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The Receptor Repertoire Defines the Host Range for Attaching *Escherichia coli* Strains That Recognize Globo-A

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***Escherichia coli* strains which colonize the human urinary tract express lectins specific for different members of the globoseries of glycolipids, e.g., globotetraosylceramide and globo-A. This study investigated the importance of globo-A expression for attachment to human uroepithelial cells, colonization of the urinary tract, and severity of urinary tract infection. The expression of receptor-active glycolipids by erythrocytes and epithelial cells was analyzed by thin-layer chromatography and bacterial overlay as well as by bacterial binding to those cells. The epithelial expression of the globo-A receptor was restricted to individuals of blood group A with a positive secretor state. Consequently, globo-A binding *E. coli* strains attached only to epithelial cells from these individuals. In contrast, globoside-recognizing strains attached in similar numbers to uroepithelial cells regardless of the ABH blood group and secretor state of the donor. The role of host receptor expression for infection with globo-A-specific *E. coli* was analyzed in 1,473 children with urinary tract infections. All those infected with strains exclusively expressing globo-A-specific adhesins were found to be of blood group A, compared with 45% in the population at large ($P < 0.006$). The inflammatory response (fever, C-reactive protein, erythrocyte sedimentation rate) of individuals infected with these strains was lower than that in individuals with infections caused by globoside binding strains. The results demonstrate the importance of fitness between host receptors and bacterial adhesins for infection and suggest that minor receptor epitope differences have profound effects on the disease process.**

Epithelial cell glycoconjugates act as receptors for attaching bacteria (3, 4, 15, 17–19, 27). Their oligosaccharide moieties vary extensively, between species, individuals, and tissues (17, 19). Molecules with receptor function for a given bacterial adhesin can be present or absent (15, 18, 35), and their availability for bacterial binding may be influenced by other constituents of the epithelial cell membrane. The susceptibility of the individual to colonization and infection with attaching bacteria can be expected to vary accordingly (35).

The globoseries of glycolipids (Table 1) mediate the attachment of *Escherichia coli* to human uroepithelial cells (15, 17–19). Their expression is determined by the P blood group (26), while the elongation of the core structure depends on the ABH blood group and secretor state (6, 7, 17, 30, 31). We recently described a group of uropathogenic *E. coli* strains which bind with high affinity to globotetraosylceramide elongated with the blood group A determinant (globo-A), but with low affinity to globotetraosylceramide (22, 36). These strains require the presence of globo-A or the Forssman glycolipid hapten to bind to target cells (22, 24, 38). In contrast, the receptor for most attaching uropathogenic *E. coli*, globotetraosylceramide, is present in all individuals except those of blood group p (17, 26). This blood group-dependent expression of the receptors provided a basis to analyze the role of receptor repertoire for the selection of the bacteria which successfully colonize and infect the human urinary tract.

The aim of the present study was to analyze the influence on bacterial adherence of the blood group-specific expression of mucosal receptors, the selection of bacteria causing urinary tract infections (UTI), and the type of disease produced by these strains.

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MATERIALS AND METHODS

Glycolipid expression in relation to blood group. (i) Cell donors. Urinary tract epithelial cells and erythrocytes were obtained from individuals of blood group A_1P_1 secretor, A_1P_1 nonsecretor, and A_2P_2 secretor. The A individuals were identified by hemagglutination with anti-A antibody (Dakopatts, Copenhagen, Denmark), and the A_1 individuals were identified with the *Dolichus biflorus* lectin (Boehringer GmbH, Mannheim, Germany) (28). The P_1 individuals were identified by agglutination with anti-P_1 antiserum (Boehringer Ingelheim). The secretor state was determined by the ability of boiled saliva samples to inhibit the agglutination of A erythrocytes by the anti-A antibodies (28). Uroepithelial cells were harvested by centrifugation at $250 \times g$ for 10 min and resuspended in phosphate-buffered saline (PBS) (pH 7.2, 0.15 M). Erythrocytes were collected from freshly drawn heparinized blood.

