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Roles of the *pap*- and *prs*-Encoded Adhesins in *Escherichia coli* Adherence to Human Uroepithelial Cells

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In this study, we reexamined the structural prerequisites for the attachment of P-fimbriated *Escherichia coli* to human urinary tract epithelial cells. The epithelial cells were obtained from A_1P_1 nonsecretor individuals, who express the globoseries of glycolipids without the ABH blood group determinants, and from A_1P_1 secretor individuals, who in addition express globo-A, a receptor for the prs_{J96} adhesin. The wild-type *E. coli* strains J96, AD110, and IA2 and the recombinant clones HB101 pap_{J96}, HB101 prs_{J96}, HB101 pap_{IA2}, and HB101 pap_{AD110} were tested for binding. They expressed P fimbriae, as defined by P blood group-dependent agglutination of human erythrocytes of the globoseries, but differed in reactivity with galactoseα1-4galactoseβ (Galα1-4Galβ)–latex beads, isolated glycolipids of the globoseries, sheep erythrocytes, and uroepithelial cells. Three different patterns of binding were represented among the recombinant clones. HB101 pap_{IA2} and HB101 pap_{AD110} agglutinated sheep erythrocytes and Galα1-4Galβ–latex beads and attached to both secretor and nonsecretor epithelial cells. HB101 prs_{J96} agglutinated sheep erythrocytes, reacted poorly with Galα1-4Galβ–latex beads, and attached to A_1 secretor but not to A_1 nonsecretor epithelial cells. HB101 pap_{J96} agglutinated Galα1-4Galβ–latex beads but not sheep erythrocytes and attached poorly to human uroepithelial cells. The receptors relevant for adhesion were analyzed by inhibition with glycolipids in suspension. The sheep erythrocyte agglutination and attachment to secretor and nonsecretor epithelial cells of HB101 pap_{IA2} and HB101 pap_{AD110} were inhibited by globotetraosylceramide, while the Forssman glycolipid had no effect. The sheep erythrocyte reactivity and attachment to secretor epithelial cells of HB101 prs_{J96} were inhibited by the Forssman glycolipid. These results permitted three conclusions. First, the expression of functionally active Galα1-4Galβ-specific adhesins, as in HB101 pap_{J96}, was not sufficient to make *E. coli* competent to attach to human uroepithelial cells. Attachment required P fimbriae of the pap_{IA2} or pap_{AD110} type. Second, the sheep erythrocyte reactivity of P-fimbriated strains could not be attributed solely to recognition of the Forssman glycolipid and may not be used to define the prs_{J96}-encoded phenotype. Third, the P-fimbrial adhesins which mediate secretor state-independent attachment to human uroepithelial cells recognized receptor epitopes provided by globotetraosylceramide.

Fimbriae on the surface of *Escherichia coli* (4, 7, 11) recognize glycoconjugate receptors on epithelial cells and erythrocytes (16). The P fimbriae-associated lectins bind to the globoseries of glycolipids (2, 13, 14), which provide a mosaic of receptor epitopes (14–19, 21, 24, 25). The adhesins encoded by the pap_{J96} DNA sequences recognize the galactoseα1-4galactoseβ (Galα1-4Galβ) disaccharide common to these glycolipids (12, 17, 22). The adhesins encoded by the prs_{J96} (*pap*-related sequence) DNA sequences require for binding a terminal GalNAcα linked to a globoseries core (18, 19, 21, 24, 25). The adhesins encoded by pap_{IA2} and pap_{AD110} differ from the previous two categories; their exact receptor requirement has not been defined (25). Among the naturally occurring glycolipids, the *pap* adhesins bind with high affinity to globotetraosylceramide, while the *prs* adhesins prefer the globo-A or Forssman glycolipid (18, 19, 21, 24, 25).

The receptor specificities of the P fimbriae have mainly been characterized by using particles coupled to purified glycolipids or glycolipids separated on thin-layer chromatogram plates (2, 6, 9, 18, 21, 28). It has been assumed that cell surface expression of these receptor-active molecules on epithelial cells is sufficient for attachment to occur. Early studies noted, however, considerable variation among P-fimbriated *E. coli* strains in their capacities to bind to a given cell type, such as sheep erythrocytes (15). The divergence in receptor specificities of the *prs*-encoded adhesins was previously discussed as a mechanism of adaptation of human strains for the infection of other species (25), since the *prs* adhesin attached to transformed cell lines of canine origin but not to those of human origin (10, 25). More recently, it was demonstrated that individuals of blood group A expressed functional receptors for the *prs*-encoded adhesin and became infected by such strains (19). These studies emphasized the importance of selecting the relevant epithelial target cell in order to understand the relative contribution of different P-fimbrial adhesins to mucosal attachment and infection.

The aim of the present study was to reexamine the contribution of different P fimbriae to the adherence of *E. coli* to human uroepithelial cells.

