The EMBO Journal vol.9 no.6 pp.2001 - 2010, 1990

Host-specificity of uropathogenic *Escherichia coli* depends on differences in binding specificity to Galα1-4Gal-containing isoreceptors

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Communicated by S.Normark

Four G adhesins, cloned from uropathogenic *Escherichia coli* strains, were examined for binding to glycolipids and various eukaryotic cells. $PapG_{AD110}$ and $PapG_{IA2}$ showed virtually identical binding patterns to Galα1-4Gal-containing glycolipids, while $PapG_{J96}$ differed slightly and $PrsG_{J96}$ markedly with respect to the effect of neighbouring groups on the binding. Their hemagglutination patterns confirmed the existence of three receptor-binding specificities. While the PapG adhesins bound to uroepithelial cells from man (T24) but not to those from the dog (MDCK II), the reverse was true of PrsG. These binding patterns were largely explained by the absence or presence of appropriate glycolipid isoreceptors, although the inability of the PapG adhesins to bind MDCK II cells was attributed to an inappropriate presentation of their receptor epitopes. The high prevalence of PrsG-like specificities observed among wild-type dog uropathogenic *E.coli* isolates, together with the determined isoreceptor composition of human and dog kidney target tissues, suggest variation in receptor specificity as a mechanism for shifting host specificity, and that this variation has evolved in response to the topography of the host cellular receptors. The receptor-binding half proposed for the predicted amino acid sequences of the four G adhesins and the corresponding adhesin of one of the dog *E.coli* isolates varied considerably among the three receptor-binding groups of adhesins, but only little within each group.

Key words: adhesin/bacterial adherence/glycolipid/receptor/tropism

Introduction

Commensal and pathogenic bacteria display a remarkable selectivity for certain hosts and tissues (Gibbons and van Houte, 1975; Beachey, 1980). This tropism is thought to be partly due to the expression of adhesins with different binding abilities for cell surfaces (Beachey, 1980; Mirelman, 1986).

Cell surface glycoconjugates, e.g. glycolipids and glycoproteins, act as receptors for a wide variety of bacterial adhesins (Mirelman, 1986), including those expressed by *Escherichia coli* causing urinary tract infections in man (Leffler and Svanborg-Edén, 1980; Källenius *et al.*, 1980; Bock *et al.*, 1985). The majority of uropathogenic *E.coli* strains bind to the Galα1-4Gal moiety present in the globoseries of glycolipids on both human erythrocytes and uroepithelial cells (Leffler and Svanborg-Edén, 1980; Källenius *et al.*, 1980; Bock *et al.*, 1985). Hemagglutination and cell adhesion are inhibited by pretreating bacteria with glycolipids or free saccharides containing a Galα1-4Gal moiety (Leffler and Svanborg-Edén, 1980; Bock *et al.*, 1985). Furthermore, human erythrocytes of blood group P^k, P and P_1, which contain the globoseries glycolipids, are agglutinated by *E.coli*, whereas p erythrocytes devoid of Galα1-4Gal are not (Källenius *et al.*, 1980).

The interaction of uropathogenic *E.coli* with Galα1-4Gal receptor determinants is mediated by thin hair-like appendages called P-fimbriae or P-pili (Hull *et al.*, 1981; Korhonen *et al.*, 1982), which are morphologically similar but of diverse serotypes. The different serotypes are due to sequence differences in the major pilus subunit, the PapA protein. The adhesin in question, the receptor-binding PapG protein, is located at the tip of the pilus structure (Lindberg *et al.*, 1987; Lund *et al.*, 1987). For the biogenesis of adhesive pili, individual *E.coli* strains contain not only one but often two chromosomal *pap* operons containing 11 coordinately regulated *pap* genes (Hull *et al.*, 1981; Normark *et al.*, 1983; Lindberg *et al.*, 1984, 1989; Norgren *et al.*, 1984). The predicted amino acid sequences of PapG adhesins of P pili of the F13 ($PapG_{J96}$), as contrasted to the $F7_2$ and F11 ($PapG_{AD110}$ and $PapG_{IA2}$ respectively) serotypes, revealed extensive sequence differences in spite of similar overall receptor-binding properties (Lund *et al.*, 1985, 1988). However, the uropathogenic *E.coli* isolate J96 contains two operons, *pap* and *prs*, each encoding pili of the F13 serotype but with adhesins, $PapG_{J96}$ and $PrsG_{J96}$, of different binding properties (Lund *et al.*, 1988; Senior *et al.*, 1989). More recently, it was sugested that dog uropathogenic *E.coli* isolates also express P-related pili but of different binding specificities from human uropathogenic strains (Garcia *et al.*, 1988a,b).

In this study, we have examined the receptor-binding specificities of cloned $PapG_{AD110}$, $PapG_{IA2}$, $PapG_{J96}$ and $PrsG_{J96}$ adhesins and compared those with clinical *E.coli* UTI strains isolated from humans and dogs. We find at least three different epitopic binding variants for Galα1-4Gal-containing isoreceptors. These receptor-binding variants are shown to influence the binding of intact bacteria to erythrocytes and uroepithelial cells from different species. The binding epitope of G adhesins among wild-type uropathogenic *E.coli* isolates appears to be dependent on the host species from which they have been isolated. The glycolipid isoreceptor composition of erythrocytes,

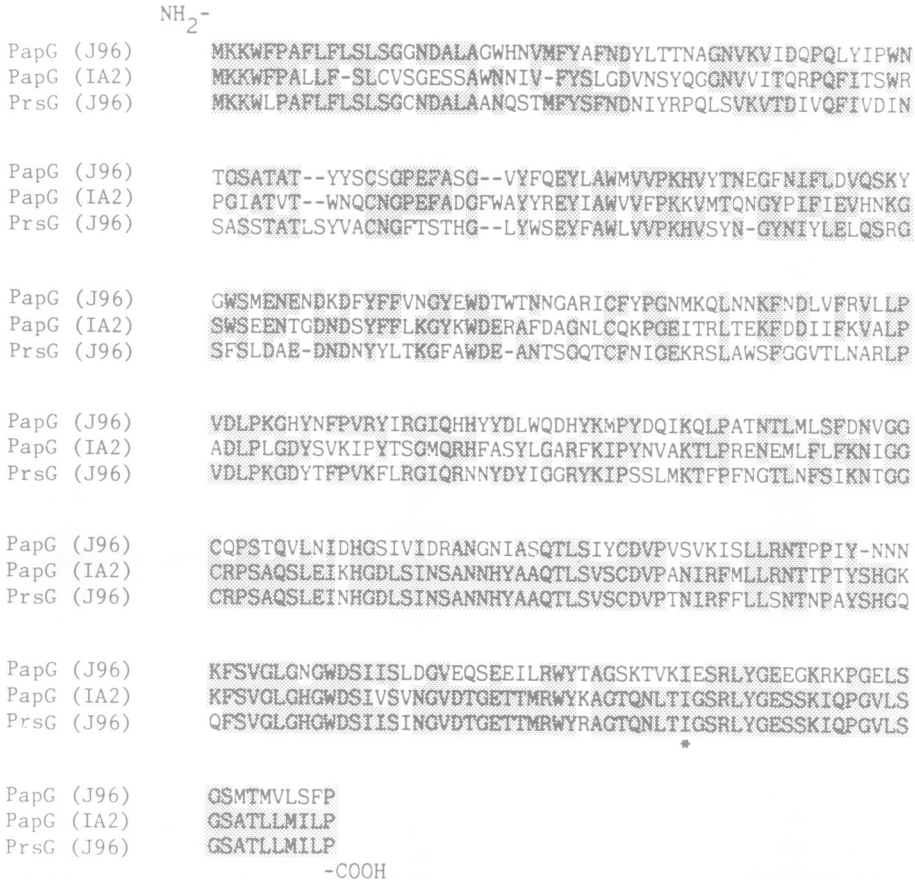

Fig. 1. Amino acid sequence comparison of PapG and PrsG adhesins from *E.coli* strains J96, IA2 and 1442. The amino acid sequences, as deduced from nucleotide sequences, are presented in the one-letter code. $PapG_{AD110}$ differs from $PapG_{IA2}$ at only five positions (Lund *et al.*, 1985). $PrsG_{1442}$ differs from $PrsG_{J96}$ only by having a threonine residue at the position marked by an asterisk. Homologous residues are shaded.

uroepithelial cells and target kidney and ureter tissues is discussed in relation to the evolution of these epitopic binding variants.

