-ml microcentrifuge tube in ice for 30 min. The tubes were centrifuged for 10 s, and the supernatant was removed. The cells were washed three times with 100 μl of saline, and the pellets were resuspended in 25 μl of saline. The appropriate FITC-labeled secondary antibody (5 μl) was mixed with each sample and incubated on ice for 30 min, followed by one wash in 100 μl of saline. The erythrocytes were suspended in 10 μl of saline and pipetted onto a glass slide over ice for inspection of hemagglutination or onto on a glass microscope slide for examination of erythrocyte staining by fluorescence microscopy.

RESULTS

Agglutination of human and animal erythrocytes. Washed erythrocytes from individuals of the O blood group with known P phenotypes were suspended at a concentration of 3% in PBS and tested for agglutination by bacterial suspensions of P678-54(pRHU845) and P678-54(pJFK102). The *pap* fimbriated clone P678-54(pRHU845) was capable of agglutinating blood of all the P phenotypes tested except the rare p phenotype, which lacks all the P blood group antigens (Table 2). The *pap-2* fimbriated clone P678-54(pJFK102) was only capable of agglutinating the P_2 and P_1 phenotypes, both of which carry the blood antigen P (globoside). These results indicated that P678-54(pJFK102) required at least the presence of the longer globoseries structure found on P, while P678-54(pRHU845) bound to any of the Galα1→4Gal-containing P blood group antigens. Interestingly, the *pap-2* clone did not agglutinate Luke-negative blood, which is a rare phenotype in which the individual lacks a P blood group-related antigen known as LKE (8, 43, 44). It has been suggested that the LKE antigen may be synthesized by the addition of two sugars onto globoside; erythrocytes lacking globoside are also always Luke negative as well (43). The results of these hemagglutination experiments indicated that the human receptor for P678-54(pJFK102) might indeed be the LKE antigen. The clinical strain J96, which carries in its chromosome both *pap* and *pap-2*, agglutinated all the P phenotypes tested except p.

P678-54(pRHU845) and P678-54(pJFK102) were titered for agglutination of human (A_2, P_1), sheep, and dog erythrocytes (Table 3). P678-54(pRHU845) agglutinated the human erythrocytes strongly, sheep erythrocytes very poorly, and dog erythrocytes not at all. Quite different results were observed with P678-54(pJFK102), which agglutinated both human and dog erythrocytes, but not strongly. Agglutination of sheep erythrocytes by the *pap-2* clone was stronger than that of either human or dog erythrocytes. Since the LKE antigen has sialic acid as its terminal residue, erythrocytes were treated with neuraminidase, but this treatment had no effect upon hemagglutination (data not shown), indicating that sialic acid is not critical for binding by *pap-2*-encoded fimbriae.

Hemagglutination inhibition. The digalactosides Galβ1→3GalNAc, GalNAcβ1→3Gal, and Galα1→4Gal were tested to determine if any could inhibit hemagglutination. Each of these disaccharides is found in one or more of the glycosphingolipids shown in Table 6. The addition of Galα1→4Gal prevented the agglutination of human erythrocytes by both clones; however, it required four times as much Galα1→4Gal to inhibit agglutination by the *pap-2* clone than was necessary for the *pap* (Table 4). Galβ1→3GalNAc and GalNAcβ1→3Gal (25 mM each) had no noticeable effect on human erythrocyte agglutination by either clone (Table 4). Agglutination of sheep erythrocytes by the *pap-2* fimbriated clone was not affected by any of the three digalactosides under the conditions of this experiment.

Studies with MAbs. MAbs against the SSEA-3, SSEA-4 (LKE), blood group A, and Forssman antigens were incubated with human A_2, P_1, sheep, or dog erythrocytes both to check for the presence of these antigens, all extended globoseries glycosphingolipids, on the surface of the erythrocytes (Tables 1, 5, 6) and to serve as a control for the specificity of the MAbs prior to their use on sections of human kidney. The indirect immunofluorescence technique proved to be a sensitive method of detecting binding of the MAbs to the surface of the erythrocytes (Table 5). The MAbs against SSEA-3, SSEA-4, and the blood group A