MATERIALS AND METHODS

Bacteria. The *E. coli* strains and recombinant clones used in this study are listed in Table 1. They were selected to represent the most extensively characterized P-fimbrial variants. The wild-type strains *E. coli* J96, *E. coli* IA2, and *E. coli* AD110 were isolated from patients with urinary tract infections (5, 12, 29). The plasmids pRHu845 and pPAP5 carried the pap_{J96} DNA sequences in the *Hin*dIII and *Eco*RI sites, respectively, of pBR322 (12, 17). The plasmids pJFK102 and pPAP601 carried the prs_{J96} sequences in the

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TABLE 1. *E. coli* wild-type and recombinant strains used in the present study[a]

E. coli strain	Abbreviation	Genotype:		P blood group-dependent hemagglutination			Source or reference
		pap	*prs*	A_1P_1	A_{1p}	OP_1	
Wild type							
J96[b]	J96	+	+	MR	−	MR	12
AD110[c]	AD110	+	−	MR	−	MR	29
IA2[c]	IA2	+	−	MR	−	MR	5
HB101[b]	HB101	−	−	−	−	−	
Transformant(s) in HB101							
HB101/pBr322[b]		−	−		−	−	
HB101/pRHu845[b]	HB101 pap_{J96}	+	−	MR	−	MR	12
HB101/pPAP5[c]	HB101 pap_{J96}	+	−	MR	−	MR	17
HB101/pDC1[c]	HB101 pap_{IA2}	+	−	MR	−	MR	5, 25
HB101/pPIL-11035[c]	HB101 pap_{AD110}	+	−	MR	−	MR	25, 29
HB101/pSN60[c]	HB101 prs_{J96}	−	+	MR	−	+[d]	21
HB101/pJFK102[b]	HB101 prs_{J96}	−	+	MR	−	+[d]	18
HB101/pRHu845[b] pJFK102	HB101 pap_{J96} + prs_{J96}	+	+	MR	−	MR	This study

[a] +, positive reactivity; MR, mannose resistant; −, negative reactivity.
[b] Kindly provided by S. and R. Hull, Baylor College of Medicine, Houston, Tex.
[c] Kindly provided by S. Normark, Washington University, St. Louis, Mo.
[d] Weakly positive reactivity.

*Bam*HI sites of pAcYc184 and pBR322, respectively (18, 22). The plasmid pPIL110-35 carried a 16-kb *Eco*RI insert from *E. coli* AD110 encoding adhesins and fimbriae of the $F7_2$ serotype (29) in the *Eco*RI site of pAcYc184. The plasmid pDC1 carried a 12.3-kb *Bam*HI fragment from *E. coli* IA2 encoding adhesins and fimbriae of the F11 antigen type in the *Bam*HI site of pAcYc184 (5). The Hu1222 derivative of *E. coli* HB101 was obtained by using transformation with the two plasmids pRHu845 and pJFK102.

Culture conditions. The wild-type *E. coli* strains were maintained on tryptic soy agar without glucose. The transformants in HB101 were maintained on tryptic soy agar supplemented with tetracycline (10 μg/ml), ampicillin (100 μg/ml), or chloramphenicol (20 μg/ml). For adherence testing, the bacteria were cultured on the same media, harvested in phosphate-buffered saline (PBS) (pH 7.2, 0.9 mM), and diluted to a concentration of 10^9 cells per ml in PBS with or without 2.5% (wt/vol) α-methyl-D-mannoside.

Hemagglutination. The ability of bacteria to agglutinate erythrocytes was tested as previously described (6, 8, 9). Briefly, freshly drawn, heparinized blood samples from human donors of blood groups AP_1, OP_1, and Ap or from sheep were washed in PBS and resuspended to 3% (vol/vol) in PBS with 2.5% α-methyl-D-mannoside. Hemagglutination was performed on microscope slides at room temperature and at 4°C by mixing equal volumes (20 μl) of the bacterial and erythrocyte suspensions. The reaction was read by the naked eye after the mixture was tilted for about 60 s and graded as + or −. The agglutination of Galα1-4Galβ–latex beads (Orion, Espoo, Finland) was performed according to the manufacturer's instructions by using the same bacterial suspensions (6). Bacteria were designated Galα1-4Galβ positive or negative.

Attachment to uroepithelial cells. Attachment to human uroepithelial cells was tested as previously described (27). Briefly, human uroepithelial cells were collected from AP_1 nonsecretor and AP_1 secretor individuals. Cells were harvested from freshly voided morning urine by using centrifugation, washed, and suspended in PBS with or without α-methyl-D-mannoside. The epithelial cells (10^5/ml) were incubated with the bacterial suspensions (10^9/ml) for 45 min at 37°C with end-over-end rotation. Unattached bacteria were removed by using repeated cycles of centrifugation and resuspension in PBS. The number of bacteria adhering to 40 epithelial cells was read by using interference contrast microscopy (Nikon), and attachment was given as the mean number of bacteria per cell. For details about the method and cell viability, etc., please see reference 27.