Results

Variant glycolipid-binding specificities of G-adhesins

Plasmids pPIL110-35, pDC1, pPAP5 and pPAP601 express P- and P-related pili from homologous gene clusters (*pap* or *prs*). The binding specificity is determined by the distal and equivalent *papG* and *prsG* genes, encoding the pilus-associated adhesins PapG and PrsG respectively (Lund *et al.*, 1985, 1988). We have recently shown that the *prsG* gene on pPAP601, unlike the *papG* gene on pPAP5, mediated hemagglutination of sheep but not human erythrocytes (Lund *et al.*, 1988), suggesting that the binding epitope of PrsG is distinctly different from that of PapG. The deduced amino acid sequences for $PapG_{J96}$ of pPAP5, $PapG_{AD110}$ of pPIL110-35 and $PapG_{IA2}$ of pDC1 have been determined before (Lund *et al.*, 1985). Whereas the two latter adhesins differed by only five amino acids, $PapG_{J96}$ showed only 46% homology with the other two proteins (Figure 1).

The *prsG* gene of pPAP601 was recently mapped and sequenced (unpublished data). The deduced amino acid sequence for $PrsG_{J96}$ was aligned to the previously sequenced PapG adhesins (Figure 1). Despite the fact that the *pap* operon of pPAP5 and the *prs* operon on pPAP601 are highly homologous and encode pili of the same serotype, their respective adhesins show only 48% homology. $PrsG_{J96}$ was strikingly homologous to $PapG_{AD110}$ and $PapG_{IA2}$ in the carboxy-terminal half of the protein, whereas the amino-terminal half was markedly different, even though short homologies were found.

Owing to the considerable sequence variation among this set of four G-adhesins, we investigated whether this affected their detailed receptor-binding specificities.

E.coli HB101 cells harbouring pPIL110-35 ($PapG_{AD110}$), pDC1 ($PapG_{IA2}$), pPAP5 ($PapG_{J96}$) or pPAP601 ($PrsG_{J96}$) were therefore metabolically labelled by [^{35}S]methionine and allowed to bind to a panel of naturally occurring glycolipids immobilized on TLC plates or coated onto microtitre wells at different concentrations (Table I and Figures 2 and 3). The four clones all bound to glycolipids having Galα1-4Gal as a common structure, although clear differences were noted with respect to neighbouring groups. Glycolipids lacking the Galα1-4Gal sequence were totally negative for binding, except for a weak interaction with some glycolipids when those were applied on TLC plates in amounts as high as 2 μg/spot (i.e. nos. 20 and 25–27). This binding was also seen in HB101 lacking a recombinant plasmid, and therefore reflects an unrelated type of binding.

The three PapG adhesins mediated very similar binding patterns in that they reacted with the terminal Galα1-4Gal moiety of globotriaosylceramide (Gb3; no. 4) and the P_1 antigen (no. 3) as well as with the internal Galα1-4Gal portion of globoside (Gb4; no. 5), the Forssman antigen

Table I. Binding of metabolically ^{35}S-labelled recombinant *E.coli* strains expressing various G adhesins, the donor strain J96 ($PapG_{J96}$ + $PrsG_{J96}$) and the host strain HB101 to glycolipids immobilized on a thin-layer plate[a]

No.	Glycolipid structure[b]	Binding[c]						Reference
		PapG (AD110)	PapG (IA2)	PapG (J96)	PrsG J96)	J96	HB101	
1	Galα1-4Gal-O-OTE[c]	–	–	–	–	–	–	synthetic
2	NeuAcα2-3(NeuAcα2-6)Galβ1-3GalNAcβ1-3Galα1-4Galβ1-4GlcCer	–	–	–	–	–	–	Kundu *et al.*, (1986)
3	Galα1-4Galβ1-4GlcNAcβ1-3Galβ1-4GlcCer	++	++	+	–	+	–	Nakai *et al.* (1975)[k]
4	Galα1-4Galβ1-4GlcCer	++	++	++	–	++	–	Hakomori (1983)
5	GalNAcβ1-3Galα1-4Galβ1-4GlcCer	++	++	++	+	++	–	Hakomori (1983)
6	GalNAcβ1-3GalNAcβ1-3Galα1-4Galβ1-4GlcCer	++	++	++	+	++	–	Angström *et al.* (1986)
7	GalNAcα1-3GalNAcβ1-3Galα1-4Galβ1-4GlcCer	++	++	++	++	++	–	Hansson *et al.* (1983)[l]
8	Galβ1-3GalNAcβ1-3Galα1-4Galβ1-4GlcCerh	++	++	++	++	++	–	Blomberg *et al.* (1982)
9	Fucα1-2Galβ1-3GalNAcβ1-3Galα1-4Galβ1-4GlcCerh	++	++	++	++	++	–	Larsson (1986)
10	GalNAcα1-3(Fucα1-2)Galβ1-3GalNAcβ1-3Galα1-4Galβ1-4GlcCerh	++	++	++	++	++	–	Breimer and Jowall (1985)
11	GlcCer(h)	–	–	–	–	–	–	Hakomori (1983)
12	GalCer(h)	–	–	–	–	–	–	Hakomori (1983)
13	Galβ1-4GlcCer(h)	–	–	–	–	–	–	Hansson *et al.* (1983)
14	NeuAcα2-3Galβ1-4GlcCer(h)	–	–	–	–	–	–	Hakomori (1983)
15	Galα1-3Galβ1-4GlcCerh	–	–	–	–	–	–	Hansson *et al.* (1983)
16	GalNAcβ1-3Galα1-3Galβ1-4GlcCer	–	–	–	–	–	–	Falk *et al.* (1986)
17	Galα1-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcCer	–	–	–	–	–	–	Eto *et al.* (1968)
18	NeuAcα2-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcCer	–	–	–	–	–	–	Hakomori (1983)
19	Galα1-3(Fucα1-2)Galβ1-3GlcNAcβ1-3Galβ1-4GlcCer	–	–	–	–	–	–	Karlsson and Larsson (1981)
20	Galβ1-4(Fucα1-3)GlcNAcβ1-3Galβ1-4GlcCerh	(+)	(+)	(+)		(+)		McKibbin *et al.* (1982)[l]
21	GalNAcα1-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcCerh	–	–	–	–	–	–	McKibbin *et al.* (1982)[f]
22	GalNAcα1-3(Fucα1-2)Galβ1-4GlcNAcβ1-3Galβ1-4GlcCerh	–	–	–	–		–	McKibbin *et al.* (1982)[l]
23	GlcNAcβ1-3Galβ1-4GlcCer	–	–	–	–	–	–	Karlsson[g]
24	Galβ1-3GlcNAcβ1-3Galβ1-4GlcCerh	–	–	–	–	–	–	Karlsson and Larsson (1979)
25	GalNAcα1-3(Fucα1-2)Galβ1-3GlcNAcβ1-3Galβ1-4GlcCerh	(+)	(+)	(+)	(+)		(+)	Karlsson and Larsson (1981)
26	GalNAcβ1-4Galβ1-4GlcCerh	(+)	(+)	(+)	(+)		(+)	Hansson *et al.* (1982)[h]
27	Galβ1-3GalNAcβ1-4Galβ1-4GlcCerh	(+)	(+)	(+)	(+)	(+)	(+)	Hansson *et al.* (1982)
28	Fucα1-2Galβ1-3GalNAcβ1-4Galβ1-4GlcCerh	-	–	–	–	–	–	Hansson *et al.* (1982)[h]