TABLE 4. Bacterial agglutination of erythrocytes in the presence of digalactoside inhibitors[a]

Strain and gene cluster	Type of blood	Agglutination in saline control[b]	Concn at which agglutination was inhibited (mM)[c]		
			Galα1→4Gal	Galβ1→3GalNAc	GalNAcβ1→3Gal
P678-54(pJFK102) *pap-2*	Human A_2, P_1	+	0.900	>25	>25
	Sheep	+	>25	>25	>25
P678-54(pRHU845) *pap*	Human A_2, P_1	+	0.225	>25	>25
	Sheep	±	ND	ND	ND

[a] The reactions were read without the aid of a microscope.
[b] +, Positive reaction; ±, weak reaction.
[c] ND, Hemagglutination of sheep erythrocytes by the *pap* clone was so weak that hemagglutination inhibition could not be unequivocally determined.

TABLE 5. Indirect immunofluorescence of MAbs bound to erythrocytes

Erythrocyte	Binding of MAb				
	Saline control	MC631 (SSEA-3)	MC813-70 (SSEA-4)	A003 (A)	M1/87 (Forssman)
Human type A_2, P_1	−	+[a]	+[b]	+[a]	−
Sheep	−	−	−	−	+[a]
Dog	−	−	−	−	−

[a] These erythrocytes were visibly agglutinated by the undiluted MAb in the absence of a secondary antibody.
[b] Weak fluorescence.

antigen all bound to human erythrocytes but not to those of either sheep or dogs. The anti-Forssman MAb bound to sheep erythrocytes, as expected, but not to either human or dog erythrocytes. Three of the MAbs, all IgM molecules, agglutinated the erythrocytes of the same species to which each bound (Table 5). Unfortunately, this agglutination prevented their use in experiments to inhibit hemagglutination by the fimbriated bacteria.

Indirect immunofluorescence was then used to detect antibody binding to frozen tissue sections (Table 7). MC631 bound to renal tubules and the uroepithelium of the renal pelvis. MC813-70 bound to glomerular cells, to Bowman's capsule, to some tubules, and to the renal pelvis uroepithelium. Purified *pap-2*-encoded fimbriae have been shown to bind to these same structures (27). MAbs 9G7 and 5A3 appeared to bind weakly to some renal tubules, but this binding might have been nonspecific because of the secondary goat anti-mouse IgM antibody which bound to the nuclei of tubules and other structures. M1/87 did not bind to the glomerulus, Bowman's capsule, or tubules of this patient's renal tissue, but M1/87 and A003 did bind focally to the uroepithelium of the renal pelvis.

We attempted to determine if any of the MAbs could inhibit fimbrial binding to the kidney (Fig. 1). MAbs 9G7, 5A3, A003, and M1/87 did not block fimbrial binding. Preincubation of MC813-70 (anti-SSEA-4) with kidney sections blocked binding of *pap-2*-encoded fimbriae to Bowman's capsule and to the tubules. MC631 (anti-SSEA-3 and anti-SSEA-4) appeared to partially block binding of the fimbriae to Bowman's capsule and to the tubules.

DISCUSSION

The receptor specificity of the adhesin encoded by P678-54(pJFK102) was studied by several different techniques. In a hemagglutination assay, the recombinant strain was capable of agglutinating human, sheep, and dog erythrocytes (Table 3). Despite the fact that globoside is the predominant glycosphingolipid found on both human and dog erythrocytes (47), the *pap* fimbriated clone P678-54(pRHU845) strongly agglutinated human but not dog erythrocytes. As the *pap-2* fimbriated clone agglutinated dog and human erythrocytes equally, it appears that *pap*- and *pap-2*-encoded fimbriae may not recognize the same epitope on human erythrocytes. The *pap*-encoded fimbriae agglutinated human A_2, P_1 blood better than they did sheep blood, while the reverse was true for *pap-2*-encoded fimbriae. The differences in binding might be explained by a difference in the density of appropriate receptors on the surface of these erythrocytes or by a difference in affinities between the fimbrial adhesins and their respective receptors.