Bacterial binding to glycolipids. Globotetraosylceramide was purified from human erythrocytes (1, 18). The Forssman glycolipid was purified from mouse intestinal cells (3). Heptaglycocylceramide with type 1 chain was purified from human meconium (24).

Bacterial binding to the glycolipids was analyzed by using thin-layer chromatography (TLC) bacterial overlay. Luria broth containing 1 mM of $CaCl_2$ and 50 μCi of [^{35}S]methionine (total volume, 500 μl) was inoculated with one bacterial colony from a fresh tryptic soy agar plate and incubated at 37°C for 15 to 18 h without shaking. The bacteria were washed three times by centrifugation at 2,000 × *g* for 10 min and were resuspended in PBS. The labeled bacteria were suspended in PBS to approximately 10^8 CFU/ml. Their specific activities ranged from 100 to 200 CFU/cpm.

The glycolipids were separated on Kieselgel 60, alumina-backed HPTLC plates (Merck, Darmstadt, Germany) by using chloroform-methanol-water (60:35:8, vol/vol/vol). The bacterial overlay was performed as previously described (2, 19). Briefly, thin-layer plates were treated with polyisobutylmethacrylate in diethyl ether-hexan (1:1, vol/vol) or in pure diethyl ether at a concentration of 0.3% (wt/vol) for 1 min, dried overnight at room temperature, and then incubated with 2% bovine serum albumin in PBS for 2 h to reduce nonspecific binding to the silica plates. Without intermediate drying, the TLC plates were subsequently overlaid with the bacterial suspension and incubated for 2 h. Unbound bacteria were removed by extensive washing with PBS, and bacterial binding to the glycolipids was detected by using autoradiography.

Inhibition of attachment by glycolipid fractions. The ability of glycolipids in suspension to inhibit bacterial attachment

TABLE 2. Difference between P-fimbriated *E. coli* strains and transformants in attachment to human uroepithelial cells

E. coli strain	Agglutination[a]		Attachment (bacteria/cell)	
	Galα1-4Galβ–latex	Sheep erythrocytes	A_1 nonsecretor	A_1 secretor
Wild type				
J96	++	MR	88 ± 11	68 ± 45
AD110	++	MR	105 ± 18	116 ± 36
IA2	++	MR	132 ± 13	142 ± 33
Transformants in HB101				
HB101	−	−	0	0
HB101/pap_{J96}	++	−	<10	<10
HB101/pap_{AD110}	++	MR	147 ± 32	107 ± 52
HB101/pap_{IA2}	++	MR	160 ± 13	169 ± 13
HB101/prs_{J96}	+	MR	<10	118 ± 1
HB101/pap_{J96} prs_{J96}	++	MR	<10	97 ± 4

[a] ++, positive reaction; MR, mannose resistant; −, negative reaction; +, weakly positive reaction.

was tested as previously described (14) by using the globotetraosylceramide and the Forssman glycolipid fractions.

For inhibition experiments, these glycolipids were taken from solutions in chloroform-methanol (2:1). The solvent was removed by evaporation in a stream of nitrogen. After the addition of 0.5 ml of PBS, the samples were sonicated in a water bath for 30 to 60 s in order to give a glycolipid suspension. Bacteria were preincubated with glycolipid suspensions (10^8 bacteria in 100 μl and 0.5 ml of glycolipids) for 30 min at 37°C. After the addition of 10^5 urinary sediment epithelial cells in 0.4 ml of PBS, the adhesion to uroepithelial cells was assayed as described above. The inhibition was given as the percentage of attached bacteria compared with that of the saline control.

RESULTS

P-fimbrial expression of the wild-type and recombinant *E. coli* strains. The wild-type and recombinant *E. coli* strains expressed P fimbriae; expression was defined as the ability to agglutinate human erythrocytes of blood group P but not those of the p phenotype (Table 1). The three wild-type strains, *E. coli* J96, *E. coli* AD110, and *E. coli* IA2, agglutinated both Galα1-4Galβ–latex beads and sheep erythrocytes. Three different patterns of binding were represented among the recombinants in *E. coli* HB101 (Table 2). HB101 pap_{J96} reacted with the Galα1-4Galβ–latex beads but not with sheep erythrocytes. HB101 prs_{J96} reacted with sheep erythrocytes but reacted weakly or not at all with the Galα1-4Galβ–latex beads. The J96 *pap* and J96 *prs* adhesins, therefore, each represented a separate P-fimbrial category. The transformants HB101 pap_{IA2} and HB101 pap_{AD110} formed a third P-fimbrial binding category (25). They retained from the wild-type strains the combined reactivity with Galα1-4Galβ–latex beads and sheep erythrocytes.