[a]Assessment of binding was done by the chromatogram binding assay as described in Materials and methods.
[b]Cer means a ceramide composed of a non-hydroxy fatty acid and a dihydroxy long-chain base; Cerh, a ceramide composed of a hydroxy fatty acid and/or a trihydroxy long-chain base; Cer(h), the presence of both ceramide types (Cer + Cerh) in the glycolipid fraction used.
[c]++, 'strong' binding (level of detection at 1–10 ng of glycolipid); +, 'moderate' binding (level of detection at 10–100 ng); (+), 'weak' binding (only detectable at 2 μg or more of glycolipid); –, no binding at all at 2 μg.
[d]Reference that reports the source, structure and/or isolation procedure of the glycolipid.
[e]OTE, 2-(octadecylthio)ethyl.
[f]Prepared by mild acidic hydrolysis of no. 22.
[g]Malignant melanoma, unpublished data.
[h]N.Strömberg, G.C.Hansson, J.Thurin and H.Leffler (unpublished data).
[k]J.Thurin, N.Strömberg and K.-A.Karlsson (unpublished data).
[l]N.Strömberg and K.-A.Karlsson (unpublished data).

(Gb5; no. 7), para-Forssman (no. 6), and substances nos. 8–10. The three adhesins were also similar in their inability to bind to the Galα1-4Gal disaccharide when directly linked to a synthetic lipid carrier (no. 1) or carrying the bulky sugar group of structure no. 2. $PapG_{AD110}$ and $PapG_{IA2}$ both mediated almost identical binding curves when assayed against serial dilutions of different glycolipids (Figure 3), whereas $PapG_{J96}$ differed by reacting ~10-fold less efficiently with the P_1 antigen (no. 3). Nevertheless, $PapG_{J96}$ mediated the same strong binding as the other PapG adhesins to globotriaosylceramide (no. 4), which like the P_1 antigen carries a terminal Galα1-4Gal moiety although on a different core structure.

Like the three PapG adhesins, the $PrsG_{J96}$ adhesin reacted only with Galα1-4Gal-containing glycolipids such as globoside (no. 5), the Forssman antigen (no. 7), para-Forssman (no. 6) and substances nos. 8–10. It did not bind to isoglobotetraosylceramide (no. 16), which is identical to globoside (no. 5) except for its Galα1-3Gal moiety. In contrast to the PapG adhesins, however, no binding was found to globotriaosylceramide (no. 4) and the P_1 antigen (no. 3), emphasizing that PrsG binding requires more than a plain Galα1-4Gal moiety. This conclusion is supported by the finding that free Galα1-4Galβ-*O*-ethyl (3 mg/ml) prevented the $PapG_{J96}$-, but not the $PrsG_{J96}$-mediated, binding to TLC-immobilized glycolipids (Figure 2). A recognition of the shared GalNAcβ1-3Galα1-4Gal sequence of the active isoreceptors cannot solely explain the prsG dependency on neighbouring groups distal to Galα1-4Gal, since GalNAcβ1-3Galβ-*O*-ethyl (3 mg/ml) did not prevent the PrsG-mediated sheep hemagglutination (not shown), and since globoside, having this trisaccharide at its terminus (no. 5), showed only partial binding activity. When the GalNAcβ terminus of globoside carried substituents such as GalNAcα1-3 (no. 7), GalNAcβ1-3 (no. 6), Galβ1-3 (no. 8), Fucα1-2Galβ1-3 (no. 9) or GalNAcα1-3(Fucα1-2)Galβ1-3 (no. 10), the binding activity increased several-fold in relation to globoside (Figure 3). This could mean a binding

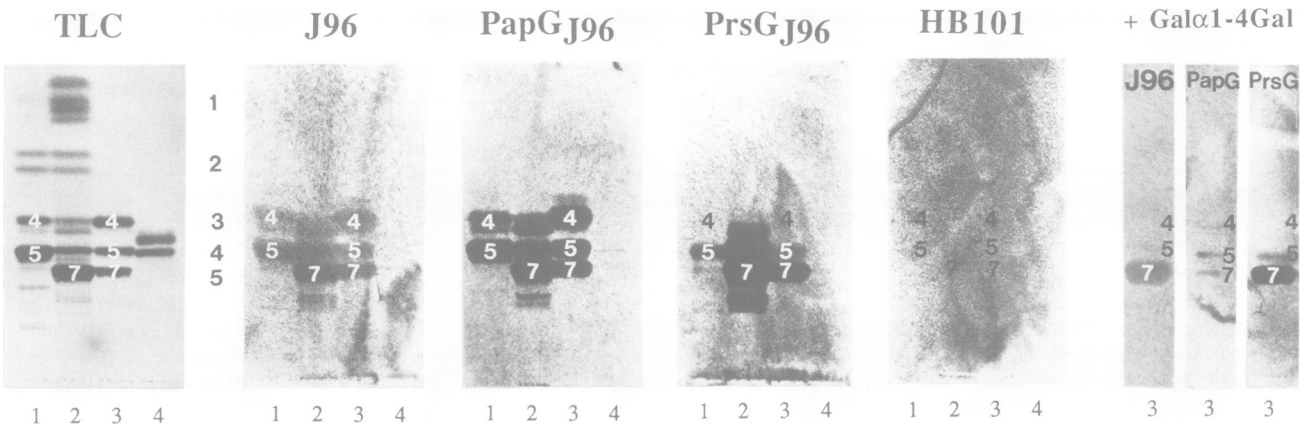

Fig. 2. Binding of metabolically ^{35}S-labelled *E.coli* wild-type strain J96, the *E.coli* cloning strain HB101, and HB101 expressing $PapG_{J96}$ (pPAP5) and $PrsG_{J96}$ (pPAP601) to glycolipid chromatograms. Five panels, each with four lanes, are shown after spray detection with anisaldehyde (to the left) and after autoradiography (15 h) from overlay with [^{35}S]methionine-labelled bacteria, as described in Materials and methods. **Lanes 1** and **2** contain total non-acid glycolipid mixtures (20–40 μg) isolated from human and sheep erythrocytes respectively. **Lanes 3** and **4** contain ~1 μg each of glycolipids nos. 4, 5, 7 and 15, 16 respectively. Numbers labelled on spots refer to Table I, and numbers to the right of the chemically developed chromatogram indicate the approximate number of sugars in the separated glycolipids. In panel 6 to the right, bacterial binding to glycolipids nos. 4, 5 and 7 was monitored after preincubation of the bacteria for 1 h in the presence of 3 mg/ml of Galα1-4Galβ-*O*-ethyl.