(ii) Glycolipid extraction method. The technique of Magnani et al. (25) combines glycolipid separation by thin-layer chromatography (TLC) and binding of biological ligands with defined specificities to characterize individual glycosphingolipids in nanomolar (microgram) concentrations. The glycolipid extraction method described here was designed for the analysis of small tissue biopsy specimens or low numbers of isolated cells, which do not permit the complete purification of glycolipids. Only those purification steps necessary to obtain a lipid fraction suitable for TLC analyses were kept from previous extraction protocols (16).

TABLE 1. Expression and receptor activity of globoseries glycolipids in human uroepithelial cells

No.	Structure	Expression as related to host blood group (P_1/P_2)				Receptor activity for *E. coli* strain	
		Secretor A_1	Secretor A_2	Nonsecretor A_1	p	506MR	1484
1	Galα1-4Galβ1→4Glcβ1-1Cer	+	+	+	−	+	−
2	GalNAcβ1-3Galα1-4Galβ1→4Glcβ1-1Cer	+	+	+	−	++	+
3	Galβ1-3GalNAcβ1-3Galα1-4Galβ1→4Glcβ1-1Cer	+	+	+	−		
4	Galβ1-3GalNAcβ1-3Galα1-4Galβ1→4Glcβ1-1Cer 2 Fucα1	+	+	−	−	+	+
5	GalNAcα1-3Galβ1-3GalNAcβ1-3Galα1-4Galβ1→4Glcβ1-1Cer 2 Fucα1	+	−	−	−	+	++
6	GalNAcα1-3GalNAcβ1-3Galα1-4Galβ1→4Glcβ1-1Cer	−	−	−	−	+	++

Packed erythrocytes or epithelial cells (1 ml) were mixed with 2 ml of methanol, sonicated for 2 min in a water bath (Branson 2200, Kebo, Sweden), incubated at 65°C for 1 h, and centrifuged at 2,000 × *g* for 10 min. The supernatant was transferred to a new tube, and the pellet was resuspended in 2 ml of methanol and treated as above. The supernatants were combined, and the pellet was resuspended in 1 ml of methanol–0.5 ml of chloroform, extracted, and combined with the previous supernatants. The supernatants (6.5 ml) were dried under nitrogen, and the lipid extract was subjected to mild alkaline degradation by treatment with 0.5 ml of 0.2 M KOH in methanol for 3 h at room temperature. After methanolysis, 5 μl of concentrated acetic acid was added to neutralize the pH and 2 ml of methanol, 0.25 ml of chloroform, and 2.25 ml of distilled water were added and the methanolysate was desalted on a prepacked 0.5-g C-18 Bond Elute column (Analytichem International, Harbor City, Calif.). The column was prewashed with 10 ml of chloroform-methanol-water (1:10:9, vol/vol/vol) and kept wet until the lipid extract dissolved in the same solvent was supplied (up to 5 mg of lipid). The lipids were finally eluted with 10 ml of chloroform-methanol (2:1, vol/vol). To eliminate the sphingomyelins and the remaining alkali-stable phospholipids from the rest of the sphingolipids, the fraction was dried and acetylated overnight in a mixture of 0.1 ml of chloroform, 0.1 ml of pyridine, and 0.1 ml of acetic acid anhydride. The solvents were evaporated after repeated addition of small volumes of methanol and toluene. The acetylated fraction was then dissolved in 1 ml of chloroform-methanol (98:2, vol/vol) and separated on a 0.1-g Si Bond Elute column. Acetylated sphingomyelins were eluted with 5 ml of chloroform-methanol (1:3, vol/vol) and 5 ml of methanol. The acetylated fraction was deacetylated in 10 μl of deacetylating reagent (10% 0.2 M KOH in methanol, 70% methanol, 20% toluene), neutralized with 1 μl of acetic acid, and analyzed without further purification.

The yield of the different steps of the extraction procedure was determined by using globoside which had been labeled with tritiated sodium borohydride (specific activity, 150 Ci/mmol) as previously described (34). One milliliter of packed human erythrocytes was mixed with tritiated globoside (45,300 cpm) and subjected to extraction. The recovery of the radiolabeled glycolipid was 98.8% after one reversed-phase chromatography step. The overall recovery was 92%. For bacterial binding studies, the purification protocol may be terminated after the first alkali degradation step.

