Identification of Target Tissue Glycosphingolipid Receptors for Uropathogenic, F1C-fimbriated *Escherichia coli* and Its Role in Mucosal Inflammation*

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Bacterial adherence to mucosal cells is a key virulence trait of pathogenic bacteria. The type 1 fimbriae and the P-fimbriae of *Escherichia coli* have both been described to be important for the establishment of urinary tract infections. While P-fimbriae recognize kidney glycosphingolipids carrying the Gal2Gal determinant, type 1 fimbriae bind to the urethelial mannosylated glycoproteins uroplakin Ia and Ib. The F1C fimbriae are one additional type of fimbria correlated with uropathogenicity. Although it was identified 20 years ago its receptor has remained unidentified. Here we report that F1C-fimbriated bacteria selectively interact with two minor glycosphingolipids isolated from rat, canine, and human urinary tract. Binding-active compounds were isolated and characterized as galactosylceramide, and globotriaosylceramide, both with phytosphingosine and hydroxy fatty acids. Comparison with reference glycosphingolipids revealed that the receptor specificity is dependent on the ceramide composition. Galactosylceramide was present in the bladder, urethers, and kidney while globotriaosylceramide was present only in the kidney. Using a functional assay, we demonstrate that binding of F1C-fimbriated *Escherichia coli* to renal cells induces interleukin-8 production, thus suggesting a role for F1C-mediated attachment in mucosal defense against bacterial infections.

Epithelial linings of the host function as very efficient barriers against microorganisms. To achieve this protective effect the mucosal lining utilizes a variety of mechanisms that engage multiple signaling pathways upon bacterial exposure. The most commonly studied mechanism for the induction of the host’s innate immune response in urinary tract infections is bacterial adhesion to uroepithelial cells. Although this is reported as one of the most important virulence trait of uropathogenic *Escherichia coli*, bacterial adhesion also leads to induction of the host’s immune system (1–3). Accordingly, adhesion to the epithelium acts as a double-edged sword for bacteria.

Adhesion of Gram-negative bacteria to epithelial cells is often mediated by fimbria or pili. These rod-shaped, proteinaceous, filamentous polymeric organelles are expressed on the surface of bacteria. P and type 1 fimbriae are the two best characterized attachment organelles, both known for their central role in urinary tract infections (4). Expression of P fimbiae is mainly associated with pyelonephritogenic isolates of uropathogenic *E. coli*. Binding of P fimbiae to Galα4Gal-carrying glycosphingolipids, an epitope present in the human kidney, is of major importance for the establishment of disease (5–7). The type 1 fimbriae are mainly associated with cystitis, and confer binding to mannosylated proteins such as uroplakin that are abundant within the lower urinary tract (8–11).

Although the biogenesis as well as binding characteristics of P fimbiae and type 1 fimbiae have been studied in detail, uropathogenic isolates of *E. coli* express other fimbiae that are less well characterized, in part because their target tissue receptors are unidentified. One such example is the F1C fimbiae, which are expressed by 14–30% of all uropathogenic strains of *E. coli* (12, 13). The F1C fimbiae are structurally related to, and genetically organized as, the type 1 fimbiae. However, comparison of the amino acid sequence reveals that F1C is more closely related to the S fimbiae (14). The S fimbiae confer binding to sia1yl-α2–3Galβ-containing receptor molecules, and are associated to sepsis and meningitis caused by *E. coli* in newborn children (15, 16).

The kidney has been reported to be the target tissue of F1C expressing *E. coli* using *in vitro* models and strictly biochemical approaches (17, 18). In the present study, we use *in vivo* and *in vitro* model systems to identify the glycosphingolipid receptor for F1C expressing strains of uropathogenic *E. coli* within the human, rat, and canine urinary tract. We present evidence that the ceramide portion of the glycosphingolipid receptor confers specificity to the binding. Moreover, we report that human renal epithelial cells produce the proinflammatory chemokine IL-81 as a consequence of F1C-mediated attachment.