Bacterial binding to glycolipids. The reactivities of the recombinant clones with the Forssman, globotetraosylceramide, and globo-A-enriched glycolipid fractions are shown in Fig. 1. The globotetraosylceramide fraction in Fig. 1, lane 1, showed one band in the tetrahexocylceramide region which reacted with HB101 pap_{J96}, HB101 prs_{J96}, HB101 pap_{AD110}, and HB101 pap_{IA2}. The Forssman glycolipid fraction in Fig. 1, lane 2, showed a double band in the pentaglucosylceramide region, which reacted with the four recombinant clones. In addition, TLC overlay with HB101 pap_{IA2} and HB101 pap_{AD110} detected the presence in this fraction of a small amount of tetrahexosylceramide. The glycolipid fraction in Fig. 1, lane 3, showed a band in the heptaglucosylceramide region which reacted with HB101 prs_{J96} and HB101 pap_{IA2}. HB101 pap_{IA2} and HB101 pap_{AD110}, in addition, detected the presence of tetraglucosylceramide in this fraction. On the basis of previous structural analyses of these fractions, the tetraglucocylceramide was identified as globotetraosylceramide, the pentaglucosylceramide fraction was identified as the Forssman glycolipid, and the heptaglucosylceramide was identified as globo-A.

Attachment of *E. coli* J96, *E. coli* HB101 pap_{J96}, and *E. coli* HB101 prs_{J96}. *E. coli* J96 attached to human A_1 secretor and A_1 nonsecretor epithelial cells (Table 2). The sheep erythrocyte reactivity and the attachment to A_1 secretor epithelial cells were explained by the prs_{J96}-encoded adhesin (Table 2). Both the sheep erythrocyte agglutination and attachment to A_1 secretor epithelial cells of HB101 prs_{J96} were inhibited by the Forssman glycolipid fraction (Tables 3 and 4). HB101 prs_{J96} did not attach to uroepithelial cells from A_1 nonsecretor individuals. The attachment of *E. coli* J96 to these cells was also not explained by the J96 *pap*-encoded adhesin. *E. coli* HB101 pap_{J96} did not attach to uroepithelial cells from A_1 nonsecretor or A_1 secretor individuals (Table 2).

The binding to A_1 nonsecretor cells of *E. coli* J96 but of neither HB101 pap_{J96} nor HB101 prs_{J96} suggested that

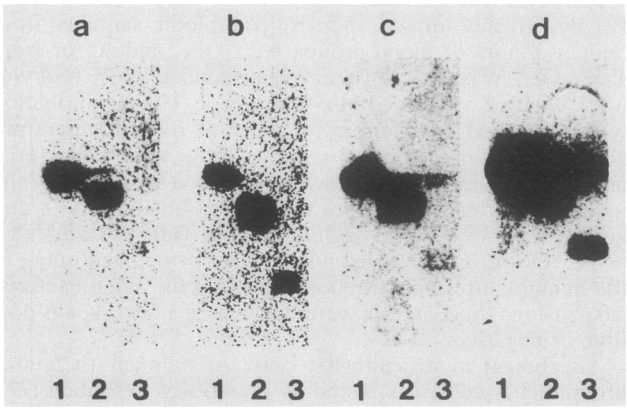

FIG. 1. Autoradiogram of radiolabeled *E. coli* HB101/pap_{J96} (a), HB101/prs_{J96} (b), HB101/pap_{AD110} (c), and HB101/pap_{IA2} (d) binding to the glycolipid fractions used for inhibition in this study. Lanes: 1, the globotetraosylceramide fraction; 2, the Forssman glycolipid fraction; 3, the A type 4 heptaglucosylceramide fraction.

TABLE 3. Inhibition of hemagglutination by soluble glycolipid fractions

E. coli strain	Inhibitor[a]	Hemagglutination[b]	
		Sheep RBCs	Human A_1P_1 RBCs
HB101/prs_{J96}	PBS	MR	MR
HB101/pap_{IA2}		MR	MR
HB101/pap_{AD110}		MR	MR
HB101/pap_{J96}		–	MR
HB101/prs_{J96}	Forssman glycolipid	–	–
HB101/pap_{IA2}		MR	MR
HB101/pap_{AD110}		MR	MR
HB101/pap_{J96}		–	MR
HB101/prs_{J96}	Globotetraosylceramide	–	–
HB101/pap_{IA2}		–	–
HB101/pap_{AD110}		–	–
HB101/pap_{J96}		–	–

[a] All inhibitors except PBS were 200 μg/ml.
[b] RBCs, erythrocytes; MR, mannose resistant; –, negative reactivity.

attachment might require the cooperative binding of both adhesins. In order to test this hypothesis, a strain with the double genotype (pap_{J96} prs_{J96}) was constructed. *E. coli* HB101 was transformed with the plasmids pRHu845 (containing the *pap* sequences) and pJFK102 (containing the *prs* sequences). The double transformant was selected for resistance to ampicillin and tetracycline and tested for the ability to agglutinate sheep erythrocytes and Galα1-4Galβ–latex beads. Transformants with the double phenotype were tested for adherence to human uroepithelial cells in vitro. Adherence to A_1 nonsecretor epithelial cells was not significantly increased compared with that of *E. coli* J96 *prs* (Table 2). The results suggested that adherence to human uroepithelial cells was not a function of synergistic recognition of the receptor epitopes for the pap_{J96}- and prs_{J96}-encoded adhesins.