To further examine the specificity of *pap-2*-type binding, we reviewed the published structures of globo-A, Forssman, para-Forssman, SSEA-4 (LKE), SSEA-3 (galactosylgloboside), and other related structures and selected three digalactosides in hemagglutination studies (Table 4). Curiously, only Galα1→4Gal prevented hemagglutination by the *pap-2* clone, even though only GalNAcβ1→3Gal blocked the binding of purified fimbriae to Bowman's capsules in human kidneys (27). This may indicate that *pap-2*-encoded fimbriae have the capacity to recognize more than one epitope or that the presentation of the epitope differs between erythrocytes and epithelial cells.

Previous studies indicated that the clone P678-54 (pJFK102) bound to the glycosphingolipids globo-A and Forssman separated on thin-layer chromatograms (29). Unfortunately, LKE, another P-related antigen, was not included in these experiments. On the basis of our results with the hemagglutination assays and of earlier studies of binding to tissues and chromatograms, we pursued the possibility that several of these structures might serve as receptors for *pap-2*-encoded adhesin recognition.

The Forssman antigen is present in the tissues and/or erythrocytes of many species (47) but has not been detected on human erythrocytes; however, a few humans are Forss-

TABLE 6. Glycosphingolipids from the human P blood group and related globoseries structures

Antigen	Structure[a]
P_1	Galα1→4Galβ1→4GlcNAcβ1→3Galβ1→4Glcβ1→1Cer
P^k (Globotriaosylceramide)	* Galα1→4Galβ1→4Glcβ1→1Cer
P (Globotetraosylceramide, Globoside)	* GalNAcβ1→3Galα1→4Galβ1→4Glcβ1→1Cer
Galactosylgloboside (SSEA-3)	* Galβ1→3GalNAcβ1→3Galα1→4Galβ1→4Glcβ1→1Cer
LKE (SSEA-4)	* NeuAcα2→3Galβ1→3GalNAcβ1→3Galα1→4Galβ1→4Glcβ1→1Cer
Forssman	* GalNAcα1→3GalNAcβ1→3Galα1→4Galβ1→4Glcβ1→1Cer
Para-Forssman	* GalNAcβ1→3GalNAcβ1→3Galα1→4Galβ1→4Glcβ1→1Cer
Globo A (A antigen, type 4 chain)	* GalNAcα1→3Gal(2←1αFuc)β1→3GalNAcβ1→3Galα1→4Galβ1→4Glcβ1→1Cer *

[a] Structure to the left of the asterisks is unique structure and to the right of asterisks is common structure.

TABLE 7. Indirect immunofluorescence of MAbs bound to cryostat sections of human urinary tissues

Tissue in kidney in which antigen was recognized	Binding of MAb[a]					
	9G7 (globoside)	5A3 (galactosylgloboside)	MC631 (SSEA-3, SSEA-4)	MC813-70 (SSEA-4)	A003 (A)	M1/87 (Forssman)
Glomerulus	−	−	±	+	−	−
Bowman's capsule	−	−	±	+	−	−
Tubules	±	±	+	+	−	−
Pelvis uroepithelium	NT	NT	+	+	+	±

[a] 9G7, 5A3, and A003 are all mouse IgM. The secondary antibody, goat anti-mouse IgM, bound to the nuclei of tubules and other structures, interfering with the interpretation of these results. Therefore, only negative results for 9G7, 5A3, and A003 could be interpreted with certainty. ± indicates a focal or weak stain. NT, Binding could not be determined.

man positive, and Forssman has been detected in the normal gastric mucosae of these individuals and in the kidneys of blood group A individuals (5, 19). Minimal amounts of para-Forssman (Table 6) have been detected on human erythrocytes, but the para-Forssman and Forssman antigens are not cross-reactive by antisera, even though they only differ by the α or β linkage of the terminal N-acetylgalactosamine (1, 5) (Table 6). It has been proposed that Forssman is the receptor for the type of P fimbriae called Prs (30, 31), which are likely to be identical to the fimbriae encoded by *pap-2* (27, 29; Karr, et al., Abstr. Annu. Meet. Am. Soc. Microbiol. 1988). This is a tempting proposal, as both *prs* and *pap-2* mediate the agglutination of sheep erythrocytes. However, on the basis of the facts that human erythrocytes are agglutinated by the *pap-2*-encoded fimbriae and that Forssman was not detected on human or dog erythrocytes or on Bowman's capsule in human kidney tissue, it appears unlikely that Forssman is the primary receptor for *pap-2*-encoded fimbriae in humans. A MAb to Forssman bound to and agglutinated sheep erythrocytes and bound weakly to kidney pelvic uroepithelium but did not bind to Bowman's capsule in the kidney or prevent the binding to human kidney of fimbriae purified from the *pap-2* clone. This suggests that the binding domain for the *pap-2*-encoded adhesin does not overlap with the epitope recognized by the MAb.