(iii) Bacteria. *E. coli* 506MR was obtained by transformation of the fecal *E. coli* 506 with pRHu845 carrying the *pap* DNA sequences inserted into the chloramphenicol site of pACYC184 (12). The *pap* DNA sequences were derived from *E. coli* J96 and encoded F13 fimbriae and adhesins specific for the Galα1-4Galβ-containing glycolipids, with globotetraosylceramide as the high-affinity receptor (22). *E. coli* 506MR was maintained on tryptic soy agar with 10 μg of tetracycline per ml.

The wild-type strain *E. coli* 1484 and the transformant HB101/pJFK102 expressed fimbriae and adhesins specific for the globoseries of glycolipids with a terminal αGalNAc, e.g., globo-A and Forssman. *E. coli* HB101 was transformed with pJFK102 containing the *prs* (*pap*-related sequence) DNA sequence from *E. coli* J96 inserted into the tetracycline site of pBR322. HB101/pJFK102 was maintained on tryptic soy agar with 100 μg of ampicillin per ml.

The receptor specificity of the adhesins was shown by agglutination of erythrocytes and Galα1-4Galβ-coated latex beads and binding to glycolipids on TLC plates. *E. coli* 506MR agglutinated human erythrocytes in a P blood group-dependent but ABH blood group-independent manner, agglutinated Galα1-4Galβ–latex beads, and bound to Galα1-4Galβ-containing glycolipids on TLC, with the highest affinity for globotetraosylceramide (22) (Table 2).

E. coli 1484 and HB101/pJFK102 agglutinated human erythrocytes in a P and A blood group-dependent manner, i.e., they agglutinated cells from donors of blood group A_1P_1 but not OP_1 or Ap (22, 36). They reacted poorly or not at all

TABLE 2. Binding of *E. coli* strains with Galα1-4Galβ- and globo-A-specific adhesins to erythrocytes and epithelial cells from donors differing in A blood group and secretor state

Blood group	Glycolipid extracts positive for globo-A on TLC overlay	*E. coli* binding	
		506MR	1484
Erythrocytes			
A_1 secretor	+	MR[a]	MR
A_1 nonsecretor	+	MR	MR
A_2 secretor	+	MR	−
O nonsecretor	−	MR	−[b]
A_1p	−	−	−
Uroepithelial cells			
A_1 secretor	+++	+	+
A_1 nonsecretor	−	+	−
A_2 secretor	−	+	−

[a] MR, Mannose resistant.
[b] Agglutination at +4°C.

with the Galα1-4Galβ–latex beads or with latex beads coupled with the blood group A trisaccharide (36). On TLC plates, they bound with high affinity to glycolipids with an internal Galα1-4Galβ disaccharide and a terminal αGalNAc, i.e., the globo-A and Forssman glycolipids, but with low affinity to globotetraosylceramide and not to the members of the globoseries with shorter oligosaccharide sequences (Table 2). In contrast to previously used blood group A-reactive monoclonal antibodies (33), this strain recognized the A terminal only when linked to a globoseries core and was therefore used as a reagent to discriminate between A type 4 and the A determinant on other type chains (4, 5, 20).

(iv) 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 resuspension in PBS. The labeled bacteria were then suspended in PBS to approximately 10^8 CFU/ml. Their specific activity ranged from 100 to 200 CFU/cpm.

Glycolipids were separated on Kieselgel 60, alumina-backed HPTLC plates (E. Merck AG, Darmstadt, Federal Republic of Germany) by using chloroform-methanol-water (60:35:8, vol/vol/vol). The bacterial overlay was performed as previously described (4, 22). 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 incubated with 2% bovine serum albumin in PBS for 2 h to reduce nonspecific binding to 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 the binding was detected by autoradiography (22). Blood group A-active glycolipids were detected with a mouse monoclonal anti-A antibody (A581; Dakopatts, Glostrup, Denmark) and iodine-labeled rabbit anti-mouse antibody (Dakopatts).

Blood group and susceptibility to UTI. (i) Patients. A total of 1,473 children, 1,200 girls (median age, 3.8 years) and 273 boys (median age, 0.5 years), were enrolled in a prospective study of UTI at the Pediatric Nephrology Unit, The Children's Hospital, Göteborg, Sweden. Diagnosis and treatment of UTI as well as follow-up were done according to a standardized protocol (14).