The possibility that the attachment of *E. coli* J96 was due to receptors other than the globoseries of glycolipids was tested by pretreatment of *E. coli* J96 with the Forssman and globotetraosylceramide fractions (Tables 3 and 4). The Forssman glycolipid did not inhibit the hemagglutination or the adhesion of *E. coli* J96. In contrast, both hemagglutination and attachment were inhibited by the globotetraosylceramide fraction.

TABLE 4. Inhibition of attachment to human uroepithelial cells by soluble glycolipids

E. coli strain	Inhibitor[a]	Attachment[b]	
		A_1 secretor	A_1 nonsecretor
HB101/prs_{J96}	PBS	58 ± 18	<10
HB101/pap_{IA2}		170 ± 31	163 ± 18
HB101/pap_{AD110}		103 ± 24	145 ± 39
J96		78 ± 22	96 ± 12
HB101/prs_{J96}	Forssman glycolipid	<10	ND
HB101/pap_{IA2}		ND	112
HB101/pap_{AD110}		ND	102
J96		ND	105
HB101/prs_{J96}	Globotetraosylceramide	<10	ND
HB101/pap_{IA2}		75	25
HB101/pap_{AD110}		52	11
J96		110	23

[a] All inhibitors except PBS were 200 μg/ml.
[b] Data expressing means ± standard deviations refer to the number of bacteria per cell. Other data are percentages. ND, not determined.

Attachment of *E. coli* IA2, *E. coli* AD110, *E. coli* HB101 pap_{IA2}, and *E. coli* HB101 pap_{AD110}. *E. coli* IA2 and AD110 and the transformants in *E. coli* HB101 attached avidly to human uroepithelial cells from A_1 secretor and A_1 nonsecretor individuals. The wild-type *E. coli* strains resembled *E. coli* J96 in that they agglutinated sheep erythrocytes and attached to human A_1 secretor and nonsecretor epithelial cells. *E. coli* HB101 pap_{IA2} and *E. coli* HB101 pap_{AD110} differed from *E. coli* HB101 pap_{J96} and *E. coli* HB101 prs_{J96} in that they retained both the sheep erythrocyte reactivity and the ability to attach to secretor and nonsecretor epithelial cells. This suggested that HB101 pap_{AD110} and HB101 pap_{IA2} differed from HB101 prs_{J96} in the mechanism of sheep erythrocyte reactivity and that the same receptor specificity might determine the binding to both sheep erythrocytes and the uroepithelial cells.

We therefore tested the abilities of the purified Forssman and globotetraosylceramide glycolipid fractions to inhibit sheep erythrocyte agglutination by *E. coli* HB101 pap_{AD110} and *E. coli* HB101 pap_{IA2} (Table 3). Pretreatment with the globotetraosylceramide fraction inhibited the agglutination of sheep erythrocytes. In contrast, pretreatment with the Forssman glycolipid fraction had no effect (Table 3). The globotetraosylceramide-enriched fraction also inhibited agglutination of human A_1P_1 erythrocytes by these strains. These results demonstrated that the sheep erythrocyte reactivities of *E. coli* HB101 pap_{IA2} and HB101 pap_{AD110} were due not to recognition of the Forssman glycolipid but to recognition of receptor epitopes provided by globotetraosylceramide.

We subsequently tested the hypothesis that the attachment to human uroepithelial cells of the pap_{IA2}- or pap_{AD110}-encoded adhesins was due to recognition of globotetraosylceramide. *E. coli* HB101 pap_{IA2} and HB101 pap_{AD110} were preincubated with the globotetraosylceramide-enriched fraction prior to the addition of human uroepithelial cells from A_1 secretor or nonsecretor individuals (Table 4). The globotetraosylceramide fraction inhibited the attachment of *E. coli* HB101 pap_{IA2} to human nonsecretor uroepithelial cells. The dose-related inhibition is shown in Fig. 2. At a concentration of 200 μg/ml, *E. coli* IA2 and AD110 were inhibited to levels of attachment less than 10% of that of the saline control. The Forssman glycolipid did not inhibit the attachment of either strain.