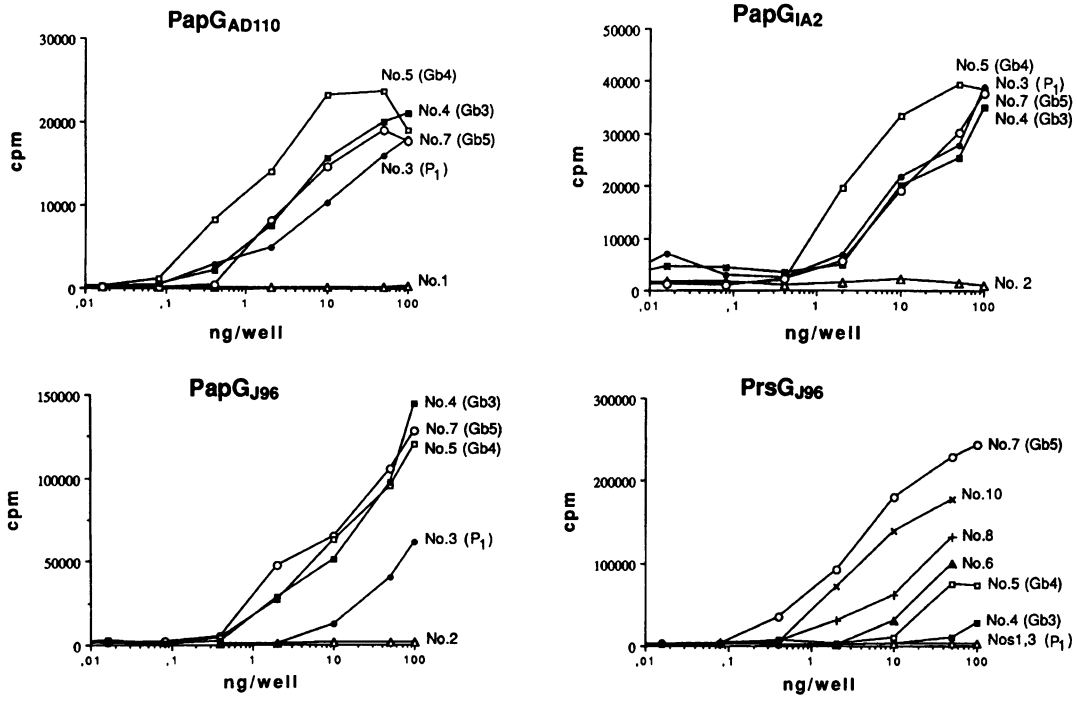

Fig. 3. Quantitative binding curves of recombinant *E.coli* strains expressing various G adhesins ($PapG_{AD110}$, $PapG_{IA2}$, $PapG_{J96}$ and $PrsG_{J96}$) to pure glycolipids coated in microtitre wells. The glycolipids (numbers to the right of each curve refer to the structures listed in Table I) were added to the microtitre wells in the amount indicated by the bottom scale (ng glycolipid/50 μl methanol). The wells were then incubated with metabolically ^{35}S-labelled bacteria, as described in Materials and methods. Data are expressed as mean values of triplicate determinations.

epitope for PrsG contained within the GalNAcβ1-3Galα1-4Gal trisaccharide but conformationally dependent on its terminal substituents (Kannagi *et al.*, 1983). Alternatively, PrsG could recognize an epitope on the GalNAcα1-3Galβ1-3Gal sequence of the active isoreceptors. However, recalling the inactivity of the GalNAcβ1-3Gal terminus (no. 16), together with the finding that the common GalNAcα1-3Gal/GalNAcβ termimus of the two most active isoreceptors, i.e. the Forssman glycolipid and substance no. 10, did not mediate binding when carried in the terminal position of substances nos 21 and 22, the latter possibility appears less likely.

In conclusion, we found three different binding specificities for Galα1-4Gal-containing glycolipids mediated by the four G adhesins. We next investigated if such differences in binding epitopes affected the recognition of bacteria to different types of eukaryotic cells.

Species-specific hemagglutination and cell-binding patterns of the G adhesins

Hemagglutination is a simple way to probe bacterium–cell interactions. For this reason, we assayed the four G adhesin clones against a panel of erythrocytes from various species (Table II). $PapG_{AD110}$ and $PapG_{IA2}$ both mediated a strong

Table II. Agglutination of different erythrocytes by recombinant *E.coli* strains expressing various G adhesins

Erythrocyte species	Hemagglutination[a]				
	$PapG_{AD110}$ (pPIL110-35)	$PapG_{IA2}$ (pDC1)	$PapG_{J96}$ (pPAP5)	$PrsG_{J96}$ (pPAP601)	HB101
Human	+++	+++	+++	−	−
Sheep	+	+	−	+++	−
Rabbit	−	−	+++	−	−
Horse	−	−	−	−	−

[a]For hemagglutination, 10 μl of erythrocytes (4%) and bacteria (10^9 cells/ml), both suspended in PBS, were mixed on a glass slide. +++, strong hemagglutination after 1 min; +, weak hemagglutination after 5 min; −, no hemagglutination after 5 min.

Table III. Adhesion of recombinant *E.coli* strains expressing various G adhesins to non-confluent uroepithelial cells from humans (T24) and dogs (MDCK)

Adhesin	Bacterial strain	Adhesion to[a]		
		T24	MDCK II	MDCK I
None	HB101	−	−	−
$PapG_{AD110}$	HB101/pPil110-35	++	−	−
$PapG_{IA2}$	HB101/pDC1	++	−	−
$PapG_{J96}$	HB101/pPAP5	+	−	−
$PrsG_{J96}$	HB101/pPAP601	−	++	−

[a]Adhesion was measured as described in Materials and methods. MDCK I and II are subclones of the same MDCK cell line (Hansson *et al.* 1986). ++, good adhesion (>25 bacteria/cell); +, weak adhesion (5−20 bacteria/cell); −, no adhesion (<5 bacteria/cell).

hemagglutination of human and a weak hemagglutination of sheep red blood cells, but not hemagglutination of rabbit and horse erythrocytes. Unlike the other two PapG adhesins, $PapG_{J96}$ mediated hemagglutination of rabbit erythrocytes, reinforcing its different receptor-binding specificity. The PrsG adhesin, in contrast to the PapG adhesins, mediated a strong hemagglutination of sheep erythrocytes but lacked reactivity with human and rabbit red blood cells.

To examine whether the PapG and PrsG adhesins also showed species specificity in adhesion to uroepithelial cells, their ability to mediate binding of *E.coli* HB101 to non-confluent monolayers of live (unfixed) human bladder carcinoma T24 cells and dog kidney-derived MDCK I and II cells was measured (Table III). All three PapG adhesins, albeit with lower efficiency for $PapG_{J96}$, mediated adherence to the T24 but not to the MDCK II cells, whereas PrsG showed the opposite binding pattern. In binding tests with polarized MDCK II cells, which retain the typical characteristics of mature uroepithelia, i.e. apical microvilli, it was confirmed that only PrsG can efficiently mediate bacterial binding to the apical surface of MDCK II cells (Table IV). $PapG_{J96}$ and $PapG_{AD110}$ did not bind at all to polarized MDCK II cells, whereas $PapG_{IA2}$ bound ~5 times less efficiently than PrsG. None of the four G adhesins allowed binding of HB101 to the MDCK subline I, which represents another differential stage of MDCK cells or a second cell type in the nephron (Hansson *et al.*, 1986).

Glycolipid receptor composition of erythrocytes, T24 and MDCK cells

The distinct differences in cell binding (Tables II−IV) could be due to different glycolipid receptor compositions (Figure 4 and Table V). Neutral glycolipid fractions were therefore prepared from human and sheep erythrocytes as well as from T24 and MDCK II cells. These fractions were separated on TLC plates, and assayed for isoreceptors using the chromatogram binding assay (Figures 2 and 4 and Table V).

Table IV. Adhesion of recombinant *E.coli* strains expressing various G adhesins to polarized MDCK II cells

Adhesin	Bacterial strain	Adhesion to polarized MDCK II cells[a]	
None	HB101	−	(1)
$PapG_{AD110}$	HB101/pPIL110-35	−	(1,3)
$PapG_{IA2}$	HB101/pDC1	(+)	(4,4)
$PapG_{J96}$	HB101/pPAP5	−	(1,6)
$PrsG_{J96}$	HB101/pPAP601	++	(19)

[a]Adhesion to polarized MDCK II cells, with the typical characteristics of mature uroepithelia (Simmons and Fuller, 1985), was measured as described in Materials and methods. An estimate of adhesion was obtained by the radioactivity counts (values within parentheses) of bound ^{35}S-labelled bacteria as well as by direct microscopic enumeration of attached bacteria (++, good adhesion; −, no adhesion). The values within parentheses are compensated for different specific incorporation of ^{35}S into different strains and given in relation to background values of HB101.

The glycolipid patterns of sheep erythrocytes and MDCK II cells were very similar, as revealed by the TLC analysis (Figure 4). Both fractions contained a major 5-sugar glycolipid species, previously characterized as the Forssman glycolipid (Momoi and Yamakawa, 1978; Hansson *et al.*, 1986), together with smaller amounts of 3- and 4-sugar species (lanes 2 and 4). Human erythrocytes and T24 cells, on the other hand, both had 3- and 4-sugar glycolipids as predominant species (lanes 1 and 3). Previous studies (Hakomori, 1983) have identified the 3- and 4-sugar glycolipids of human erythrocytes as globotriaosylceramide (no. 4) and globoside (no. 5) respectively.