The host response to each bacteriuria episode was characterized by the rectal body temperature (°C) (afebrile patients were assigned the value of 37°C), C-reactive protein in serum (CRP) (milligrams per liter), and erythrocyte sedimentation rate (ESR) (millimeters per hour). Leukocyte excretion was quantitated in uncentrifuged urine by direct microscopy with a Fuchs-Rosenthal chamber (cells per cubic millimeter). The maximal renal concentrating capacity was measured as the highest urine osmolality in two consecutive samples obtained either after fluid deprivation for 15 to 18 h or after intranasal administration of a vasopressin analog (Minirin; Ferring AB, Malmö, Sweden) and given as the standard deviation score in relation to age-matched controls without UTI. A diagnosis of acute pyelonephritis was based on bacteriuria, a temperature of at least 38.5°C, elevated CRP (>20 mg/liter) and/or ESR (>25 mm/h), and/or a temporary decrease in renal concentrating capacity. Acute cystitis was defined by burning and frequency of urination in patients with a temperature of <38°C and without changes in CRP, ESR, or concentrating capacity. Asymptomatic bacteriuria was defined as significant bacteriuria (>10^5/ml) with the same strain of bacteria present in at least two consecutive cultures in children without symptoms or abnormal laboratory findings.

(ii) Bacteria. Urine cultures were obtained from clean catch specimens in children older than 2 years. In infants, positive bag samples were confirmed by suprapubic bladder aspiration. Positive cultures were defined as >10^5 bacteria per ml of voided urine or as any growth in suprapubic aspirates.

Members of the family *Enterobacteriaceae* were identified by routine methods and stored in deep agar cultures. A total of 2,504 *E. coli* isolates were saved from the 1,473 children. Adhesin expression was defined by hemagglutination and reactivity with the Galα1-4Galβ-coupled latex beads. The Galα1-4Galβ adhesins caused a P blood group-dependent but ABH blood group-independent agglutination of human erythrocytes and agglutinated the Galα1-4Galβ-coupled latex beads. Globo-A specificity was defined by blood group A- and P-dependent agglutination of human erythrocytes and by the lack of reactivity with the Galα1-4Galβ–latex beads. In addition, the adhesins of strains inducing mannose-resistant agglutination of human erythrocytes of blood group p were defined as MRp, those of strains causing only mannose-reversible agglutination of guinea pig erythrocytes were defined as MS, and those lacking reactivity with human or guinea pig erythrocytes were defined as adhesin negative.

(iii) Statistical methods. Correlation coefficients were calculated by the Pearson product moment correlation method. Linear regressions were analyzed by the GLM procedure of the Statistics Analysis System program. The correlation coefficients were compared between episodes caused by Galα1-4Galβ-positive and globo-A strains by the method of Morrison (29).

RESULTS

Uroepithelial glycolipids. Glycolipids were extracted from uroepithelial cells of donors of blood group A_1P_1 nonsecretor, A_2P_2 secretor, and A_1P_1 secretor, using the new simplified glycolipid extraction protocol (see Materials and Methods). The extracts were fractionated by TLC, and specific glycolipids were detected by overlay with radiolabeled bacteria or monoclonal antibodies of defined specificity (Fig. 1).

E. coli 506MR bound with high affinity to globotetraosylceramide (structure 1, Table 1) and bound to other Galα1-4Galβ-containing glycolipids with lower affinity, albeit sufficient for detection by TLC analysis (22). Staining of the epithelial cell glycolipid extracts with this strain showed the presence of glycolipids with three and four sugar residues corresponding to globotria- and globotetraosylceramide (structures 1 and 2, Table 1). These components occurred in all the urinary sediment samples (A_1P_1 secretor, A_1P_1 nonsecretor, and A_2P_1 secretor). In addition, the A_1 nonsecretor sample contained a weakly staining pentaglycosylceramide proposed to be structure 3 in Table 1. The A_2 sample contained a hexaglycosylceramide interpreted as structure 4 in Table 1.

