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Glycolipid Receptors for Uropathogenic *Escherichia coli* on Human Erythrocytes and Uroepithelial Cells

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A specific family of glycolipids, the globoseries, was shown to act as receptors on human uroepithelial cells and erythrocytes for the majority of uropathogenic *Escherichia coli* strains attaching to or hemagglutinating those cells. This was demonstrated in three different ways: (i) correlation between the natural presence of glycolipid in the target cell (erythrocytes of different species) and binding of bacteria; (ii) inhibition of attachment to human uroepithelial cells by preincubation of bacteria and glycolipid; and (iii) induction of binding to unreactive cells by coating of these cells with glycolipid. Strains reacting with the receptor agglutinated guinea pig erythrocytes in a mannose-resistant way after, but not before, coating of the cells with globotetraosylceramide. Unrelated glycolipids were not recognized. The reaction was made independent of simultaneous occurrence of mannose-sensitive adhesins on the strains by addition of D-mannose. The receptor-coated cells were used as a tool to screen for prevalence of receptor recognition in a collection of 453 *E. coli* strains isolated from patients with urinary tract infection or from the stools of healthy children. Of 150 strains attaching to human uroepithelial cells and agglutinating human erythrocytes, 121 bound to globotetraosylceramide (81%). Globoside recognition was especially frequent among pyelonephritis strains (74/81). The glycolipid composition of the urogenital epithelium and kidney tissue and the ability of uropathogenic *E. coli* to bind to these glycolipids may be a determinant in host-parasite interaction leading to urinary tract infection.

The mechanism of cell-cell interaction and the role of complex carbohydrates in this recognition process are currently discussed (8, 14). One example of cell-cell interaction is the adhesion of bacteria to host cells (9, 16), which has been implicated as a virulence factor in the establishment of infection (9, 16, 36, 40, 40a, 41).

The ability of bacteria to associate with mammalian cells varies between cells from different species, tissues, and even individuals (16, 36, 39, 40a). Each bacterial strain has a special range of cells to which it can bind. The binding characteristics of each bacterial strain may be experimentally classified by the pattern of agglutination of erythrocytes from various species and individuals (3) and by the differential attachment to epithelial cells of various origin (9, 16, 40a). In addition, bacterial binding reactions are classified as mannose sensitive (MS) when reversed in the presence of mannose (3, 34), or as mannose resistant (MR).

Many uropathogenic *Escherichia coli* strains show an MR attachment to urinary tract epithelial cells and agglutination of human erythrocytes (19, 40a). MR attachment to human urinary tract epithelial cells in vitro is related to severity of urinary tract infection in vivo (40, 41). Some of the *E. coli* strains in addition show MS binding to other cells and thus simultaneously carry more than one type of adhesive abilities (10, 40a). Recent results (18, 26, 43) have shown that the MR hemagglutination and epithelial cell attachment of pyelonephritis *E. coli* strains may be explained by the recognition of glycolipids of the globoseries (indicated in Table 1) at the host cell surface.

Glycosphingolipids are anchored in the exterior part of the plasma membrane by their lipophilic part, ceramide, with the carbohydrate chains sticking out from the cell surface (20, 37, 45, 49). The variable carbohydrate chain structures confer the ability to some glycolipids to act as specific receptors. For example, GM_1 ganglioside is the receptor for cholera toxin, and other glycolipids of the ganglioseries may act as receptors for other bacterial toxins and virus (13, 48). Glycolipids of the lactoseries act as blood group A, B, and H and Lewis antigens (11), and the blood group P and P^k (30, 49) and Forssman (11) antigens are found in the globoseries.

The species, tissue, and individual variations of glycolipids (45, 49) make them possible candidates as determinants of tropisms in host-pathogen interactions. A role as host receptors for attaching *E. coli* bacteria has been indicated by both inhibition and direct binding experiments. Thus, glycolipids (or glycolipid-derived saccharides) inhibited the binding of bacteria to cells (7, 18, 26, 43). The range of cells to which bacteria bound correlated with target cell glycolipid composition (18, 23, 26). Direct binding of an *E. coli* strain to a glycolipid (globotetraosylceramide, or globoside) at the cell surface was demonstrated by the agglutination of glycolipid-coated but not of uncoated erythrocytes (26). The aim of the present study was to investigate how general recognition of globotetraosylceramide is among uropathogenic *E. coli* and to what extent this property can account for the adhesive and hemagglutinating abilities of the strains.