When glycolipid chromatograms of sheep erythrocyte and MDCK II origin were overlaid with *E.coli* HB101 expressing PrsG, binding occurred in both cases to the 5-sugar Forssman glycolipid (no. 7) as well as to a series of less abundant glycolipid species ranging from 4 to 8 sugars in size (lanes 2 and 4 in Figure 4; lane 2 in Figure 2). The binding of PrsG to glycolipids obtained from human erythrocytes and T24 cells was, however, restricted to globoside (no. 5) on human erythrocytes and to a corresponding band on T24 cells (lanes 1 and 3 in Figure 4).

HB101 expressing $PapG_{J96}$, unlike $PrsG_{J96}$, recognized the two major human glycolipids, globotriaosylceramide (no. 4) and globoside (no. 5), as well as the two corresponding major glycolipid bands on T24 cells (lanes 1 and 3 in Figure 4). The very weak binding of $PrsG_{J96}$ to globoside (compare nos. 5 and 7 in Figure 3 and Table I) and the data given above explain why $PapG_{J96}$, but not $PrsG_{J96}$, reacts with human erythrocytes and T24 cells. $PapG_{J96}$ also mediated strong binding to the Forssman glycolipid (no. 7) and to the less predominant 3- and 4-sugar glycolipid bands on sheep erythrocytes and MDCK II cells. It is therefore surprising that *E.coli* HB101 cells expressing $PapG_{J96}$ neither agglutinates sheep erythrocytes nor binds to MDCK II cells. It may be that the binding epitopes on the Forssman glycolipid for $PapG_{J96}$ and $PrsG_{J96}$ are differently exposed on the cell surface as a result of sterical interferences from neighbouring molecules.

Total glycolipids prepared from MDCK I cells was also analysed against the $PapG_{J96}$- and $PrsG_{J96}$-expressing HB101 cells (lane 5 in Figure 4). Neither $PrsG_{J96}$ nor $PapG_{J96}$ recognized any glycolipids in this fraction, thus explaining why these adhesins failed to mediate binding to the cells (Table III).

Receptor-binding specificities of dog UTI E.coli isolates in relation to the glycolipid composition of dog and human kidney tissues

It has recently been shown that dog UTI *E.coli* isolates frequently exhibit hemagglutination of sheep erythrocytes and bind to MDCK II cells rather than to T24 cells (Garcia *et al.*, 1988b), which is similar to the cell-binding pattern mediated by the PrsG adhesin (Tables II–IV). Sixteen wild-type UTI dog *E.coli* were isolated and assayed for binding to a panel of Galα1-4Gal-containing glycolipids exposed on thin-layer chromatograms (Figure 5 and Table VI). Seven out of the 16 strains reacted strongly with the Forssman glycolipid (no. 7) but only weakly with globoside (no. 5) and not at all with globotriaosylceramide (no. 4). All these seven strains specifically bound to MDCK II cells and not to T24 cells (Garcia *et al.*, 1988b). None of the 16 strains bound to globotriaosylceramide (no. 4) or to the P_1 antigen (no. 3). These findings establish the high prevalence among dog UTI *E.coli* isolates of pili adhesins with binding specificities similar to that of PrsG and the low prevalence of PapG-like specificities.

The G gene from one of the seven dog UTI isolates (strain 1442) that bind strongly to the Forssman glycolipid has been cloned and sequenced (unpublished data). The nucleotide sequence of $prsG_{1442}$ was highly homologous to that of $prsG_{J96}$, and the deduced amino acid sequences of the respective proteins differed at only one position (Figure 1).

If the Forssman glycolipid or related isoreceptors act as the true receptors for dog UTI *E.coli* strains, these structures should also be present in the dog kidney and/or uroepithelium. To test this, we analysed PrsG-expressing

Table V. Difference in isoreceptor composition between various cells and target tissues[a]

Cell or tissue	Isoreceptors (% of total neutral glycolipids[b])			Reference[c]
	Globotriaosylceramide (no. 4)	Globoside (no. 5)	Forssman (no. 7)	
Human erythrocytes	14	61	–	Hakomori (1983)
Human T24 cells	27	26	–	
Human kidney	32	37	–	Breimer and Karlsson (1983)
Human ureter	5	21	–	Breimer *et al.* (1985)
Sheep erythrocytes	8	7	55	Momoi and Yamakawa (1978)
Dog MDCK II cells	5	4	35	Hansson *et al.* (1986)
Dog kidney	21	2	11	Sung *et al.* (1973)
Dog ureter	2	2	20	

[a]The deduced isoreceptor glycolipid compositions are based on the thin-layer chromatographic and bacterial-binding analyses shown in Figure 4, as well as on structural data (see references).
[b]The individual isoreceptors were quantified by scanning densitometry, as described in Materials and methods.
[c]Selected references presenting structural data on the glycolipid composition of the corresponding cells or tissues.

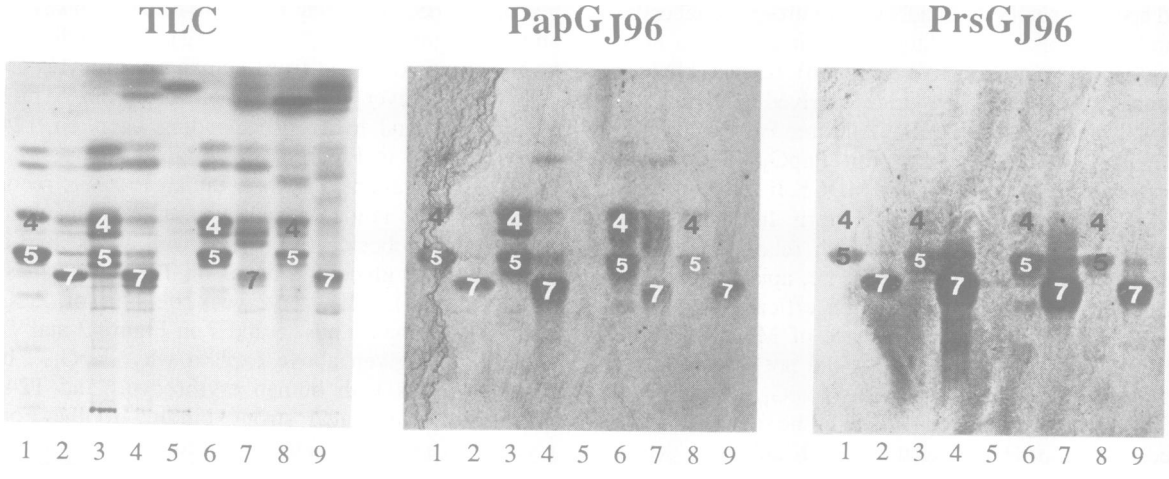

Fig. 4. Detection of receptor glycolipids for *E.coli* HB101 expressing the $PapG_{J96}$ and $PrsG_{J96}$ adhesins on various cells and tissues by binding of metabolically ^{35}S-labelled bacteria to glycolipid chromatograms. Representative autoradiograms after binding of the recombinant strains are shown, as well as the total glycolipid pattern after spray detection with anisaldehyde (see Materials and methods). Total non-acid glycolipid fractions (20–40 μg) used were from human erythrocytes (**lane 1**), sheep erythrocytes (**lane 2**), human bladder carcinoma T24 cells (**lane 3**), MDCK II cells derived from the dog (**lane 4**), MDCK I cells (**lane 5**), human kidney (**lane 6**), dog kidney (**lane 7**), human ureter (**lane 8**) and dog ureter (**lane 9**). The vertical numbers denote the approximate number of sugars in glycolipids with the corresponding mobility, and the numbers used to label glycolipids bands refer to Table I and the text.