Globo-A is an A blood group antigen with a type 4 chain, which makes this A antigen a globoseries glycolipid (Table 6). The globo-A antigen has been detected in the kidneys of blood group A individuals (7) and as a minor component of human erythrocytes of the A_1 phenotype but not on erythrocytes from individuals of the A_2 phenotype (11). Globo-A has also been identified in the human ureteral transitional epithelium (6). Since P678-54(pJFK102) binds to globo-A on thin-layer chromatography, globo-A might be another possible receptor. However, the *pap-2* recombinant strain also agglutinates human erythrocytes of the O and A_2 phenotypes (Tables 2 and 3), neither of which expresses globo-A antigen, so globo-A or any other A antigen cannot be the sole receptor for the *pap-2*-encoded adhesin. Furthermore, as was seen with an anti-Forssman MAb, an anti-A MAb failed to exhibit the same binding pattern to human kidney as the purified *pap-2*-type fimbriae and failed to prevent the *pap-2*-type fimbriae from binding to Bowman's capsule (Table 7).

Other structures that could possibly be receptors for this adhesin include SSEA-3 (galactosylgloboside) and globoside. SSEA-3 has been detected on umbilical vein endothelial cells and smooth muscle cells (17), human teratocarcinoma cell lines and A, B, or O erythrocytes (40), and in the distal tubules and collecting ducts of the adult mouse kidney (16). One glycolipid isolated from human ureteral transitional cells is probably SSEA-3 (6). Globoside has been found in extremely high concentrations on human erythrocytes (45, 47). Globoside has also been detected in the cells within the glomerulus and in proximal convoluted tubules of the human kidney (33) and on human umbilical vein smooth muscle cells and endothelial cells (17). P678-54(pJFK102) agglutinated all the human erythrocytes containing globoside; however, MAbs to galactosylgloboside and to globoside failed to bind to Bowman's capsule in sections of human kidney, unlike purified fimbriae prepared from P678-54(pJFK102) (Tables 2 and 7).

Tippett et al. (43) proposed that the antigen present on most human erythrocytes detected by Luke serum (44) and by the MAb MC813-70 (25) should be called LKE. MC813-70, raised against a human teratocarcinoma cell line, identifies SSEA-4 on the surface of this cell line (Table 1). The SSEA-4 ganglioside and LKE are thought to be the same structure (43), but this has not been proven. MC813-70, in combination with another MAb known as MC631, confirms the presence of SSEA-4 on cell surfaces, while MC631 alone indicates the presence of SSEA-3, SSEA-4, or possibly globoside (25, 26). SSEA-4 has been detected on human teratocarcinoma cells and erythrocytes (25, 43) and on human umbilical vein endothelial cells and smooth muscle cells (17). The primary structure of LKE (SSEA-4) is that of globoside with an attached terminal NeuAcα2→3Galβ1→3 (Table 6). The results of hemagglutination (Table 2) suggest LKE as a potential receptor recognized on human erythrocytes. Furthermore, the MAb to SSEA-4, MC813-70, bound to the same structures in the human kidney as the fimbriae purified from P678-54(pJFK102) did, including Bowman's capsule (Table 7) (27), and also prevented the binding of the purified fimbriae to human kidney (Fig. 1).