The inhibition of attachment was further tested by using uroepithelial cells from an A_1 secretor donor (Fig. 3). As expected, the globotetraosylceramide fraction did not inhibit the attachment of *E. coli* HB101 prs_{J96} to these cells. The attachment to A_1 secretor epithelial cells of *E. coli* HB101 pap_{IA2} and HB101 pap_{AD110} was only partially inhibited by globotetraosylceramide. When globotetraosylceramide was at a concentration of 200 μg/ml, HB101 pap_{AD110} was inhibited to about 45% of the saline control. HB101 pap_{IA2} changed from a pattern of adherence in which the bacteria were distributed randomly over the cell surface to a patchwise attachment, which was difficult to quantitate.

DISCUSSION

The P-fimbrial family of adhesins recognize isoreceptor epitopes provided by the globoseries oligosaccharides. This

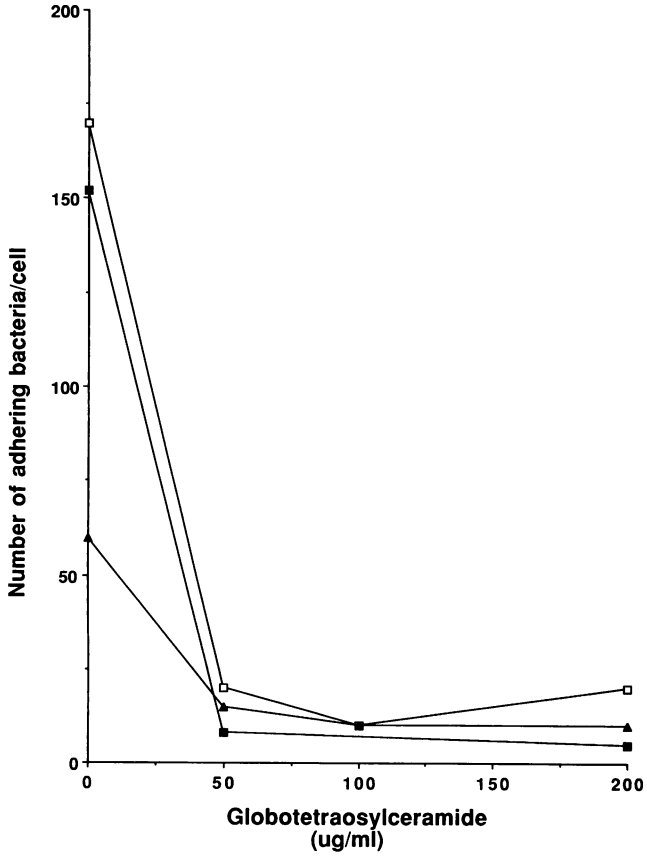

FIG. 2. Globotetraosylceramide inhibition of attachment of bacteria to human uroepithelial cells from an A_1P_1 nonsecretor individual. □, *E. coli* IA2; ▲, *E. coli* J96; ■, *E. coli* AD110.

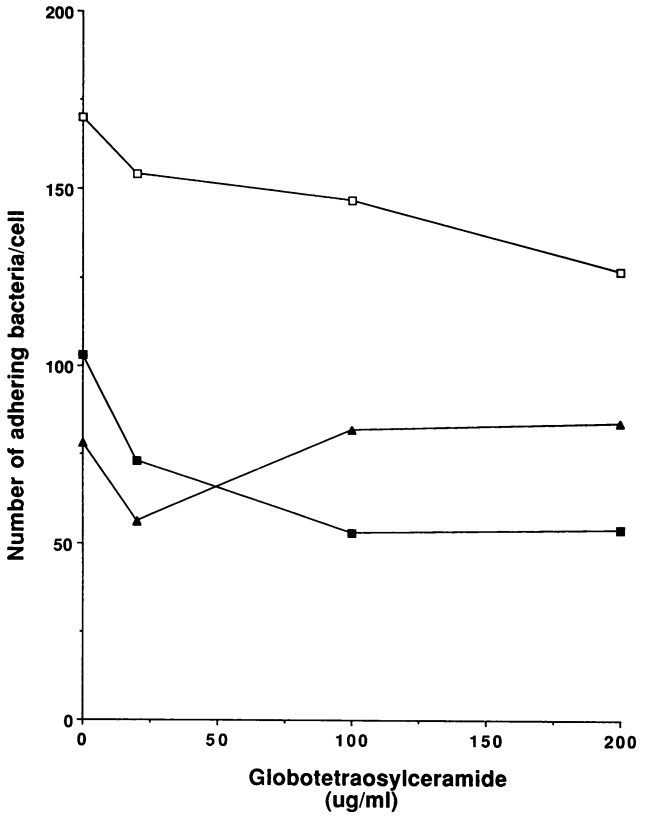

FIG. 3. Globotetraosylceramide inhibition of attachment of bacteria to human uroepithelial cells from an A_1P_1 secretor donor. □, *E. coli* IA2; ▲, *E. coli* J96; ■, *E. coli* AD110.