E. coli 1484 bound with high affinity to structures 5 (globo-A) and 6 (Forssman antigen) (Table 1) and with low affinity to several other Galα1-4Galβ-containing glycolipids (22). This strain stained only one band in the A_1 secretor sample, corresponding to a heptaglycosylceramide (Fig. 1). This band had blood group A reactivity, as seen by staining with the anti-A monoclonal antibody A581. There was no reactivity with glycolipids from the other donors or in the

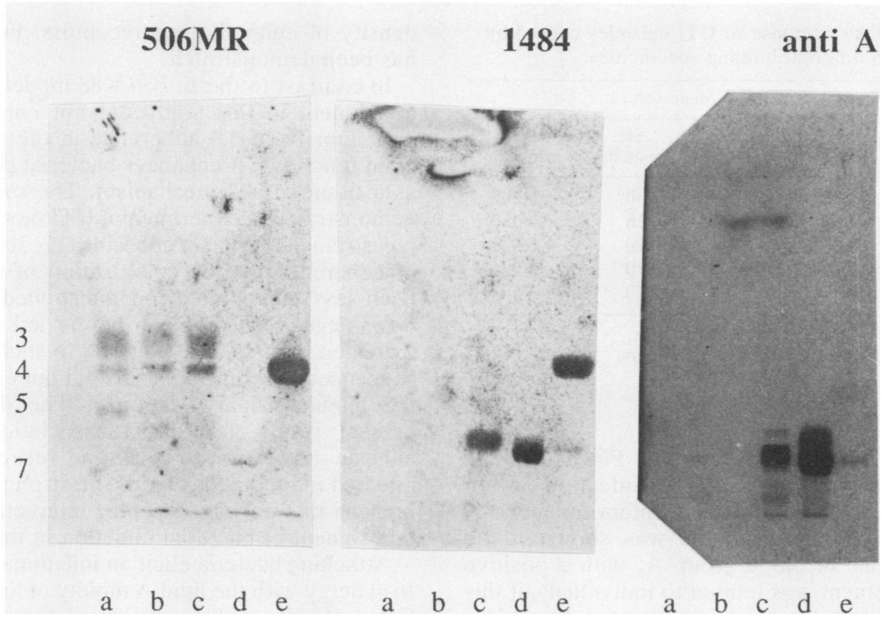

FIG. 1. Glycolipid extracts from uroepithelial cells. The glycolipids were extracted from donors of different blood groups. Lanes: a, A_1 nonsecretor; b, A_2 secretor; c, A_1 secretor; d, the references A hexaglycosylceramide on a type 2 chain and A heptaglycosylceramide on a type 1 and type 4 chain; and e, the reference glycolipid globoside. The numbers on the left indicate the numbers of saccharides in the glycolipids. The reagents used for overlay were *E. coli* 1484 and 506MR and the anti-A antibody A581. The extraction procedure is described in the Materials and Methods.

pentaglycosylceramide region, corresponding to the Forssman antigen.

(i) Bacterial adherence in relation to blood group. The receptor function of the glycolipids in the intact cell membrane and the role of blood group for this function were analyzed by bacterial adherence (Table 2). The Galα1-4Galβ-specific strain *E. coli* 506MR attached in similar numbers to cells from the three donors. In contrast, *E. coli* 1484 attached only to the epithelial cells from the blood group A donor with a positive secretor state.

Blood group and susceptibility to UTI. The 2,504 *E. coli* strains from the 1,473 children were analyzed for adhesin expression by hemagglutination and agglutination of Galα1-4Galβ–latex beads. Nine infections in eight individuals were caused by strains exclusively recognizing globo-A but negative for the other adhesins. Six individuals infected with seven of these strains were available for ABH blood group determination. All of them were A positive, compared with 45% in the population at large (22). The probability of this outcome is less than 0.006.

(i) Reduced inflammatory response to globo-A-specific strains. During the study, it became apparent that the majority of globo-A binding strains had not caused acute pyelonephritis. This was in contrast to strains with the Galα1-4Galβ-positive phenotype, which are enriched in those infections (8). We therefore analyzed the types of infection caused by *E. coli* strains with the different receptor specificities (Table 3). The Galα1-4Galβ-specific strains caused episodes diagnosed as acute pyelonephritis in 48% of the cases. In contrast, only one of nine (11%) of the globo-A strains caused acute pyelonephritis. This frequency was similar to that observed for strains with MRp or MS adhesin or without detectable adhesins.