HB101 *E.coli* cells for binding to thin-layer chromatograms containing neutral glycolipids prepared from whole-dog kidney and ureter tissues (Figure 4 and Table V). In both fractions (lanes 7 and 9 in Figure 4), the cells reacted weakly with a 4-sugar glycolipid band and strongly with a 5-sugar glycolipid, corresponding to globoside (no. 5) and the Forssman glycolipid (no. 7) respectively (Sung *et al.*, 1973). In comparison, glycolipid chromatograms of the corresponding human tissues were also analysed against the PrsG adhesin (lanes 6 and 8 in Figure 4; Table V). In this case, the PrsG-expressing cells bound weakly to globoside (no. 5), the major glycolipid in both human kidney and ureter (Breimer and Karlsson, 1983; Breimer *et al.*, 1985).

Discussion

Escherichia coli commonly causes urinary tract infections (UTI) in man as well as in dogs, provided that it expresses adhesins that interact with specific receptors on the host. Human UTI *E.coli* isolates (Leffler and Svanborg-Edén, 1980; Källenius *et al.*, 1980; Bock *et al.*, 1985) frequently express so-called P-pili containing a tip-associated adhesin that recognizes the Galα1-4Gal moiety of the globoseries of glycolipids. Dog UTI *E.coli* isolates often express pili serologically classified as P-pili but with an adhesin recently predicted to have a different, although chemically undefined, receptor structure (Garcia *et al.*, 1988a,b). In the present study, four cloned UTI *E.coli* P-pili adhesins—collectively referred to as G adhesins—were shown to have three variant receptor specificites for Galα1-4Gal isoreceptors, as deduced from their binding patterns to glycolipids as well as to erythrocytes and uroepithelial cells from various species. The variant specificity conferred by the G adhesin, which exclusively mediated binding to dog-derived uroepithelial cells, was the only one expressed among dog UTI *E.coli* isolates. These findings suggest that the host specificity of uropathogenic *E.coli* strains depends on the detailed binding specificity of their pili adhesins for Galα1-4Gal-containing isoreceptors.

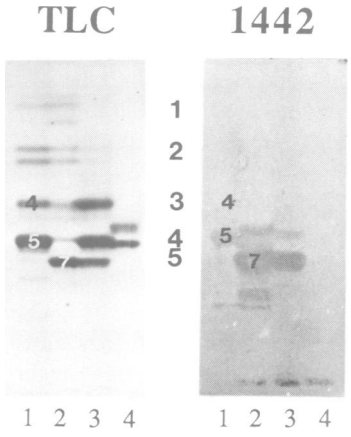

Fig. 5. Binding of the clinical dog *E.coli* UTI strain 1442 to glycolipid chromatograms. Shown are a representative autoradiogram (20 h) after overlay with metabolically ^{35}S-labelled bacteria and the total glycolipid pattern as visualized by spray detection with anisaldehyde (see Materials and methods). **Lanes 1** and **2** contain total non-acid glycolipids (20–40 μg) from human and sheep erythrocytes respectively. **Lanes 3** and **4** contain 1 μg each of glycolipids 4, 5, 7 and 3, 16 respectively. The numbers used to label glycolipid bands refer to Table I, and the vertical numbers denote the approximate number of sugars in glycolipids with the corresponding mobility.

All four G adhesins mediated an exclusive binding to Galα1-4Gal-containing glycolipids (Figures 2 and 3 and Table I) but depending on the effect of neighbouring groups distal or proximal to this disaccharide, three receptor-binding types were distinguished (Figure 3 and Table I). Thus, whereas $PapG_{AD110}$ and $PapG_{IA2}$ showed almost identical glycolipid-binding profiles, the $PapG_{J96}$ adhesin differed in its poor ability to recognize the Galα1-4Gal terminus of the P_1 antigen (no. 3). Earlier work on the interaction of two *Propionibacterium* species with lactosylceramide (Strömberg *et al.*, 1988b) and influenza viruses with sialic acid conjugates (Pritchett *et al.*, 1987) has shown similar influences on the receptor activity by proximal groups. The PrsG adhesin, representing the third binding type, differs from the PapG adhesins in its distinct dependency on neighbouring groups distal to the Galα1-4Gal moiety for binding. Similarly, a neighbouring GalNAcβ residue was recently suggested to be necessary for optimal binding of *N.gonorrhoeae* to lactosylceramide (Strömberg *et al.*, 1988a). Since a structural variety of proximal additions allowed PrsG binding, one may in this case argue against a strict neighbouring group requirement for receptor function. The PrsG adhesin might recognize a specific conformation of the GalNAcβ1-3Galα1-4Gal trisaccharide, which is only attained when certain terminal substituents are present (Kannagi *et al.*, 1983). In Figure 6, a computer-based molecular model of globoside is projected to visualize a possible conformationally dependent binding epitope for PrsG. At present, however, we cannot completely exclude the possibility that the PrsG adhesin has both a primary and

Table VI. Binding of clinical dog UTI *E.coli* isolates to glycolipid chromatograms[a]

Bacterial strains	Binding to	
	Globotriaosylceramide (no. 4) P_1 (no. 3)	Forssman (no. 7) Globoside (no. 5)
7 dog UTI *E.coli* strains[b]	−	+
9 dog UTI *E.coli* strains[c]	−	−
$PrsG_{J96}$[d]	−	+
$PapG_{AD110+IA2+J96}$[e]	+	+

[a]The chromatogram binding assay was conducted as described in Materials and methods. A representative result is shown in Figure 5 for dog UTI *E.coli* strain 1442. +, positive binding; −, negative binding.
[b]Strains 221, 304, 1013, 1083, 1442, 1520 and 1655 (Garcia *et al.*, 1988a,b).
[c]Strains 152, 154, 233, 664, 1389, 1565, 1612, 3056 and 3084 (Garcia *et al.*, 1988a,b).
[d]Recombinant *E.coli* strain HB101/pPAP601.
[e]Recombinant *E.coli* strains HB101/pPIL110-35 + pDC1 + pPAP5.

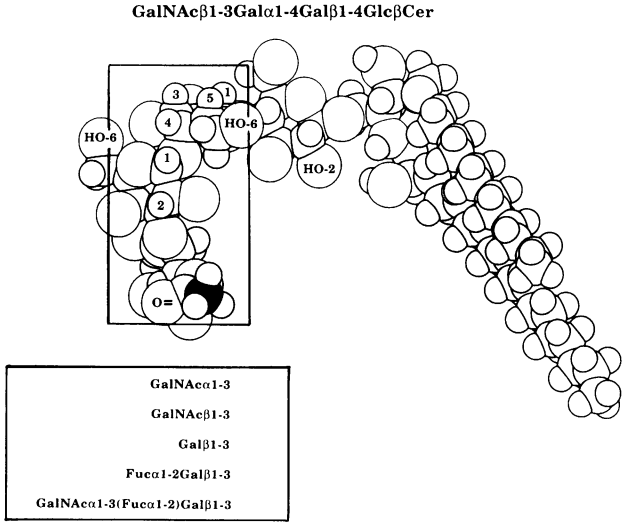

Fig. 6. Computer-based molecular modelling of isoreceptors (Karlsson, 1989) to propose a model for the interaction of $PrsG_{J96}$ with the GalNAcβ1-3Galα1-4Galβ sequence (upper box) of globoside (no. 5 of Table I). The model assumes that $PrsG_{J96}$ interacts with the acetamido group of GalNAcβ (carboxyl oxygen and methyl carbon indicated) and with the non-polar bend of the Galα1-4Gal moeity (H-1, H-3, H-4, H-5 and HO-6 of Galβ and H-1, H-2 and HO-6 of Galα indicated). The model accounts for the fact that structurally and conformationally different groups substituted on GalNAcβ in position 3 (lower box) have similar improving effects on the binding of PrsG by assuming that they influence the torsion angles at the β-linkage of GalNAc to Galα, optimally positioning the acetamido group for protein interaction. The non-polar bend of Galα1-4Gal has previously been shown to contain the binding epitope(s) for PapG adhesins (Bock *et al.*, 1985; Magnusson *et al.*, 1989). The weak interaction of $PapG_{J96}$ with the Galα1-4Galβ1-4GlcNAc sequence of the P_1 antigen (no. 3 of Figure 3) may be due to sterical hindrances from the acetamido group of the proximal GlcNAc. In the corresponding Galα1-4Galβ1-4Glc sequence of globoside, a strong binder of $PapG_{J96}$, the acetamido group is replaced by the HO-2 of Glc. The minimum energy conformation of the saccharides was calculated using the HSEA (Lemieux *et al.*, 1980; Thorgersen *et al.*, 1982) and GESA (Paulsen *et al.*, 1984) programs. The conformational features of the ceramide part were adopted from the crystal structures of galactosylceramide (Pascher and Sundell, 1977) and other membrane lipids (Pascher *et al.*, 1987).

a secondary binding site, where the latter is capable of accommodating a variety of structures, or an optimal binding epitope contained in the GalNAcα1-3Galβ1-3Gal sequence.