In conclusion, the receptor epitope recognized by *pap-2*-encoded fimbriae appears to be complex, possibly including the long internal structure Galβ1→3GalNAcβ1→3Gal α1→4Gal of SSEA-4 (LKE). We have identified SSEA-4 as a likely receptor on Bowman's capsule in the human kidney and on human erythrocytes for the *pap-2*-encoded fimbrial adhesin. *pap-* and *pap-2*-encoded fimbriae, both shown to bind to bladder and renal pelvis uroepithelia and to smooth muscles of the bladder wall (27), may bind to distinct but related receptors (for example, P1 versus SSEA-4) located within the same morphological structures. Alternatively, the *pap-2*-encoded fimbriae might have the unique capacity to bind to SSEA-4 and also to some other receptors also recognized by the *pap*-encoded fimbriae. We are investigating the possibility that the *pap-2*-type fimbriae may have two adhesins, as previously suggested (29). The presence of two adhesins could explain why only the fimbriae encoded by *pap-2* bind to Bowman's capsule

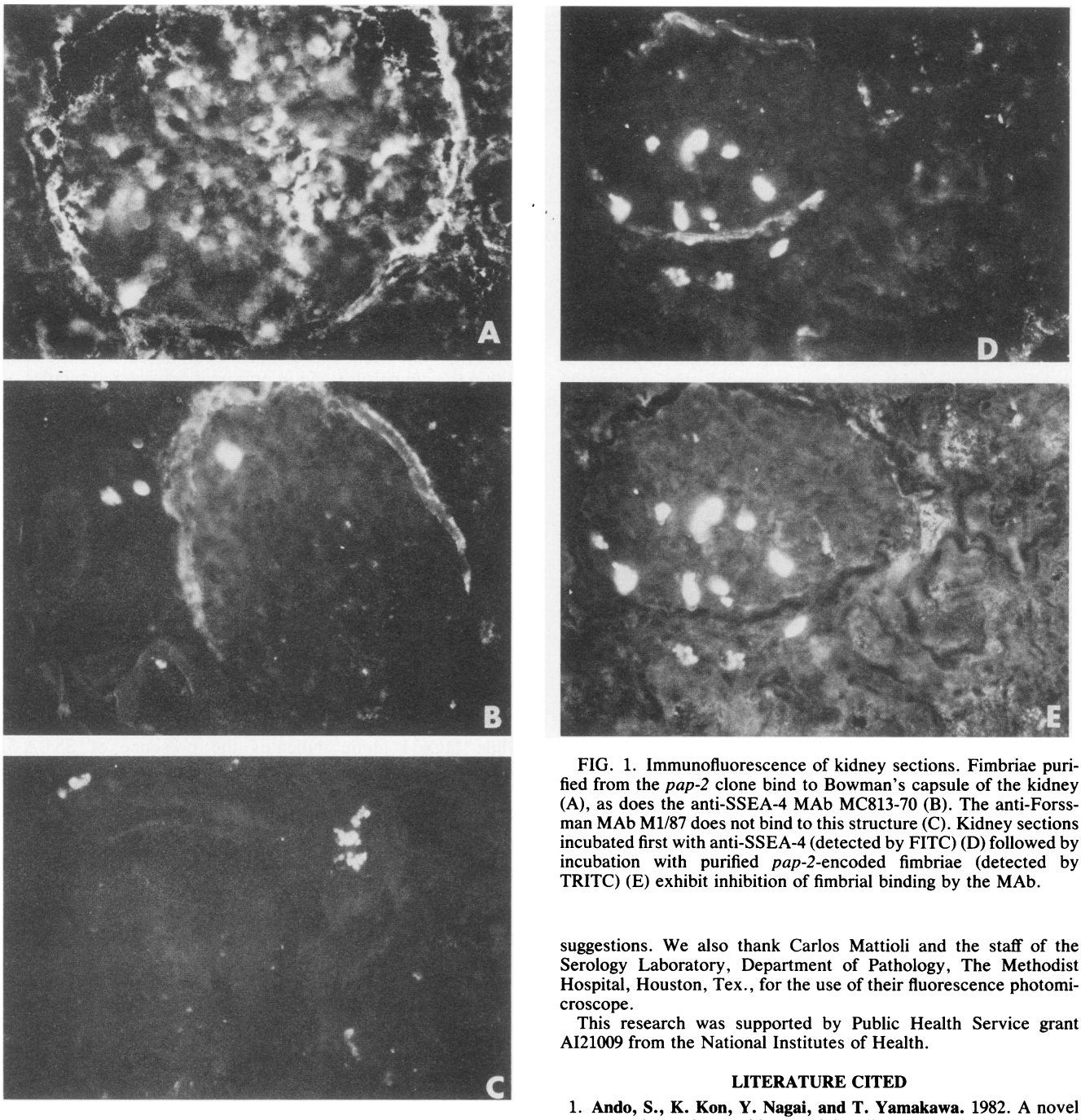

FIG. 1. Immunofluorescence of kidney sections. Fimbriae purified from the *pap-2* clone bind to Bowman's capsule of the kidney (A), as does the anti-SSEA-4 MAb MC813-70 (B). The anti-Forssman MAb M1/87 does not bind to this structure (C). Kidney sections incubated first with anti-SSEA-4 (detected by FITC) (D) followed by incubation with purified *pap-2*-encoded fimbriae (detected by TRITC) (E) exhibit inhibition of fimbrial binding by the MAb.

and why Galα1→4Gal inhibits hemagglutination but not binding to Bowman's capsule, while GalNAcβ1→3Gal blocks binding to Bowman's capsule but not hemagglutination.