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§ Both authors contributed equally to this work.

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1 The abbreviations used are: IL, interleukin; HPLC, high performance liquid chromatography; EI, electron ionization; FAB, fast atom
The glycosphingolipids were chromatographed on aluminum-backed silica gel plates and visualized with anisaldehyde (A). Duplicate chromatograms were incubated with radiolabeled F1C-fimbriated E. coli strain ARD6 (B) and P-fimbriated E. coli strain HB101/pPIL291 (C), followed by autoradiography for 12 h, as described under "Materials and Methods." The solvent system used was chloroform/methanol/water (60:35:8, by volume), as described under "Materials and Methods." The lanes were: reference globoside (GalNAcβ3Galα4Galβ1GlcP1Cer) of human erythrocytes, 4 μg (lane 1); non-acid glycosphingolipids of infant rat kidney, 40 μg (lane 2); acid glycosphingolipids of infant rat kidney, 40 μg (lane 3); non-acid glycosphingolipids of canine kidney, 40 μg (lane 4); reference galactosylceramide (GalP1Cer), 4 μg (lane 5).

**Materials and Methods**

**Bacterial Strains, Culture Conditions, and Labeling**

The human and rat pyelonephritogenic E. coli strain ARD6 (serotype O6:K13:H1, World Health Organization designation Su 4344/41) was used in this study (19). The non-fimbriated E. coli strain HB101 was transformed with the plasmid L40 carrying the foc operon from the pyelonephritogenic strain KS71 (20). The Gal4G4 binding recombinant E. coli strain HB101/pPIL291-15, carrying a plasmid-borne pap gene cluster with a class II papG allelic, was obtained from Dr. I. van Die (Vrije University, Amsterdam, The Netherlands). E. coli strains were cultured (37 °C, 12 h) on Luria-agar plates supplemented with 10 μl of 35S-methionine (400 mCi; Amersham Biosciences, UK). The glycosphingolipids were separated on aluminum-backed silica gel plates and visualized with anisaldehyde (A). The solvent system used for borate-impregnated plates was chloroform/methanol/water (60:35:8, by volume).

**Reference Glycosphingolipids**

Total acid and non-acid glycosphingolipid fractions were obtained by standard procedures (21). The individual glycosphingolipids were isolated by repeated chromatography on silicic acid columns of the native glycosphingolipid fractions or acetylated derivatives thereof. The identity of the purified glycosphingolipids was confirmed by mass spectrometry (22), proton NMR spectroscopy (23), and degradation studies (24, 25). Reference galactosylceramide was obtained from Sigma.

**Thin-layer Chromatography**

Mixtures of glycosphingolipids (40 μg) or pure glycosphingolipids (0.1–4 μg) were separated on glass- or aluminum-backed silica Gel 60 HPTLC plates (Merek, Darmstadt, Germany), using chloroform/methanol/water (60:35:8, by volume) as solvent system. Borate-impregnated bombardment; HPTLC, high performance thin-layer chromatography.

The glycosphingolipid nomenclature follows the recommendations by the IUPAC-IUB Commission on Biochemical Nomenclature (CBN for Lipids: Eur. J. Biochem. (1977) 79, 11–21; J. Biol. Chem. (1982) 257, 3347–3351; and J. Biol. Chem. (1987) 262, 13–18). It is assumed that Gal, Glc, GlcNAc, GalNAc, and NeuAc are of the D-configuration, Fuc of the L-configuration, and all sugars present in the pyranose form.