study analyzed the receptor specificities and attachment to human uroepithelial cells of three groups of P fimbriae encoded by the pap_{J96}, prs_{J96}, and pap_{IA2}-pap_{AD110} DNA sequences. They shared the ability to agglutinate human erythrocytes in a P blood group-dependent manner but differed in reactivities with Galα1-4Galβ–latex beads, sheep erythrocytes, and uroepithelial cells from secretor and nonsecretor donors. The pap_{IA2}- and pap_{AD110}-encoded adhesins agglutinated Galα1-4Galβ–latex beads and sheep erythrocytes and attached to epithelial cells from secretor and nonsecretor individuals. These binding reactions were inhibited by globotetraosylceramide but not by the Forssman glycolipid. The prs_{J96}-encoded adhesins agglutinated sheep erythrocytes and attached to secretor epithelial cells. The sheep erythrocyte and uroepithelial cell binding was inhibited by the Forssman glycolipid. The pap_{J96}-encoded adhesins agglutinated Galα1-4Galβ–latex beads but not sheep erythrocytes and attached poorly to human uroepithelial cells. The results emphasize that the pap_{IA2} sequences encode P fimbriae which mediate attachment to urinary tract epithelial cells.

The identification of the globoseries of glycolipids as receptors for P fimbriae was based on two techniques: inhibition and coating. Glycolipid extracts from uroepithelial cells were shown to inhibit attachment, unless the extracted cells were derived from an individual of blood group p (14). Coating receptor-negative cells such as p erythrocytes with purified glycolipids of the globoseries induced binding to those cells. Since Galα1-4Galβ is the common structural denominator among the globoseries of glycolipids (14) and the only structure shared among natural glycolipids which bound P-fimbriated *E. coli* on TLC plates, it was proposed to be the minimal receptor site (2). Indeed, when coupled with latex beads, synthetic Galα1-4Galβ functioned as a receptor, as shown by bacterial agglutination of the beads (6). However, it was apparent from early studies that different members of the globoseries vary in receptor function (13–16). Globotetraosylceramide-coated erythrocytes or latex beads were agglutinated more strongly and rapidly than the Galα1-4Galβ–latex beads and reacted with a larger number of uropathogenic *E. coli* strains (9, 15). Globotetraosylceramide was a more efficient inhibitor of attachment than the Galα1-4Galβ disaccharide or globotriaosylceramide (14, 16). Qualitative differences in receptor function were further suggested by the variable reactivities of clinical isolates with sheep erythrocytes (15). Although these contain the Forssman glycolipid with an internal Galα1-4Galβ residue, only a fraction of the globoside-recognizing strains reacted with the sheep erythrocytes (15).

Recently, these variations in receptor recognition have been partially explained. The pap_{J96}, pap_{IA2}, and prs_{J96} DNA sequences show extensive sequence homologies, with the exception of *papA* encoding the antigenically variable fimbrial subunit and *papE* and *papG* encoding the adhesin (17, 21). The $papG_{IA2}$ and $papG_{IA2}$ sequences show a high degree of homology but differ by at least 50% from $papG_{J96}$ (21, 25). The adhesins encoded by $papG_{J96}$, $prsG_{J96}$, and $papG_{IA2}$ were assigned to three separate classes on the basis

TABLE 5. Summary of the binding assays and glycolipid inhibition

Binding assay	Binding of recombinants in *E. coli* HB101[a]			
	pap_{J96}	prs_{J96}	pap_{IA2}	pap_{AD110}
TLC binding				
Globotetraosylceramide	+	+	+	+
Forssman glycolipid	+	+	+	+
Globo-A	−	+	+	+[b]
Galα1-4Galβ–latex	+	+[b]	+	+
Sheep erythrocytes	−	+[c]	+[d]	+[d]
Human uroepithelial cells				
A_1P_1 secretor	−	+[c]	+	+
A_1P_1 nonsecretor	−	−	+[d]	+[d]

[a] +, positive binding in TLC, agglutination of latex beads or erythrocytes, and attachment to uroepithelial cells; −, negative reaction.
[b] Weakly positive reaction.
[c] Inhibited by the Forssman glycolipid fraction.
[d] Inhibited by the globotetraosylceramide fraction.

of their binding to transformed cell lines and erythrocytes (25). pap_{J96} was proposed to encode adhesins preferring a terminal Galα1-4Galβ residue, such as globotriaosylceramide. The prs_{J96}-encoded adhesin required for binding a terminal GalNAcα residue linked to a globoseries core (18, 19, 21, 24, 25). The exact specificity of the $papG_{IA2}$ adhesin class and the degree of phenotypic homology with the $papG_{AD110}$-encoded adhesin have not been determined.