ACKNOWLEDGMENTS

We thank Don Marcus and Baiba Gillard, Departments of Medicine and Microbiology and Immunology, Baylor College of Medicine, Houston, Tex., and Davor Solter, The Wistar Institute for Anatomy and Biology, Philadelphia, Pa., for the donation of some of the MAbs used in these experiments and for their helpful advice and suggestions. We also thank Carlos Mattioli and the staff of the Serology Laboratory, Department of Pathology, The Methodist Hospital, Houston, Tex., for the use of their fluorescence photomicroscope.

This research was supported by Public Health Service grant AI21009 from the National Institutes of Health.

LITERATURE CITED

1. **Ando, S., K. Kon, Y. Nagai, and T. Yamakawa.** 1982. A novel pentaglycosyl ceramide containing di-β-N-acetylgalactosaminyl residue (para-Forssman glycolipid) isolated from human erythrocyte membrane. Adv. Exp. Med. Biol. **152**:71–81.
2. **Bock, K., M. E. Breimer, A. Brignole, G. C. Hansson, K.-A. Karlsson, G. Larson, H. Leffler, B. E. Samuelsson, N. Strömberg, C. Svanborg Edén, and J. Thurin.** 1985. Specificity of binding of a strain of uropathogenic *Escherichia coli* to Galα1→4Gal-containing glycosphingolipids. J. Biol. Chem. **260**: 8545–8551.
3. **Bollgren, I., and J. Winberg.** 1976. The periurethral aerobic bacterial flora in healthy boys and girls. Acta Paediatr. Scand. **65**:74–80.
4. **Bollgren, I., and J. Winberg.** 1976. The periurethral aerobic flora in girls highly susceptible to urinary infections. Acta Paediatr. Scand. **65**:81–87.
5. **Breimer, M. E.** 1985. Chemical and immunological identification