**Glycosphingolipid Receptors for F1C-fimbriated E. coli**

**Comparison of glycosphingolipid recognition by F1C-fimbriated and P-fimbriated E. coli.** The glycosphingolipids were chromatographed on aluminum-backed silica gel plates and visualized with anisaldehyde (A). Duplicate chromatograms were incubated with radiolabeled F1C-fimbriated E. coli strain ARD6 (B) and P-fimbriated E. coli strain HB101/pPIL291-15 (C), followed by autoradiography for 12 h, as described under "Materials and Methods." The solvent system used was chloroform/methanol/water (60:35:8, by volume). The lanes were: reference globoside (GalNAcβ3Galα4Galβ1GlcP1Cer) of human erythrocytes, 4 μg (lane 1); non-acid glycosphingolipids of infant rat kidney, 40 μg (lane 2); acid glycosphingolipids of infant rat kidney, 40 μg (lane 3); non-acid glycosphingolipids of canine kidney, 40 μg (lane 4); reference galactosylceramide (GalP1Cer), 4 μg (lane 5).

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**Isolation of Binding Active Glycosphingolipids from Rat, Canine, and Human Kidneys**

Acid and non-acid glycosphingolipids were isolated from rat, canine, and human kidneys by standard methods (21). In addition, non-acid glycosphingolipid fractions were isolated from samples of canine urethra, urinary bladder, the trigonum area of the urinary bladder, urethers, and kidney. The amounts obtained are summarized in Table I. HPLC separation of the total non-acid glycosphingolipid fractions of rat, canine, and human kidneys was performed using a Kromasil 5 Silica column (1 × 25 cm inner diameter, particle size 5 μm; Phenomenex, Torrence, CA). The fractions obtained were analyzed by thin-layer chromatography using anisaldehyde for detection, and the glycosphingolipid-containing fractions were tested for binding of F1C-fimbriated E. coli using the chromatogram binding assay.

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binding-active monoglycosylceramide (designated fraction R1).

**Human Kidney**—Isolation of non-acid glycosphingolipids from 520 g dry weight human kidneys has been described previously (29). The non-acid glycosphingolipid fraction was subjected to repeated silicic acid chromatography, and the mono- to triglycosylceramides were pooled, giving 700 mg. This fraction was further separated by HPLC by an isocratic elution with chloroform/methanol/water (80:25:0.5, by volume) during 180 min and a flow rate of 2 ml/min. Pure binding-active monoglycosylceramide (5.0 mg; designated fraction H1) eluted in tube 5, while tube 12 (158 mg) contained the binding-active compound migrating in the tri- to tetraglycosylceramide region. Since the latter fraction also contained several non-binding compounds, this fraction was further separated by HPLC eluted with a linear gradient of chloroform/methanol/water (80:20:1 to 60:35:8 (by volume)) during 180 min, and with a flow rate of 2 ml/min. Pooling of tubes 70–170 resulted in 2.0 mg of pure binding-active glycosphingolipid, which was designated fraction H2.

**Canine Kidney**—Part of the total non-acid glycosphingolipid fraction of canine kidney (15.0 mg) was separated on a 20-g Iatrobeads column (Iatron Laboratories Inc., Tokyo, Japan) eluted stepwise with increasing amounts of methanol and water in chloroform (29). Pure binding-active monoglycosylceramide (1.0 mg; designated fraction C1) and triglycosylceramide (1.3 mg; designated fraction C2) was thereby obtained.

**Enzymatic Hydrolysis**

Hydrolysis of glycosphingolipids with β-galactosidase from *Streptococcus pneumoniae* (Oxford Glycosystems Ltd., Abingdon, UK) was performed according to the manufacturer’s instructions. Glycosphingolipids were also treated with green coffee bean α-galactosidase (Glyko, Inc., Novato, CA) according to the protocol of the manufacturer.

**Mass Spectrometry**

Negative ion FAB mass spectra were recorded on a JEOL SX-102A mass spectrometer (JEOL, Tokyo, Japan). The ions were produced by 6 keV xenon atom bombardment, using triethanolamine (Fluka, Buchs, Switzerland) as matrix, and an accelerating voltage of ~10 kV. EI mass spectrometry was performed on permethylated aliquots of the isolated glycosphingolipids (30). The derivatized samples were analyzed on a JEOL SX-102A mass spectrometer, using the in beam technique (31). The analyses was performed with an electron energy of 70 eV, trap current of 300 μA, and acceleration voltage of 10 kV. The temperature was raised from 150 to 410 °C, by increases of 10 °C/min.