The diagnosis of UTI is based on the intensity of the inflammatory response. Patients infected with Galα1-4Galβ-positive strains are known to mount a higher inflammatory response than those infected with other strains (8). Here, the level of fever, CRP, ESR, and leukocytes was compared between episodes caused by *E. coli* expressing adhesins of the globo-A, Galα1-4Galβ, MRp, or MS specificity. The inflammatory response to the globo-A strains was not different from that to the MS, MRp, or adhesin-negative strains but was significantly lower than that to the Galα1-4Galβ-positive strains (Table 4).

DISCUSSION

The term fitness describes the ability of bacteria to establish and maintain a population in a specific ecological habitat. Adherence enhances fitness by promoting bacterial persistence at mucosal surfaces (10, 11). For host receptor expression to influence colonization and/or disease, adherence must significantly contribute to fitness. The classic example is piglet diarrhea caused by *E. coli* expressing the K88 adhesin (37). Adherence enhanced the colonization of the small intestine, and both adherence and toxin production were required for disease (37). The susceptibility to disease was further controlled by the presence or absence of small

TABLE 3. Clinical diagnosis of 2,504 UTI episodes in relation to the adhesin expression of the strains

Diagnosis	Adhesin expression (%) for				
	Galα1-4Galβ (n = 1,240)	Globo-A (n = 9)	p (n = 163)	MS (n = 593)	Negative (n = 499)
Pyelonephritis	48[a]	11	15	14	16
Asymptomatic bacteriuria	24	44	35	52	57
Other	28	44	50	34	27

[a] $P < 0.05$ compared with the globo-A binding strains.

TABLE 4. Inflammatory response to UTI episodes caused by *E. coli* with different binding specificities

Binding specificity	No.	Mean values			
		Fever (°C)	CRP (mg/liter)	SR (mm/h)	Leukocytes (cells/mm^3)
Galα1-4Galβ	1,327	38.2[a]	42[b]	30[a]	1,203
Globo-A	9	37.5	17	18	676
MRp	175	37.5	17	20	1,326
MS	646	37.5	15	20	725
Negative	560	37.4	19	20	653

[a] $P < 0.01$ compared with the globo-A binding strains.
[b] $P < 0.001$ compared with the globo-A binding strains.

intestinal receptors for the K88 adhesin. Piglets lacking receptors were resistant to colonization and infection (35). In the present study, the expression of receptors for globo-A binding *E. coli* on uroepithelial cells was shown to be restricted to individuals of blood group A_1 with a positive secretor state. Attachment was limited to individuals of this phenotype. In accordance with the receptor expression, strains recognizing globo-A preferentially colonized individuals of blood group A. This finding confirms in humans the importance of fitness between the receptor specificity of bacterial adhesins and mucosal receptor expression for colonization and infection.

There were some interesting discrepancies between bacterial binding to glycolipids on TLC plates and to intact cells. Besides its high affinity for globo-A, *E. coli* 1484 also bound with lower affinity to some glycolipids (22) (e.g., globotetraosylceramide, structure 2, Table 1). This low-affinity binding was not sufficient to mediate attachment to the globotetraosylceramide-containing epithelial cells or to allow detection of this structure in uroepithelial cell glycolipid extracts. Bacteria bound on TLC to extracts from erythrocytes, in which globotetraosylceramide was much more abundant, but did not mediate hemagglutination (22). The Galα1-4Galβ-specific strain bound to the Forssman antigen on TLC plates but did not agglutinate sheep erythrocytes. These examples demonstrated that the presence or absence of receptor-active glycolipids in target cells cannot be deduced from bacterial binding to the cells and that binding to cells cannot be predicted from results of bacterial binding to extracted glycolipids on TLC plates. This is analogous to the results with lectins specific for the GalNAc residue (39).