MATERIALS AND METHODS

Bacteria. The collection of 453 *E. coli* strains used has been extensively described (10, 40, 40a). The bacteria had been isolated from the urine of girls with acute pyelonephritis (111 strains), acute cystitis (103 strains), and asymptomatic bacteriuria (119 strains) or from the stools of healthy children (120 strains) and kept in deep agar stab cultures until used. For diagnostic criteria, serology, adhesion, and hemagglutination in relation to clinical origin of the strains, see the literature (10, 40, 40a). For testing, the bacteria were cultured in static Luria broth (28), harvested by centrifugation, and suspended in phosphate-buffered saline (PBS) (pH 7.2, 300 mosmol/liter) (39).

The adhesive and hemagglutinating properties of the 453 *E. coli* strains had been examined previously (10, 40). The majority of strains had multiple binding specificities (10). Two main classes of binding reactions were distinguished: MR adhesion to uroepithelial cells and agglutination of human and other erythrocytes, or MS agglutination of guinea pig and other erythrocytes. The strains were classified into groups A through E according to their combination of these binding properties: group A included strains with only MR agglutination of human erythrocytes; group B were strains with MR agglutination of human and MS agglutination of guinea pig erythrocytes; group C were strains with MR agglutination of both human and guinea pig erythrocytes; group D were strains exhibiting only MS agglutination of guinea pig erythrocytes; and group E were non-agglutinating strains. A strain carrying colonization factor I (CFA I) (kindly provided by T. Wadström) was included in some experiments for comparison.

Hemagglutination. A dense bacterial suspension (10^9 bacteria per ml) was mixed on a glass slide with a 3% erythrocyte suspension (20 μl of each). After gentle mixing for 5 min at room temperature, agglutination was read by the naked eye and graded as −, +, ++, or +++. Erythrocytes were harvested from freshly drawn, heparinized blood from guinea pigs, oxen, sheep, rabbits, and horses and suspended in PBS with or without D-mannose (25 mg/ml). Human blood of group A Rh^+ P_1 was obtained from the Blood Bank, Salgrenska Hospital, Göteborg, Sweden, and blood of groups A_1 Rh^+ p and O Rh^+ P_1^k was obtained from the Finnish Red Cross Blood Center, Helsinki.

Adhesion. Attachment to epithelial cells from the urine of one healthy female donor was tested as described earlier (39). The adhesive capacity of a strain is given as the mean number of bacteria attached to 40 epithelial cells. Ability of glycolipids to inhibit attachment was tested by preincubation of bacteria and glycolipid suspensions for 30 min at 37°C, followed by addition of the epithelial cells without washing of the bacteria (26, 43).

Glycolipids. The glycolipids used are shown in Table 1. The glycolipids were prepared by chloroform-methanol extraction, mild alkaline hydrolysis, dialysis, diethylaminoethyl-cellulose chromatography, and silicic acid chromatography (22). The glycolipids were further purified as their acetylated derivatives (12) and were at least 95% pure as judged by thin-layer chromatography. For the preparation of lactoneotetraosylceramide, the major ganglioside of human erythrocytes, *N*-acetylneuraminosyllactoneotetraosylceramide (45), was isolated and degraded by *Vibrio cholerae* neuraminidase (Calbiochem, San Diego, Calif.). The known glycolipid structures were confirmed by mass spectrometry, nuclear magnetic resonance spectroscopy, and degradative methods (5, 6, 21; also references in [2]).

For testing, the glycolipids were taken from solutions in chloroform-methanol, cleared of solvent in a stream of nitrogen, desiccated overnight, and suspended in PBS by sonication in a water bath.

Glycolipid coating of erythrocytes. The technique for erythrocyte coating was originally adapted to the study of blood group antigens (46; B. E. Samuelsson, unpublished data). Erythrocytes were mixed with a glycolipid suspension and incubated with shaking for 3 h at 37°C (50 μl of a 20% erythrocyte suspension and 500 μl of glycolipid suspension). We used 100 μg of glycolipid if not otherwise stated (Table 2). After repeated washing to eliminate unbound glycolipid, the erythrocytes were suspended to a final concentration of 3% in PBS with or without D-mannose (25 mg/ml). During the procedure about 25% of the erythrocytes were lost. This was the same whether erythrocytes were preincubated in PBS only or with different concentrations of glycolipid.