**Proton NMR Spectroscopy**

$^1$H NMR spectra were acquired on a Varian 500 MHz spectrometer at 30 °C. Samples were dissolved in dimethyl sulfoxide/D$_2$O (98:2, by volume) after deuterium exchange.

**Cell Stimulation**

The human renal epithelial cell line A498 (ATCC HTB-44) was grown in 24-well cell culture plates in RPMI 1640 medium supplemented with 10% fetal calf serum, 25 mM HEPES, and 2 mML -glutamine (Invitrogen, Stockholm, Sweden) at 37 °C in 5% CO$_2$. At confluency, cells were washed before control medium (no additives) or medium containing $2 \times 10^5$ colony forming units of HB101 or HB101/L40 was added. Supernatants were collected 6 and 25 h post-infection and were analyzed by enzyme-linked imunosorbent assay for IL-8 (Diacline, Besancon, France).

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**Fig. 3.** Migration of the isolated F1C-binding monoglycosylceramide on borate-impregnated silica gel plates. The glycosphingolipids were chromatographed on borate-impregnated silica gel plates and visualized with anisaldehyde. The solvent system used was chloroform/methanol/water (100:30:4, by volume). The lanes were: glucosylceramide of porcine intestine, 4 μg (lane 1); galactosylceramide (Sigma), 4 μg (lane 2); monoglycosylceramide isolated from rat kidney, 4 μg (lane 3); monoglycosylceramide isolated from human kidney, 4 μg (lane 4); monoglycosylceramide isolated from canine kidney, 4 μg (lane 5).

**Fig. 4.** Negative ion FAB mass spectrum of the binding-active monoglycosylceramide isolated from rat kidney. The series of molecular ions (M-H$^-$/m/z 788–844) indicate a glycosphingolipid one hexose and phytosphingosine and hydroxy 20:0–24:0 fatty acids. A series of ceramide ions, obtained by elimination of the carbohydrate unit, is found at m/z 626–682 (M-Hex-H$^-$/m/z 654). Thus, the glycosphingolipid was identified as a monohexosylceramide with phytosphingosine and hydroxy 20:0–24:0 fatty acids.
ARD6 Binds to Monoglycosylceramide and Tri- or Tetraglycosylceramide Isolated from Rat, Canine, and Human Kidney—

The uropathogenic E. coli strain ARD6 was originally isolated from a child suffering from pyelonephritis (19), and it has also been shown to cause pyelonephritis in rat (32). To identify renal receptor(s) to which ARD6 binds, non-acid and acid glycosphingolipids were isolated from kidneys of 20-day-old, non-infected rats. Similar preparations were also isolated from canine and human tissues. A binding assay of 35S-labeled ARD6 to non-acid glycosphingolipids separated on thin-layer chromatography showed specific binding to a novel glycosphingolipid, which was isolated and purified for structural analysis.

**RESULTS**

**ARD6 Binds to Monoglycosylceramide and Tri- or Tetraglycosylceramide Isolated from Rat, Canine, and Human Kidney**—The uropathogenic E. coli strain ARD6 was originally isolated from a child suffering from pyelonephritis (19), and it has also been shown to cause pyelonephritis in rat (32). To identify renal receptor(s) to which ARD6 binds, non-acid and acid glycosphingolipids were isolated from kidneys of 20-day-old, non-infected rats. Similar preparations were also isolated from canine and human tissues. A binding assay of 35S-labeled ARD6 to non-acid glycosphingolipids separated on thin-layer chromatography showed specific binding to a novel glycosphingolipid, which was isolated and purified for structural analysis.