of the Forssman pentaglycosylceramide in human kidney. Glycoconjugate J. 2:375–385.
6. **Breimer, M. E., G. C. Hansson, and H. Leffler.** 1985. The specific glycosphingolipid composition of human ureteral epithelial cells. J. Biochem. **98**:1169–1180.
7. **Breimer, M. E., and P.-A. Jovall.** 1985. Structural characterization of a blood group A heptaglycosylceramide with globo-series structure. FEBS Lett. **179**:165–172.
8. **Bruce, M., A. Watt, G. S. Gabra, R. Mitchell, D. Lakhesar, and P. Tippett.** 1988. LKE red cell antigen and its relationship to P_1 and P^k: serological study of a large family. Vox Sang. **55**:237–240.
9. **Chen, H.-T., and E. A. Kabat.** 1985. Immunochemical studies on blood groups: the combining site specificities of mouse monoclonal hyridoma anti-A and anti-B. J. Biol. Chem. **260**: 13208–13217.
10. **Chen, H.-T., E. A. Kabat, A. Lundblad, and R. M. Ratcliffe.** 1987. Nucleotide and translated amino acid sequences of cDNA coding for the variable regions of the light and heavy chains of mouse hybridoma antibodies to blood group A and B substances. J. Biol. Chem. **262**:13579–13583.
11. **Clausen, H., K. Watanabe, R. Kannagi, S. B. Levery, E. Nuedelman, Y. Arao-Tomono, and S. Hakomori.** 1984. Blood group A glycolipid (A^x) with globo-series structure which is specific for blood group A_1 erythrocytes: one of the chemical bases for A_1 and A_2 distinction. Biochem. Biophys. Res. Commun. **124**:523–529.
12. **Cox, C. E.** 1966. The urethra and its relationship to urinary tract infection: the flora of the normal female urethra. Sout. Med. J. **59**:621–626.
13. **Dakour, A., A. Lundblad, and D. Zopf.** 1987. Separation of blood group A-active oligosaccharides by high-pressure liquid affinity chromatography using a monoclonal antibody bound to concanavalin A silica. Anal. Biochem. **161**:140–143.
14. **Fihn, S. D., C. Johnson, and W. E. Stamm.** 1988. *Escherichia coli* urethritis in women with symptoms of acute urinary tract infection. J. Infect. Dis. **157**:196–199.
15. **Fowler, J. E., Jr., and T. A. Stamey.** 1977. Studies of introital colonization in women with recurrent urinary infections. VII. The role of bacterial adherence. J. Urol. **117**:472–476.
16. **Fox, N., L. Shevinsky, B. B. Knowles, D. Solter, and I. Damjanov.** 1982. Distribution of murine stage-specific embryonic antigens in the kidneys of three rodent species. Exp. Cell Res. **140**:331–339.
17. **Gillard, B. K., M. A. Jones, and D. M. Marcus.** 1987. Glycosphingolipids of human umbilical vein endothelial cells and smooth muscle cells. Arch. Biochem. Biophys. **256**:435–445.
18. **Hagberg, L., R. Hull, S. Hull, S. Falkow, R. Freter, and C. Svanborg Edén.** 1983. Contribution of adhesion to bacterial persistence in the mouse urinary tract. Infect. Immun. **40**:265–272.
19. **Hakomori, S., S.-M. Wang, and W. W. Young, Jr.** 1977. Isoantigenic expression of Forssman glycolipid in human gastric and colonic mucosa: its possible identity with "A-like antigen" in human cancer. Proc. Natl. Acad. Sci. USA **74**:3023–3027.
20. **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.
21. **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.
22. **Iwahi, T., Y. Abe, M. Nakao, A. Imada, and K. Tsuchiya.** 1983. Role of type 1 fimbriae in the pathogenesis of ascending urinary tract infection induced by *Escherichia coli* in mice. Infect. Immun. **39**:1307–1315.
23. **Källenius, G., R. Möllby, S. B. Svenson, I. Helin, H. Hultberg, B. Cedergren, and J. Winberg.** 1981. Occurrence of P-fimbriated *Escherichia coli* in urinary tract infections. Lancet **ii**:1369–1372.
24. **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.
25. **Kannagi, R., N. A. Cochran, F. Ishigami, S. Hakomori, P. W. Andrews, B. B. Knowles, and D. Solter.** 1983. Stage-specific embryonic antigens (SSEA-3 and -4) are epitopes of a unique globo-series ganglioside isolated from human teratocarcinoma cells. EMBO J. **2**:2355–2361.