RESULTS

The role of glycolipids in the binding of bacteria to cells was investigated in three types of experiments: (i) agglutination of glycolipid-coated erythrocytes; (ii) agglutination by bacteria of erythrocytes from various species and individuals in relation to the glycolipid composition of the erythrocytes; (iii) inhibition of attachment to epithelial cells by glycolipids.

Agglutination of glycolipid-coated erythrocytes. Erythrocyte membranes absorb or incorporate glycolipids from the outside (29, 38, 46). By the addition of glycolipid to a previously

unreactive erythrocyte a new target cell may be constructed, suitable for study of the role of the added glycolipid as receptor.

Guinea pig erythrocytes were coated with different glycolipids. Ten strains in group A (only MR hemagglutination of human erythrocytes) were used to test the coating method (Table 2). After coating with globotetraosylceramide, the guinea pig erythrocytes became agglutinable by those strains. The degree of agglutination increased with the amount of globoside used for coating (Table 2). A weak agglutination was found after coating with lactoneotetraosylceramide for some bacterial strains. Erythrocytes

TABLE 1. *Name, source, and structure of glycolipids discussed or used in the present investigation*

Name[a]	Source[b]	Structure
Lactosylceramide	Human brain	Galβ1 → 4Glcβ1 → 1Cer
Globotriaosylceramide[c] (P^k antigen)	Human erythrocytes	Galα1 → 4Galβ1 → 4Glcβ1 → 1Cer
Globotetraosylceramide[c] = "globoside" (P antigen)	Human erythrocytes	GalNacβ1 → 3Galα1 → 4Galβ1 → 4Glcβ1 → 1Cer
Globopentaosylceramide[c] = "Forssman pentaglycosylceramide" (Forssman antigen)		GalNacα1 → 3GalNAcβ1 → 3Galα1 → 4Galβ1 → 4Glcβ1 → 1Cer
Lactoneotetraosylceramide = "paragloboside"	Human erythrocytes	Galβ1 → 4GlcNAcβ1 → 3Galβ1 → 4Glcβ1 → 1Cer
IV^3-*N*-Acetylneuraminosyllactoneotetraosylceramide = "sialylparagloboside"	Human erythrocytes	NeuAcα2 → 3Galβ1 → 4GlcNAcβ1 → 3Galβ1 → 4Glcβ1 → 1Cer
II^3-*N*-Acetylneuraminosylgangliotetraosylceramide = GM_1 ganglioside (cholera toxin receptor)	Human brain	Galβ1 → 3GalNAcβ1 → 4Galβ1 → 4Glcβ1 → 1Cer 3 ↑ NeuAc2

[a] Nomenclature and abbreviations used in this paper. The glycolipid nomenclature and symbols follow a recent recommendation (15). The terms globoseries, ganglioseries, and lactoseries refer to families of glycolipids as described in reference 45. Trivial names are indicated according to reference 45 and 49, and the abbreviation GM_1 ganglioside is as in reference 44.

[b] The source is given for the glycolipid material used in experiments.

[c] These compounds belong to the globoseries.

TABLE 2. *Hemagglutination by E. coli of guinea pig erythrocytes coated with different glycolipids*[a]

E. coli strain[b]	PBS	Hemagglutination						
		Globoside				Paragloboside (100 μg)	Sialylparagloboside (100 μg)	GM_1 ganglioside (100 μg)
		10 μg	20 μg	50 μg	100 μg			
3669	−	(+)	++	++	+++	(+)	−	−
1444	−	(+)	+	++	+++	+(+)	−	−
1445	−	+	++	++	++	−	−	−
3976	−	−	+	++	+++	+	−	−
364	−	−	+	++	++	−	−	−
4283	(+)	−	+	++	+++	(+)	−	−
547	−	(+)	+	++	+++	+	−	−
1680	−	−	+	+	++	+	−	−
1682	−	−	+	++	+++	++	−	−
3333	−	−	+	+	++	−	−	−

[a] Erythrocytes were preincubated in glycolipid suspensions (see the text). The figures under each glycolipid name show the amount (per 0.5 ml) used for preincubation of 50 μl of 20% cell suspension.