**EI mass spectrum of the permethylated binding-active triglycosylceramide isolated from human kidney.** The spectrum was recorded at 280 °C. The series of molecular ions at m/z 1308–1365 indicated a triglycosylceramide with phytosphingosine and hydroxy 20:0–24:0 fatty acids. Immonium ions, containing the complete carbohydrate chain together with the fatty acid, were found at m/z 1052 and 1080, and also gave evidence of a saccharide part composed of three hexoses, in combination with hydroxy 22:0 and 24:0 fatty acids. The ions at m/z 1096 and 1124, also indicated a trihexosylceramide with phytosphingosine in combination with hydroxy 22:0 (1336/11002) and hydroxy 24:0 (1365/11002) fatty acids. Ceramide ions of phytosphingosine with hydroxy 20:0–24:0 were found at m/z 666–722. Terminal hexose was indicated by the ions at 219 and 187 (219–32).

**Proton NMR spectrum of the binding-active triglycosylceramide isolated from human kidney.** The spectrum was recorded at 500 MHz and 30 °C. Three H1 anomeric signals are found at 4.78 ppm (α), 4.25 ppm (β), and 4.21 ppm (γ), respectively, and the compound was thus identified as Galα4Galβ4Glcβ1Cer through comparison with earlier published spectra (34).

**Effects of β-galactosidase and α-galactosidase hydrolysis on binding of FIC-fimbriated E. coli to non-acid kidney glycosphingolipids.** The glycosphingolipids were chromatographed on aluminum-backed silica gel plates and visualized with anisaldehyde (A and C). Duplicate chromatograms were incubated with radiolabeled FIC-fimbriated E. coli strain HB101/pL40 (B), followed by autoradiography for 12 h, as described under “Materials and Methods.” The solvent system used was chloroform/methanol/water (60:35:8, by volume). The lanes on chromatograms (A) and (B) were: non-acid glycosphingolipids of human kidney, 40 μg (lane 1); non-acid glycosphingolipids of human kidney after hydrolysis with β-galactosidase, 40 μg (lane 2); triglycosylceramide (fraction H2) isolated from human kidney, 2 μg (lane 3); and the lanes on chromatograms (C) and (D) were: non-acid glycosphingolipids of human blood group A erythrocytes, 40 μg (lane 1); non-acid glycosphingolipids of canine kidney after hydrolysis with β-galactosidase, 40 μg (lane 2); non-acid glycosphingolipids of canine kidney, 40 μg (lane 3). The band marked with “X” is a non-glycosphingolipid contaminant.
The binding pattern of the GalCer Glc1Cer (t18:0/h16:0/24:0) was obtained. In comparison to this distinct binding with the monoglycosylceramide region, the diglycosylceramide region was also obtained in the canine and human kidney samples along with binding to the tri- to tetraglycosylceramide region in the rat, canine, and human kidney samples along with binding to the monoglycosylceramide region in the canine kidney sample. Binding to the monoglycosylceramide region in the canine kidney sample. Binding to the monoglycosylceramide region and tri- to tetraglycosylceramide region was also obtained when using non-acid glycosphingolipids from human kidney (see Fig. 2B, lane 3). Occasionally, a band migrating in the diglycosylceramide region was detected in the human and canine kidney samples. No binding to the acid glycosphingolipid fractions of rat (lane 1) and canine (lane 2) kidney is seen, together with a band migrating in the tri- to tetraglycosylceramide region in the canine kidney sample. Binding to the monoglycosylceramide region and tri- to tetraglycosylceramide region was also obtained when using non-acid glycosphingolipids from human kidney (see Fig. 2B, lane 3). Occasionally, a band migrating in the diglycosylceramide region was detected in the human and canine kidney samples. No binding to the acid glycosphingolipid fractions of rat (Fig. 1B, lane 3), canine, or human kidney (data not shown) was obtained. In comparison to this distinct binding pattern, the GalGlc4Gal-binding P-fimbriated E. coli (Fig. 1C) displayed a broader binding pattern with several binding-active compounds in each tissue sample.