The present study was made possible by the dual function of the globoseries of glycolipids as bacterial receptors and blood group antigens. Once the combined requirement of P and A blood group for globo-A expression was shown on erythrocytes (22, 36) and the selective epithelial globo-A expression by secretors was demonstrated in this study, we could deduce the individual variation in receptor expression from the blood group. In theory, it should be possible to evaluate the role of epithelial receptor expression for susceptibility to UTI by analysis of blood group p individuals, who lack the globoseries of glycolipids and consequently the receptor for Galα1-4Galβ-positive *E. coli*. The group of individuals with this metabolic error is, unfortunately, too small for epidemiologic analysis. Other P blood group variables, however, show epidemiologic association with disease. Children of blood group P_1 have an increased relative risk for recurrent pyelonephritis compared with those of the P_2 phenotype (23, 23a). This was proposed to depend on the density of epithelial cell receptors, but no such difference has been demonstrated.

In contrast to the *E. coli* K88 model, the globo-A-specific attachment in this study did not enhance the severity of infection. Bacterial adherence in the urinary tract has two main functions: it enhances bacterial persistence and serves as a tissue attack mechanism. The role in persistence was demonstrated in experimental UTI models, using isogenic *E. coli* strains differing in adhesins (11, 30). In humans, the role of adherence for the colonization of the urinary tract has been less clear and even questioned. Phenotypically adhesin-negative *E. coli* can be carried asymptomatically for extended periods. The addition to such a strain of the DNA sequences encoding adhesins did not enhance persistence in the human urinary tract (1). The demonstration in the present study of a direct association between bacterial adhesin expression in vitro and receptor repertoire of the infected individual provides the strongest evidence so far in humans that adhesin-receptor interaction influences the establishment of bacterial infection in the urinary tract.

Attaching bacteria elicit an inflammatory response (8, 21). In synergy with the lipid A moiety of lipopolysaccharide, the Galα1-4Galβ-specific adhesins activate mucosal cytokines, e.g., interleukin-6, and recruit polymorphonuclear cells to the mucosa. The cascade of cytokines can, in turn, give rise to fever and elevated CRP and ESR. In this study, the globo-A-specific strains caused a lower inflammatory response than the Galα1-4Galβ-specific strains. Consequently, these infections were diagnosed as asymptomatic rather than as acute pyelonephritis. This may have several explanations. First, the amount of receptor may determine the strength of the bacterial interaction with the host and, indirectly, the host response. Globo-A is a minor component of uroepithelial glycolipids compared with globotetraosylceramide, the high-affinity receptor strains for the Galα1-4Galβ-specific adhesins. Second, although the receptors are part of the same group of glycolipids, the globo-A strains bind to an epitope further out from the ceramide portion than the Galα1-4Galβ disaccharide. Consequently, the approximation to the cell surface of other bacterial products required for activation of inflammation may be less effective. Third, globoside but not globo-A may act as a signal transducer after lectin binding and activate the acute-phase response. Fourth, there are possible explanations for the lower inflammatory response to the globo-A binding strains other than qualitative differences in ligand-receptor interaction. The globo-A binding strains may lack other virulence-associated factors which explain the inflammatogenicity of the Galα1-4Galβ binding strains.

The affinity of the globo-A-specific adhesins for the A blood group-positive hosts may be of greater quantitative importance than suggested by the low frequency of exclusively globo-A binding strains. About 10 to 30% of UTI strains contain more than one copy of the *pap* homologous DNA sequences (2, 13, 32), which encode Galα1-4Galβ-specific adhesins and which cross-hybridize with *prs*, the sequence encoding the globo-A-specific adhesin. Such strains have been shown to coexpress the Galα1-4Galβ- and globo-A-specific adhesins. In the present study these strains were included in the Galα1-4Galβ-positive group. The affinity for globo-A may thus contribute to the selection of hosts also for such strains. In this case, we predict that the A blood group frequency would be increased among patients infected with *E. coli* binding to globo-A. Strains which coexpress several adhesins may also be more inflammatogenic than strains with a single binding specificity.

The strains with specificity for globo-A and the Forssman glycolipid were first isolated from the urinary tract of dogs (9, 36). The shift from binding to globoside to binding to Forssman antigen and globo-A has been proposed to represent an adaptation for the colonization of a new species, i.e., the dog, rather than for UTI of humans (38). This was based on bacterial binding to epithelial cells in culture. The present study demonstrated the presence in humans of receptors for the *prs* adhesins. The proportion of A secretor individuals is about 30%, suggesting a sufficient host population size for the *prs*-encoded phenotype to be maintained in humans.

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