[b] The results are shown for 10 *E. coli* strains isolated from patients with urinary tract infection and selected from the larger collection (see text) by the property to agglutinate human erythrocytes but not normal guinea pig erythrocytes (as for group A of Table 3).

coated with other glycolipids (Table 2; structures in Table 1) were not agglutinated.

In contrast to the MS agglutination of guinea pig erythrocytes induced by many *E. coli* strains (groups B and D), the agglutination of globotetraosylceramide-coated guinea pig erythrocytes was not reversed by mannose (Table 3). Thus, if a bacterial strain showed MR agglutination of erythrocytes after but not before globoside coating, the strain was considered to recognize this glycolipid at the cell surface. The strains in group D showed only MS agglutination of both normal and globoside-coated guinea pig erythrocytes, indicating that these strains bound to mannose residues on guinea pig erythrocytes but not to globoside (Table 3). The agglutination by strains in group B of normal guinea pig erythrocytes was MS but the agglutination of globoside-coated erythrocytes was MR, covering the MS hemagglutination.

Hemagglutination induced by glycolipid coating could also be demonstrated with ox erythrocytes for strains of groups A and B (Table 4, strains 3669, 1682, 3048).

Some strains (group C, Table 3; 3155, Table 4) already showed MR agglutination of uncoated guinea pig and ox but not of sheep erythrocytes. Sheep erythrocytes remained unreactive after coating with globoside, and therefore strain 3155 did not recognize this receptor but another one. The CFA I-positive strain (Table 4) did not induce MR agglutination of any of the coated erythrocytes and thus also did not recognize globoside.

Relation between natural occurrence of receptor substance and binding of *E. coli* to target cells. The role of globoseries of glycolipids for bacterial binding reactions was evaluated from results using erythrocytes of different blood groups or from different species with varying glycolipid composition (Table 4). The globoseries of glycolipids are antigens in the P blood group system (49). Thus, human erythrocytes of the blood group p lack those glycolipids, group P_1^k erythrocytes lack globoside but possess globotriaosylceramide, and erythrocytes of blood groups P_1 and P_2 possess both globotriaosylceramide and globoside (25, 30). Erythrocytes of these blood groups were used to evaluate the role of globoseries glycolipids (see footnote *c* in Table 1) for bacterial binding, measured as hemagglutination (Table 4).

Strains 3669, 1682, and 3048 all gave MR hemagglutination of blood group P_1 or P_2 erythrocytes (containing globotetraosylceramide), but not of p erythrocytes lacking globoseries glycolipids (25, 30). P_1^k erythrocytes were, however, agglutinated. This indicated that the bacteria recognized globoside and globotriaosylceramide found in the P_1^k cells (18) and shows that this recognition is necessary for the hemagglutination of human erythrocytes by these bacteria.

Strain 3155 and the CFA I-positive strain induced MR agglutination of all the human erythrocytes, without relation to their content of globoseries glycolipids, indicating that these strains recognized receptors other than globoseries glycolipids on the cell surface. When tested against a panel of erythrocytes from various species, strains 3669, 1682, and 3048 all gave MR hemagglutination of sheep erythrocytes (containing globoseries glycolipids; 32) but not of guinea pig, ox, or horse cells (lacking globoseries glycolipids; 35, 47). They also attached to human uroepithelial cells, which possibly contain globoseries glycolipids (26) (Table 5).

Adherence to uroepithelial cells and inhibition by receptor analogs. All strains inferred to recognize globoseries of glycolipids by the above experiments (groups A and B in Table 3 and strains 3669, 1682, and 3048 in Table 4) adhered to human uroepithelial cells (Tables 3

TABLE 3. *Agglutination of glycolipid-coated erythrocytes, demonstration of recognition of globoside for E. coli strains with different hemagglutination patterns, and relation to adhesion to human urinary tract epithelial cells*

E. coli group[a]	No. of strains	Mean adhesion (bacteria per cell)	Agglutination of erythrocytes[b]			Globoside recognition
			Human	Guinea pig	Guinea pig + globoside	
A	10	82	MR		MR	Yes
B	10	57	MR	MS	MR	Yes
C	1	40[c]	MR	MR	MR	No
D	10	6		MS		No
E	7	0				No

[a] Groups of uropathogenic bacteria defined as in the text.