The reactivities of the four cloned G adhesins against a panel of erythrocyte species revealed striking differences between the three adhesin receptor-binding types (Table II), thereby suggesting that even slight variations in detailed receptor specificity may have profound biological consequences.

Among the 16 dog uropathogenic *E.coli* isolates analysed in this study, seven were found to express PrsG-like specificities, whereas none of them showed a pattern of binding similar to any of the three PapG adhesins (Table VI). Previous studies (Garcia *et al.*, 1988a,b) have shown that many of these dog UTI isolates express pili of the $F7_2$ and F13 serotypes, as well as hemagglutination and cell-binding patterns similar to those observed here for PrsG (Tables II–IV). Collectively, these data point to an involvement of the PrsG binding specificity in the development of urinary tract infections in dogs.

The shiga toxin also recognizes Galα1-4Gal but, as opposed to the G adhesins, in most cases it does not tolerate sugar additions distal of this disaccharide (Lindberg *et al.*, 1987). Similar receptor-binding variants have been reported among the large groups of bacteria classified as lactose binders (Holgersson *et al.*, 1985; Strömberg *et al.*, 1988a,b) and mannose binders (Firon *et al.*, 1984), as well as among the sialic acid-recognizing influenza (Rogers and Paulson, 1983) and Sendai viruses (Holgersson *et al.*, 1985). Whereas the NeuAcα2-3Gal-binding influenza virus variant shows tropism for avian and equine hosts, the NeuAcα2-6Gal variant binds specifically to human hosts (Rogers and Paulson, 1983). In view of these previous findings and our present data, it may be that variation in detailed receptor-binding specificity represents a rather common mechanism for generating a shifted host or tissue specificity.

In parallel to three receptor-binding types of G adhesins ($PapG_{AD110}$ and $PapG_{IA2}$—$PapG_{J96}$—$PrsG_{J96}$ and $PrsG_{1442}$), three antigenic types were distinguished (Figure 1). Thus, whereas $PapG_{AD110/IA2}$ and $PrsG_{J96/1442}$ only differ at five and one amino acid positions respectively, the three adhesin groups differ markedly from each other (Figure 1). A remarkable sequence homology was found in the carboxy-terminal regions between $PrsG_{J96}$ and $PrsG_{1442}$ as compared to $PapG_{AD110}$ and $PapG_{IA2}$. It has recently been shown that the $PapG_{J96}$ adhesin contains separate regions required for receptor binding and assembly into the pilus (unpublished data). These regions are located in the amino-terminal and carboxy-terminal halves of the protein respectively. It is likely that the receptor-binding properties of the other G adhesins are also specified by the amino-terminal half of the respective adhesin protein. The three groups of G adhesins therefore differ significantly in their proposed receptor-binding region, and amino acids constituting part of the receptor-binding domain can therefore not be localized. The receptor-binding variants of influenza virus correlate with single amino acid substitutions in the receptor-binding pocket of their hemagglutinins (Rogers *et al.*, 1983; Weiss *et al.*, 1988), which like the G adhesins show large overall antigenic variation (Wilson *et al.*, 1981). Thus, the variation in binding among G adhesins, and in particular the slight difference between $PapG_{J96}$ and $PapG_{AD110/IA2}$, could be due to specific amino acid replacements in the binding site. To resolve the molecular background to these differences in receptor-binding specificity, however, X-ray crystallographic studies need to be performed on adhesin receptor complexes.

Human erythrocytes and human bladder carcinoma T24 cells contain high-affinity receptors for PapG (globoside no. 5) but mainly low-affinity receptors for PrsG (Figure 4 and Table V), thereby providing a likely explanation why only PapG-expressing bacteria attach to these cells. Sheep erythrocytes and dog kidney-derived MDCK II cells both contain large quantities of the Forssman glycolipid (no. 7) to which all four G adhesins bind with high affinity (Figure 3 and Table I). Nevertheless, the various PapG adhesins, as opposed to $PrsG_{J96}$, did not mediate adherence to these cells (Tables II and III). The PapG-binding epitope(s) on the Forssman glycolipid may therefore be shielded by neighbouring molecules in the membrane (Lampio *et al.*, 1986). Thus, bacterial adhesion may be determined not only by the presence or absence of appropriate receptors but also by their accessibility to the bacteria in the natural membrane. Hypothetically, the hook-like conformation of the receptor glycolipids, with their sugar chains bending back towards

the membrane bilayer, may screw the molecule to expose the epitope for PrsG selectively, while shielding the epitope(s) for PapGs. In this context, it is noteworthy that the glycolipid isoreceptor composition of rabbit erythrocytes, agglutinated by $PapG_{J96}$ only (Table II), differs from that of human and sheep erythrocytes (unpublished data). The fact that dog kidney and ureter tissues, similarly to dog kidney-derived MDCK II cells, contain the Forssman glycolipid as the major isoreceptor (Figure 4 and Table V) suggests that sterical interferences in the presentation of the PapG-binding epitope on the Forssman glycolipid might have led to the evolution of the PrsG specificity among dog urinary tract *E.coli* isolates.

Materials and methods

Bacteria, growth conditions and labelling

Uropathogenic *E.coli* strains, isolated either from humans (J96; Hull *et al.*, 1981) or dogs (listed in Table VI; Garcia *et al.*, 1988a,b), and the recombinant *E.coli* strains HB101/pPAP5 (Lindberg *et al.*, 1984), HB101/pPAP601 (Lund *et al.*, 1988), HB101/pDC1 (Clegg, 1982) and HB101/pPIL110-35 (van Die *et al.*, 1984) were used. The recombinant plasmid pPAP5 consists of the vector pBR322 and a 9.7 kb *Eco*RI−*Bam*HI chromosomal fragment derived from *E.coli* J96 and encodes adhesive P-pili (Lindberg *et al.*, 1984). Plasmid pPAP601 is a pACYC derivative carrying another *E.coli* J96 chromosomal fragment encoding serologically identical F13 pili but with another adhesive capacity (Lund *et al.*, 1988). Plasmid pDC1 is a pACYC184 derivative carrying an 8.0 kb *Cla*I−*Bam*HI chromosomal fragment of *E.coli* IA2 (Clegg, 1982), whereas pPIL110-35 is a pACYC184 derivative containing a 16 kb *Eco*RI fragment of $E.coli_{AD110}$ (van Die *et al.*, 1983). pDC1 and pPIL110-35 both encode P-pili but of serotypes F11 and $F7_2$, respectively.