**FIC Fimbriae Expressed by ARD6 Are Responsible for Glycosphingolipid Binding**—To investigate which attachment organelles are expressed by ARD6, PCR analysis was performed, using primers for detection of P pilus, type 1 fimbriae, FIC fimbriae, and afimbrial adhesins. This analysis showed that ARD6 harbors the genes for type 1 fimbriae and FIC fimbriae but not P fimbriae or afimbrial adhesin (data not shown). This was further verified in agglutination studies. ARD6 did not agglutinate human erythrocytes due to lack of P fimbriae expression. In contrast, ARD6 induced mannose-sensitive agglutination of yeast, which is the hallmark for type 1 fimbriae expression (data not shown). However, type 1 fimbriae are not likely to be responsible for binding of ARD6 to glycosphingolipid receptors, because the binding assays are routinely performed in the presence of 1% mannose, which inhibits binding via the type 1 fimbriae. To investigate whether the F1C fimbriae are responsible for the observed bacterial binding to glycosphingolipids, the FIC-expressing plasmid L40 was introduced into the non-fimbriated E. coli strain HB101. When the resulting strain, HB101/L40, was tested in the chromatogram, an identical binding pattern was observed as for the resulting strain, HB101/L40, was tested in the chromatogram, an identical binding pattern was observed as for the resulting strain, HB101/L40, was tested in the chromatogram, an identical binding pattern was observed as for the resulting strain, HB101/L40, was tested in the chromatogram, an identical binding pattern was observed as for the resulting strain, HB101/L40, was tested in the chromatogram, an identical binding pattern was observed as for the resulting strain, HB101/L40, was tested in the chromatogram, an identical binding pattern was observed for the resulting strain, HB101/L40, was tested in the chromatogram, an identical binding pattern was observed.
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Characterization of the minor binding-active triglycosylceramides isolated from human (fraction H2) and canine (fraction C2) kidney demonstrated globotriaosylceramide (Galα3Galβ4Glcβ1Cer) with sphingosine and hydroxy 20:0 fatty acids. This conclusion was based on the following four properties. (i) EI mass spectrometry of the permethylated fraction H2 and fraction C2 resulted in almost identical mass spectra (exemplified in Fig. 5), identifying a trihexosylceramide with sphingosine and hydroxy 20:0-24:0 fatty acids. (ii)
The proton NMR spectra of the triglycosylceramides of human (Fig. 6) and canine (data not shown) kidney both had three H1 anomeric signals at 4.78 ppm (α), 4.25 ppm (β), and 4.21 ppm (β), respectively, and the compounds were thus identified as Galα4Galβ3Glcβ1Cer through comparison with previously published spectra (34). (iii) Treatment of the total non-acid glycosphingolipid fractions of canine and human kidneys with β-galactosidase had no influence on the binding of F1C-fimbriated E. coli to the compounds migrating in triglycosylceramide region (exemplified in Fig. 7, B, lane 2). (iv) When the total non-acid glycosphingolipid fractions of canine and human kidneys were treated with α-galactosidase, a binding-active compound migrating in the diglycosylceramide region appeared (exemplified in Fig. 7D, lane 2).

The F1C-binding Specificity Depends on the Ceramide Composition—To investigate the specificity of F1C binding to glycosphingolipids, we next examined the binding characteristics of ARD6 and HB101/L40 to a library of pure reference glycosphingolipids (summarized in Table II). This experiment shows that in addition to galactosylceramide and globotriaosylceramide, the F1C-fimbriae mediate binding to glucosylceramide (No. 2 in Table II), lactosylceramide (No. 7), and iso-globotriaosylceramide (No. 11). The detection limit for these five compounds in the chromatogram binding assay was ~0.2 μg, while non-binding compounds were not recognized even when 2 μg was applied on the TLC. An attempt to estimate the relative affinity of binding was made by performing densitometry of autoradiograms obtained by binding of F1C-fimbriated bacteria to serial dilutions of glycosphingolipids on TLC (Fig. 8). However, with the exception of a slightly less binding affinity to glucosylceramide, no obvious preference for any of the other binding-active glycosphingolipids was found.