[b] MS signifies hemagglutination reversible by D-mannose (25 mg/ml); MR signifies hemagglutination unaffected by D-mannose.

[c] Attached only to squamous epithelial cells.

TABLE 4. *Agglutination by various E. coli strains*[a]

Target cell	Content of globoseries glycolipids[b]	Binding of *E. coli* strain[c]:					
		3369(A)	1682(A)	3048(B)	3155(C)	6013(D)	CFA I
Untreated erythrocytes							
Human							
A Rh^+ p	−	—[d]			MR		MR
O Rh^+ P_1^k	+	MR	MR	MR	MR		MR
A Rh^+ P_1 or P_2	+	MR	MR	MR	MR		MR
Guinea pig	−			MS	MR	MS	MS
Sheep	+	MR	MR	MR			
Ox	−				MR		
Horse	−			MS	MS	MS	MS
Erythrocytes coated with globotetraosylceramide							
Guinea pig		MR	MR	MR	MR	MS	MS
Sheep		MR	MR	MR			
Ox		MR	MR	MR	MR		

[a] Susceptibility of erythrocytes of different blood groups and species to agglutination by various *E. coli* strains and the relation to natural glycolipid composition and the effect of globotetraosylceramide coating.

[b] +, Natural presence of globoseries glycolipids (marked *c* in Table 1) as major glycolipid components. The data are from references 25, 30, 32, 35, and 47.

[c] Parentheses indicate group designation as defined in the text. CFA I, The CFA I-positive strain.

[d] *E. coli* 3669 and its isolated pili have been suggested to react weakly with certain p erythrocytes (Korhonen et al., manuscript in preparation). The interaction of pili on this and other strains with the receptor will be the subject of a separate report (C Svanborg-Edén, E. Schoolnic, H. Leffler, T. Korhonen, and E. C. Gotschlich, manuscript in preparation).

TABLE 5. *Inhibition of attachment to human urinary sediment epithelial cells by glycolipids*[a]

E. coli strain	Mean no. of bacteria/cell		
	PBS	Globoside[b]	Paragloboside[b]
3669	75	0	55
1682	130	16	130
3048	64	0	64
3155[c]	15	15	16
CFA I positive	0	0	0

[a] Bacteria were preincubated in glycolipid or PBS, and then epithelial cells were added. Attachment was recorded as described in the text.

[b] A 200-μg portion mixed with 200 μg of lactosylceramide.

[c] This strain only bound to squamous epithelial cells, in contrast to the others which bound to both squamous and transitional epithelial cells.

and 5). Strain 3155 (group C), recognizing another receptor, also adhered (Table 5), but neither strain 6013 (not shown) nor the CFA I-positive strain did.

The adhesion of strains 3669, 3048, and 1682, but not 3155, was inhibited by preincubation of bacteria with a suspension of globotetraosylceramide. This inhibition suggested that the added glycolipid competed with the natural receptor for a specific binding site on the bacterial surface. Other factors explaining the inhibition of adhesion, such as decreased viability (trypan blue exclusion; 39) of bacteria or cells or agglutination of bacteria by the added glycolipids (as seen by light microscopy), were not observed. Lactoneotetraosylceramide did not inhibit the attachment of any strain (not shown). In a previous study the addition of lactosylceramide was shown to increase the inhibitory efficiency of globotetraosylceramide (26). This information was used to design the experiments of Table 5, in which globotetraosylceramide combined with lactosylceramide was used for inhibition. The degree of adhesion inhibition of a given concentration of globoside varied with the different strains. For strains 3669 and 3048 it was complete, for strain 1682 it was partial, and for strain 3155 there was none. The results of Table 5 indicated that globoseries glycolipids are receptors for the adhesins of strain 3669, 1682, and 3048 bacteria on urinary tract epithelial cells but not for those of strain 3155. In contrast to the other strains adhering to both transitional and squamous epithelial cells, strain 3155 adhered only to the squamous epithelial cells. This also supports the hypothesis that a different receptor is recognized by this strain.