26. **Kannagi, R., S. B. Levery, F. Ishigami, S. Hakomori, L. H. Shevinsky, B. B. Knowles, and D. Solter.** 1983. New globoseries glycosphingolipids in human teratocarcinoma reactive with the monoclonal antibody directed to a developmentally regulated antigen, stage-specific embryonic antigen 3. J. Biol. Chem. **258**:8934–8942.
27. **Karr, J. F., B. Nowicki, L. D. Truong, R. A. Hull, and S. I. Hull.** 1989. Purified P fimbriae from two cloned gene clusters of a single pyelonephritogenic strain adhere to unique structures in the human kidney. Infect. Immun. **57**:3594–3600.
28. **Korhonen, T. K., M. V. Valtonen, J. Parkkinen, V. Väisänen-Rhen, J. Finne, F. Ørskov, I. Ørskov, S. B. Svenson, and P. H. Mäkelä.** 1985. Serotypes, hemolysin production, and receptor recognition of *Escherichia coli* strains associated with neonatal sepsis and meningitis. Infect. Immun. **48**:486–491.
29. **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.
30. **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.
31. **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.
32. **Marcus, D. M., S. Gilbert, M. Sekine, and A. Suzuki.** 1988. Monoclonal antibodies that bind to galactosylgloboside (SSEA-3 antigen). Arch. Biochem. Biophys. **262**:620–625.
33. **Marcus, D. M., and R. Janis.** 1970. Localization of glycosphingolipids in human tissues by immunofluorescence. J. Immunol. **104**:1530–1539.
34. **Marre, R., and J. Hacker.** 1987. Role of S- and common-type 1-fimbriae of *Escherichia coli* in experimental upper and lower urinary tract infection. Microb. Pathog. **2**:223–226.
35. **Marrie, T. J., C. A. Swantee, and M. Hartlen.** 1980. Aerobic and anaerobic urethral flora of healthy females in various physiological age groups and of females with urinary tract infections. J. Clin. Microbiol. **11**:654–659.
36. **Nowicki, B., C. Svanborg-Edén, R. Hull, and S. Hull.** 1989. Molecular analysis and epidemiology of the Dr hemagglutinin of uropathogenic *Escherichia coli*. Infect. Immun. **57**:446–451.
37. **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.
38. **O'Grady, F. W., B. Richards, M. A. McSherry, S. M. O'Farrell, and W. R. Cattell.** 1970. Introital enterobacteria, urinary infection, and the urethral syndrome. Lancet **ii**:1208–1210.
39. **Plos, K., T. Carter, S. Hull, R. Hull, and C. Svanborg-Eden.** 1990. Frequency and organization of *pap* homologous DNA in relation to clinical origin of uropathogenic *Escherichia coli*. J. Infect. Dis. **161**:518–524.
40. **Shevinsky, L. H., B. B. Knowles, I. Damjanov, and D. Solter.** 1982. Monoclonal antibody to murine embryos defines a stage-specific embryonic antigen expressed on mouse embryos and human teratocarcinoma cells. Cell **30**:697–705.
41. **Springer, T., G. Galfré, D. S. Secher, and C. Milstein.** 1978. Monoclonal xenogeneic antibodies to murine cell surface antigens: identification of novel leukocyte differentiation antigens. Eur. J. Immunol. **8**:539–551.
42. **Springer, T. A.** 1980. Characterization of "jumping" and "lineage" antigens using xenogeneic rat monoclonal antibodies, p. 197–198. *In* R. H. Kennett, T. J. McKearn, and K. B. Bechtol (ed.), Monoclonal antibodies—hybridomas: a new dimension in biological analysis. Plenum Publishing Corp., New York.

43. **Tippett, P., P. W. Andrews, B. B. Knowles, D. Solter, and P. N. Goodfellow.** 1986. Red cell antigens P (globoside) and Luke: identification by monoclonal antibodies defining the murine stage-specific embryonic antigens -3 and -4 (SSEA-3 and SSEA-4). Vox Sang. **51**:53–56.
44. **Tippett, P., R. Sanger, R. R. Race, J. Swanson, and S. Busch.** 1965. An agglutinin associated with the P and the ABO blood group systems. Vox Sang. **10**:269–280.
45. **Von dem Borne, A. E. G., M. J. E. Bos, N. Joustra-Maas, J. F. Tromp, R. Van Wijngaarden-du Bois, and P. A. T. Tetteroo.** 1986. A murine monoclonal IgM antibody specific for blood group P antigen (globoside). Br. J. Haematol. **63**:35–46.
46. **Wray, S. K., S. I. 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.
47. **Yamakawa, T.** 1983. Glycolipids of the red blood cells, p. 37–41. *In* N. S. Agar and P. G. Board (ed.), Red blood cells of domestic mammals. Elsevier Science Publishing, Inc., New York.