There are major problems involved in the definitions of receptor specificity. By using TLC overlay, it was shown that the adhesins of all three classes bind to several Galα1-4Galβ-containing glycolipids, including globotetraosylceramide and the Forssman glycolipid (Table 5). The $papG_{J96}$ adhesin recognized internal in addition to terminal Galα1-4Galβ residues. The prs_{J96}-encoded adhesin bound to globotetraosylceramide with a single GalNAc residue and not only to the Forssman and globo-A glycolipids. HB101 pap_{IA2} bound the globo-A structure, as well as globotetraosylceramide and the Forssman glycolipid. The results of the TLC overlays therefore permit the speculation that the receptor specificity is a function of affinity rather than of all-or-none recognition of different receptor epitopes. While the results of the TLC assay describe the propensity of the adhesins to interact with a given glycolipid under conditions of ideal exposure and/or availability, they only partly predict the outcome of the same interaction on the surface of a receptor-bearing cell (26). Although *E. coli* HB101 pap_{J96} recognized the globotetraosylceramide and Forssman glycolipids by TLC, it did not bind the same structures in human uroepithelial cells of the nonsecretor or secretor phenotype or sheep erythrocytes. In analogy, HB101 prs_{J96} recognized globotetraosylceramide and the Forssman glycolipid on TLC plates but bound only to cells expressing the Forssman or the globo-A glycolipid receptors.

Globotetraosylceramide fulfilled the criteria for a receptor for HB101 pap_{IA2} and HB101 pap_{AD110}. It occurred on the epithelial cells and erythrocytes to which the bacteria bound, and the globotetraosylceramide fraction inhibited their attachment and hemagglutination. The results therefore suggested that globotetraosylceramide is one relevant receptor for these adhesins. The TLC analysis also suggested that HB101 pap_{IA2} and HB101 pap_{AD110} had higher affinities for globotetraosylceramide than the HB101 prs_{J96} and HB101 pap_{J96} adhesins. Both detected minor amounts of globotetraosylceramide in the Forssman and globo-A fractions. HB101 pap_{J96} and HB101 prs_{J96} did not. The increased adhesive capacity compared with those of HB101 of pap_{J96} and HB101 prs_{J96} may be due to this high affinity. The relative nature of the receptor specificity is also shown by the finding that globotetraosylceramide inhibited the attachment of the HB101 *prs*J96 strain. This indicates differences in conformation between the soluble and membrane-bound forms of this receptor.

The comparison between the secretor and nonsecretor epithelial cells provided interesting information. Whereas globotetraosylceramide blocked the attachment of HB101 pap_{IA2} and HB101 pap_{AD110} to nonsecretor cells, it only partially inhibited binding to the secretor cells. The partial inhibition by globotetraosylceramide suggested that HB101 pap_{IA2} and HB101 pap_{AD110} recognized additional receptor structures in those cells. Evidence for additive binding to uroepithelial cells has previously been presented (20). *E. coli* strains attached in higher numbers when they coexpressed two adhesins for which there were receptors in the target cell. The nature of the receptor molecules in addition to globotetraosylceramide which mediate the attachment to the secretor epithelial cells remains to be defined.

The sheep erythrocyte has been used to identify P-fimbriated *E. coli* strains with specificity for the Forssman glycolipid. This strategy was based on the clear-cut results from HB101 prs_{J96} (22). The present study demonstrated, however, that HB101 pap_{IA2} and HB101 pap_{AD110} agglutinated sheep erythrocytes in a Forssman glycolipid-independent manner. Our continued studies have shown variable sheep erythrocyte reactivities among wild-type strains carrying DNA sequences homologous with those of pap_{IA2} and prs_{J96} (Plos et al., submitted for publication). The sheep erythrocyte reactivity can therefore not be used to define the *prs*-encoded phenotype.

The pap_{J96} gene cluster is a model system for studies of the biogenesis and function of P fimbriae (17, 21, 22, 25). Positive reactions with erythrocytes and Galα1-4Galβ–latex beads have been equated with attachment to uroepithelial cells. The defective binding to epithelial cells was noted but not explained. It has recently become clear that the $papG_{J96}$ DNA sequences are unique to the *E. coli* J96 parent strain. Screening of clinical isolates with a $papG_{J96}$-specific probe did not detect a single strain with sequence homology. The $papG_{IA2}$ and $prsG_{J96}$ sequences were, however, frequent among P-fimbriated wild-type strains.

The results of the present study emphasized the importance of using the pap_{IA2}-pap_{AD110} gene cluster for the continued elucidation of the determinants of attachment. Comparisons between the pap_{J96} and pap_{IA2} gene clusters may reveal sequence differences which explain the deficient binding of pap_{J96}. This question may also be addressed by analysis of the wild-type *E. coli* strain J96, which attached in a globoside-reversible manner and thus apparently contained the sequences required for functional attachment.

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