Agglutination of globotetraosylceramide-coated erythrocytes in relation to clinical origin of the *E. coli* strains. The collection of 453 *E. coli* strains contained 150 strains

(Table 6) with capacity to attach to human uroepithelial cells and to induce MR agglutination of human erythrocytes. Of the 150 strains, 121 bound to globoside, i.e., induced MR agglutination of guinea pig erythrocytes after, but not before, coating with globoside (Table 6). Twenty strains did not bind to globoside. The remaining nine strains (group C) were not tested (see below and footnote *c* in Table 6).

As previously reported, adhesion to sedimented epithelial cells and MR hemagglutination of human erythrocytes are properties common in pyelonephritic *E. coli* strains, but less common for strains isolated from cases of cystitis and asymptomatic bacteriuria and rare among normal fecal strains (Table 6). In all diagnosis groups most strains with adhesive capacity and MR hemagglutination show "globoside recognition." The proportions were 92% of acute pyelonephritis strains, 80% of acute cystitis strains, and 64% of asymptomatic bacteriuria strains and four of eight normal fecal isolates.

Nine strains gave MR hemagglutination of both human and guinea pig erythrocytes (group C). The ability of these bacteria to recognize globoside was not tested, except for strain 3155 as discussed above. Interestingly, such strains were more common among isolates from patients with acute cystitis than from patients with other diagnoses.

The 150 strains were also tested for capacity to agglutinate sheep and ox erythrocytes (Table 6). A variable degree of agglutination of sheep erythrocytes was seen for less than 50% of the strains possessing the property to recognize globoside but not for other strains in groups A, B, and D. A few strains gave agglutination of ox erythrocytes, and this property was carried by most (six of nine) of the strains in group C.

DISCUSSION

The species, individual, and tissue specificity of bacterial binding reactions have implied that bacteria carry ligands (adhesins) binding to specific receptors on the cell surface (9, 16, 40a). One receptor substance has so far been isolated from the target cell (26). In this report we present further data that globoseries glycolipids act as receptors for uropathogenic *E. coli* attaching to human uroepithelial cells and agglutinating human erythrocytes. Three different experimental approaches provided evidence for this conclusion: (i) binding of bacteria to a previously unreactive target cell could be induced by coat-

TABLE 6. *Recognition of globoside, hemagglutination, adhesion, and clinical origin of bacterial strains*

Diagnosis	No. of strains tested	Hemagglutination pattern[a]			No. of strains with this pattern[b]	No. of strains recognizing globoside	No. of strains agglutinating sheep erythrocytes	Mean adhesion (bacteria per epithelial cells)
		Human	Guinea pig	Group				
Acute pyelonephritis	111	MR		A	11	10	3	70
		MR	MS	B	69	64	17	62
		MR	MR	C	1	NT[c]	1	54
		Others			30	0	NT	4
Acute cystitis	103	MR		A	1	1	1	81
		MR	MS	B	32	28	17	61
		MR	MR	C	6	NT	3	60
		Others			64	0	NT	11
Asymptomatic bacteriuria	119	MR		A	1	0	0	64
		MR	MS	B	20	14	5	62
		MR	MR	C	1	NT	0	54
		Others			97	0	NT	8
Normal fecal isolates	120	MR		A	1	1	1	48
		MR	MS	B	6	3	0	52
		MR	MR	C	1	NT	1	29
		Others			112	0	NT	12

[a] MR, Hemagglutination unaffected by D-mannose; MS, hemagglutination reversible by D-mannose. Bacterial groups A, B, and C are defined by the hemagglutination patterns of human and guinea pig erythrocytes as described in the text.

[b] Only strains showing MR hemagglutination of human erythrocytes were tested. Discrepancies compared to the results in reference 10 are due to variations in individual broth cultures.

[c] NT, Not tested because the guinea pig erythrocytes were agglutinated (MR) already before coating.

ing of the unreactive cell with globoseries glycolipids; (ii) the adhesion of *E. coli* strains to uroepithelial cells was inhibited by globoseries glycolipids; (iii) binding of bacteria to epithelial or erythrocyte target cells was related to their content of globoseries glycolipids. Binding to the globoseries glycolipid receptors was common among strains causing acute pyelonephritis, suggesting a role in the pathogenesis of urinary tract infection.