For most binding assays, the bacteria were cultivated overnight at 37°C in Luria broth or on Luria agar plates (Bertani, 1951) or CFA plates. Growth media for the recombinant strains were supplemented with carbenicillin, chloramphenicol, kanamycin and tetracyclin (100, 200, 20 and 10 μg/ml respectively). Metabolically labelled bacteria were obtained by addition of [^{35}S]methionine (Amersham, 600 Ci/mmol, 150−200 μCi/plate or tube). After growth, the bacteria were harvested by centrifugation, washed twice in PBS, pH 7.3, and finally resuspended in the same medium to give suitable radioactivity counts. About 10^7 c.p.m. was incorporated in 10^9 cells, giving 100 bacteria/c.p.m.

Preparation of total glycolipid fractions

Total non-acid glycolipids from the sources given in Table I and Figures 2, 4 and 5 were prepared as previously described (Karlsson, 1987). This included extraction of tissues with chloroform/methanol (2:1, 1:9, v/v), treatment of the lipid extract with 0.2 M KOH in methanol and dialysis to degrade and remove phosphoglycerolipids, and silicic acid column chromatography to remove non-polar lipids. Non-acid and acid glycolipids were then separated by DEAE−cellulose chromatography, followed by removal of sphingomyelin from the neutral fraction by acetylation and a second silicic acid column chromatography.

Isolation of individual glycolipids

A number of individual glycolipids (Table I) were isolated by repeated Iatrobeads column chromatography (6RS-8060, Iatron Laboratories Inc., Tokyo, Japan) applied for both acetylated and native derivatives using continuous gradients of chloroform/methanol (0−4%) and chloroform/methanol/water (65:24:4−50:40:10) respectively. For the preparation of other glycolipids (e.g nos. 6, 9, 10, 24 and 25), see Table I.

Identity of isolated glycolipids

The purity (and identity) of isolated compounds were initially assessed using TLC. Chemical structures (Table I) were confirmed and established by MS and proton NMR spectroscopic analyses of permethylated and permethylated-reduced substances, and through GC analyses of the permethylated alditol acetates obtained after acid hydrolysis of the permethylated glycolipids (see references accompanying Table I).

Densitometric analysis

High-performance TLC plates (Si-60 on glass sheets; Merck, Darmstadt, FRG) were stained with copper acetate reagent (Fewster *et al.*, 1969) and scanned at 500 nm using a Shimadzu C5930 TLC scanner. Signals were integrated and plotted on a Shimadzu DR-2 recorder using base-line correction for optimal calculations of peak areas.

Chromatogram binding assay

The assay was conducted as described elsewhere (Hansson *et al.*, 1985; Karlsson and Strömberg, 1986). Two similar sets of glycolipids were chromatographed in parallel on silicic gel 60 coated on aluminium sheets (HPTLC nano-plates, Merck, Darmstadt, FRG). The reference chromatogram was sprayed with anisaldehyde (Stahl, 1962) for visualization of the glycolipid bands. The other chromatogram, intended for bacterial binding, was treated with 0.5% (w/v) polyisobutylmethacrylate in diethyl ether for 1 min, dried and soaked with 2% (w/v) BSA in PBS, pH 7.3, for 2 h. Subsequently, this plate was overlaid with [^{35}S]methionine-labelled bacteria (2 ml of 10^7 c.p.m./ml, 10^9 cells/ml) for 2 h, washed 5 times, dried and exposed to X-ray film (XAR-5, Eastman, Kodak) to detect bound bacteria.

Microtitre well binding assay

The assay (Karlsson and Strömberg, 1986) was performed as follows: serial dilutions of glycolipids in methanol (50 μl/well) were dried overnight in microtitre wells (Cooks M24, Nutacon, Holland), followed by blocking with 2% BSA in PBS, pH 7.3, for 2 h. The wells were then incubated with [^{35}S]methionine-labelled bacteria (50 μl/well with 10^9 cells/ml, 10^7 c.p.m./ml) for 4 h, washed 5 times with PBS, dried and measured for radioactivity in a scintillation counter.

Cell cultures

The T24 cell line is an established cell line of human bladder carcinoma (Bubenik *et al.*, 1973). MDCK I and II cells (Simmons and Fuller, 1985; Hansson *et al.*, 1986) were obtained from Dr Kai Simons at the EMBL laboratory, Heidelberg. Cells were grown in DMEM, supplemented with 10% calf serum (Gibco) for 3 (T24) or 5 (MDCK I and II) days in 5% CO_2 at 37°C. To obtain polarized uroepithelial cells (Simons and Fuller, 1985), MDCK II cells were grown in Eagle's MEM as 3 day old monolayers on 3 μm pore size premounted polycarbonate filters (Transwell and Transwell-COL; Costar, Cambridge, MA) but otherwise using the same incubation conditions as described above. The resultant monolayer showed an electrical resistance of ~100−200 Ω/cm^2, as measured by the Millicell-ERS epithelial voltohmmeter (Millipore).

Cell-binding assays

For bacterial binding to non-polarized uroepithelial cells, non-confluent layers of cells in 5 cm diameter Petri dishes were washed twice with adhesion buffer (PBS containing 10 mM Ca^{2+}). Bacteria, suspended in adhesion buffer (0.5 ml, 10^9/ml), were then added to the cell layer followed by incubation for 2 h at 37°C. After removal of the unbound bacteria by four or five consecutive washes, the cell layer was stained with May−Grünwald−Giemsa, rinsed with distilled water and dried for microscopic enumeration of attached bacteria. For bacterial binding to polarized uroepithelial cells, MDCK II cells grown on filter dishes were washed twice with Eagle's MEM (without calf serum and antibiotics) and incubated with 0.2 ml suspensions of unlabelled or ^{35}S-labelled bacteria for 2 h at 37°C. After removal of unbound bacteria by six consecutive washes with adhesion buffer, the number of attached bacteria was estimated either with fluorescence microscopy (Transwell-COL) or by radioactivity counting of the washed MDCK cell layer.

Hemagglutination assay

For hemagglutination, 10 μl of fresh bacteria (10^9 cells/ml) and 10 μl of erythrocyte suspension (4%), both suspended in PBS of pH 7.3, were mixed on a glass slide. During gentle mixing for 5 min at room temperature, agglutination was examined by the naked eye and graded (−, + to 3+).

DNA sequencing

The *papG* sequences of plasmids pPIL110-35, pDC1 and pPAP5 have been reported before (Lund *et al.*, 1985). For sequencing of the *prsG* DNA, relevant fragments of plasmid pPAP601 were cloned into phage M13 cloning vectors M13 mp18 and M13 mp19 (Messing and Vieira, 1982) and transformed into *E.coli* TG1. Single-stranded template DNAs isolated from the phage (Messing *et al.*, 1981) were then sequenced by the dideoxy chain termination method (Sanger *et al.*, 1977) using the E20 sequencing primer (Symbicom AB, Umeå). To clone and sequence the adhesin gene of the dog uropathogenic isolate 1442, chromosomal DNA was partially digested with *Sau*3A1, and the fragments >10 kb were ligated into a dephosphorylated EMBL vector. Recombinant phages in strain *E.coli* LE392

containing the G gene were detected in a plaque hybridization with an 80 nt long *prs*G derived probe. Fragments representing the G gene were subcloned in the vector Bluescript, and sequenced using the T7 sequencing kit from Pharmacia.

Acknowledgements

We are indebted to Drs M.E.Breimer and B.E.Samuelsson for providing total non-acid glycolipids of human kidney and ureter tissues (Breimer and Karlsson, 1983; Breimer *et al.*, 1985), to Dr G.C.Hansson for providing total non-acid glycolipids of MDCK I and II cells (Hansson *et al.*, 1986), and to Drs M.E.Breimer (Breimer *et al.*, 1985) and G.Larsson (Larsson, 1986) for the kind gift of glycolipid substances nos. 10 and 9 respectively. P.Falk is acknowledged for kind help with scanning densitometry. This work was supported by grants from the Swedish Medical Research Council (4769, 9126 and 3967), the Swedish Natural Sciences Research Council (3373-112), the Swedish Board for Technological Development (81-3384 and 89-513), and the Swedish Medical Society (748).

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Received on January 16, 1990; revised on March 19, 1990