The coating of erythrocytes and other cells with glycolipids has earlier been used to study blood group antigens (29, 46), the Forssman antigen (38), and the cholera toxin receptor (13, 33). The glycolipids incorporated in the cell membrane showed biological receptor activity, i.e., mediated specific hemagglutination (29, 46), capping by antibodies (38), and stimulation of adenyl cyclase by cholera toxin (13, 33). The effect of all cases was due to the specific recognition and binding of antibody or toxin to the carbohydrate chain of the glycolipid. The present results may also be explained by the binding of bacterial surface ligands to the tetrasaccharide portion of globotetraosylceramide.

The recognition by bacteria of the glycolipid receptor, demonstrated as the property to agglutinate glycolipid-coated erythrocytes, was specific in two ways. First, it could be shown for some bacterial strains (group A and B and strains 3669, 1682, and 3048), but not others (strains 3155 and 6013 and the CFA I-positive strain). Second, it could be demonstrated with one glycolipid, globoside, but not others with different carbohydrate chains (Table 2). This property was probably due to the binding of a ligand, carried by the active strains, to the carbohydrate chain of globoside. All strains recognizing globoside coated onto erythrocytes also carried the capacity to adhere to urinary sediment epithelial cells and to agglutinate human erythrocytes. Globoside recognition thus seemed decisive for the adhesion and hemagglutination of these strains. For three strains (3669, 3048, and 1682) selected for detailed studies there are two other lines of evidence suggesting that globotetraosylceramide is important as a receptor in these reactions. First, the attachment to uroepithelial cells was inhibited after pretreatment of bacteria with globoside (but not with paragloboside, another glycolipid). This indicated that the added globoside either competed for the ligand binding sites or otherwise hindered their function. Second, as shown in Table 4, bacterial hemagglutination correlated with the natural presence of globoside and other glycolipids of the globoseries in the erythrocytes (see footnote *c*, Table 1).

Blood group P_1^k erythrocytes lack globoside but contain globotriaosylceramide. The fact that such cells were agglutinated indicated that the bacteria could recognize also the latter glycolipid (see also references 18 and 26) and possibly other structurally related glycolipids (see below and references 18 and 26) such as the Forssman pentaglycosylceramide, the major glycolipid of sheep erythrocytes (32). The glycolipid fractions isolated from natural sources may be extremely complex (see, for example, references 1, 2, 11, 45, 49), and the complete separation of individual components is rarely possible. Although the presence of minor glycolipid contaminants in the purified samples cannot be ruled out, the globoside was most probably the active component. Therefore, the results taken together with those of hemagglutination studies (Table 4; 18) suggest that bacteria recognize a common feature of the globoseries glycolipids oligosaccharide chains, i.e., the —Galα1→4Galβ1— part (18, 26). The latter is a terminal part of globotriaosylceramide and the P_1 antigen and an internal part of globoside and the Forssman glycolipid hapten (Table 1). The disaccharide Galα1→4Gal has been synthesized and found to inhibit hemagglutination (G. Källenius, S. B. Svensson, R. Mollby, J. Winberg, H. Hultberg, P. Garegg, and A. Lundblad, FEMS Symposium on Microbial Enve-

TABLE 7. *Possible recognition of various receptors in different cells by E. coli strains as interpreted from the present results*

Cells	Receptors recognized			
	Globoseries glycolipids	Mannose residues	Unknown I	Unknown II
Bacterial strain				
3669	+			
1682	+			
3048	+	+		
3155		+	+	
6013		+		
CFA I positive		+		+
Target cells				
Erythrocytes				
Human[a]	+	(+)[b]	+	+
Sheep	(+)			
Ox			(+)	
Horse		+		
Guinea pig		+	+	
Urinary sediment epithelial cells	+		+	

[a] Erythrocytes of the rare blood group p lack globoseries glycolipids (25, 30).

[b] (+), Weaker or variable recognition; compare Tables 4 and 6 and text.

lopes, Saimaanranta, Finland, abstr. no. 113, 1980). Furthermore, the tetrasaccharide released from globotetraosylceramide recently was shown to inhibit attachment to uroepithelial cells (Leffler et al., Scand. J. Infect. Dis. Suppl., in press).

To define the chemical specificity and mechanisms of the interaction between bacterial ligands and epithelial surface receptors, the purified interacting components must be studied (compare the much more well-developed knowledge of the interaction of antibodies and antigens in reference 17). The present investigation is a step towards better knowledge of the receptor side.

The nature of the bacterial ligand(s) and the structural basis for the interaction with the receptor are presently being investigated. Pili isolated from *E. coli* strains 3669 and 3048 have been shown to interact with the tetrasaccharide of globotetraosylceramide (Leffler et al., in press). The functional binding site on pili is likely to be shared by different strains, also in the absence of serological or amino acid sequence relatedness of the pili. Heterogeneity in the specificity of these ligands was indicated since only about 30% of strains recognizing globotetraosylceramide also agglutinated sheep erythrocytes and showed differential behavior towards blood group p erythrocytes (Korhonen et al., Scand. J. Infect. Dis. Suppl., in press).

Some of the strains tested recognized receptors unrelated to the globoseries of glycolipids. One such group of receptors was likely to contain D-mannose, since many bacterial reactions were inhibited in the presence of D-mannose (3, 10, 34). The range of erythrocytes involved in MS agglutination was in agreement with that earlier described (3) except for the weaker hemagglutination of human and sheep erythrocytes reported. The property to recognize mannose occurred independently of the property to recognize globoseries glycolipids. For strains carrying both properties, the hemagglutination pattern and adhesive abilities were the sum of those for strains carrying either property alone. Thus, for strain 3048 the recognition of globoseries glycolipids resulted in the agglutination (MR) of human and sheep erythrocytes and adhesion to sediment epithelial cells, and in addition to this the mannose recognition resulted in hemagglutination (MS) of guinea pig and horse erythrocytes. Table 7 shows the recognition of various receptors by different bacterial strains and the hypothetical occurrence of the receptors in various erythrocytes and epithelial cells. Of the receptors proposed to exist, only globoseries glycolipids have been isolated from target cells (45, 49). Mannose residues are found in cell surface glycoproteins (24), but it is not known which of these are recognized by bacteria.

The existence of non-globoseries, non-mannose-containing receptors (unknown receptors I and II, Table 7) was indicated by the MR hemagglutination by strain 3155 and the CFA I-positive strain of human erythrocytes despite the fact that both strains lacked the ability to recognize globoside coated onto erythrocytes. The agglutination was independent of P blood group, and strain 3155 also gave MR agglutination of guinea pig and ox erythrocytes. *E. coli* 3155, but not the CFA I-positive strain, attached to human urinary tract epithelial cells, and globoside did not inhibit the attachment of strain 3155. A different receptor for the adhesion of strain 3155 to epithelial cells was also indicated by the fact that this strain adhered only to squamous epithelial cells, in contrast to the globoside-recognizing strains, which adhered to both transitional and squamous epithelial cells. In adhering only to squamous epithelial cells *E. coli* 3155 resembles *Proteus mirabilis* strains (42).

The ability to attach to human sediment epithelial cells and to agglutinate human erythrocytes is related to virulence for *E. coli* causing human urinary tract infection (40, 40a, 41). These properties were common for pyelonephritic *E. coli* but rare for normal fecal *E. coli* isolates. The results of the present study show that for most strains the adhesive and hemagglutinating capacities are paralleled by and probably due to the recognition of globoside.

How does the binding of globoside increase virulence of bacteria in the urinary tract? Globotetraosylceramide and globotriaosylceramide are at most minor components of human urinary tract epithelial cells (26, 43; Leffler, unpublished data). In the human kidney globoside is, however, the major glycolipid (31). Of the strains recognizing globoside in the present material, 60% were isolated from cases of pyelonephritis. In contrast to this, six of the nine strains of group C (Tables 3 and 6) recognizing another receptor on the epithelial cells were from cases of cystitis.

On the host side, individual variations of receptor density were indicated to correlate with frequency and severity of infection (27). The density of host receptors and the capacity of the bacteria to bind to globoseries glycolipids may thus determine the likelihood of urinary tract infection resulting from, and the location in the kidney of, *E. coli* entering the urinary tract. Screening for the latter property by use of glycolipid-coated erythrocytes or receptor analogs

bound to other particles could prove clinically useful.

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