A common feature of all the binding-active glycosphingolipids is the presence of a ceramide with phytosphingosine and hydroxy fatty acids. However, a ceramide with phytosphingosine and hydroxy fatty acids does not always allow F1C binding, since other glycosphingolipids such as gangliotetraosylceramide (No. 19) and blood group active pentaglycosylceramide (Nos. 21, 22, and 24) all contain this ceramide composition, but are not recognized by the F1C-fimbriated E. coli. Taken together the binding data indicates that the minimum binding epitope for the F1C fimbriae is the galactose or glucose unit linked to the ceramide part, with tolerance for some extensions of the carbohydrate chain. The requirement for a specific ceramide suggests that this ceramide gives a correct presentation of the binding epitope. Alternatively, part of the ceramide is involved in the binding process.

Binding-active Glycosphingolipids Are Distributed throughout the Ascending Urinary Tract—The tissue distribution of receptors used for bacterial attachment may be an important virulence determinant for the bacteria, facilitating their ascension through the urinary tract. When selected compartments of the canine urinary tract were analyzed for their expression of binding-active glycosphingolipids we used preparations of non-acid glycosphingolipids from urethra, urinary bladder, urethers, and kidney that were separated on thin-layer chromatograms. Binding experiments using F1C-fimbriated E. coli demonstrated the presence of binding-active monoglycosylceramide in urinary bladder, urethers, and kidney, but not in the sample from urethra (Fig. 9B). Binding-active triglycosylceramide was found only in the kidney. Again, comparative studies showed that the Galα4Gal binding F1C-fimbriated E. coli (Fig. 9C) displayed a broader binding pattern with several binding-active compounds in each tissue sample.

F1C-mediated Bacterial Adhesion Triggers the Proinflammatory Response—To investigate whether F1C-mediated adhesion induces a proinflammatory response in renal epithelial cells, A498 cells were infected with E. coli strain HB101 and the F1C-expressing strain HB101/L40. Cellular activation was monitored by examination of the presence of the chemokine IL-8 in the supernatant 6 and 24 h post-infection (Fig. 10). Supernatants from cells infected by F1C-expressing bacteria showed an ~3-fold increase of IL-8 as compared with supernatants from cells infected by the isogenic non-fimbriated strain. These data suggest a similar role for F1C in inflammation as previously described for other fimbriae (2).

**DISCUSSION**

Although expression of P fimbriae are considered as one of the major determinants for the establishment of pyelonephritis, this disease can also be caused by non-P-fimbriated E. coli strains (19, 32). Here, we report an alternative mechanism for bacterial adhesion. We report that the F1C fimbriae confer binding to glycosphingolipids isolated from human, canine, and rat kidney. These F1C-binding compounds were identified as galactosylceramide and globotriaosylceramide. Galactosylceramide was present in all tissues within the ascending urinary tract except for the urethra, while globotriaosylceramide was specifically expressed in renal tissue. The ceramide portion of both binding-active galactosyl- and globotriaosylceramide consists of phytosphingosine and hydroxy fatty acids. The presence of these fatty acids of the ceramide composition was overlooked.

A large number of commensal as well as pathogenic bacteria preferentially bind to lactosylceramide with phytosphingosine and/or hydroxy fatty acids, while the same bacteria are unable to bind to galactosylceramide and glucosylceramide (35–38). This binding deficiency is independent of phytosphingosine and/or hydroxy fatty acids in the ceramide portion of the receptor. Furthermore, globotriaosylceramide with phytosphingosine and hydroxy fatty acids is not recognized by the lacto- or globotriaosylceramide binding Helicobacter pylori (38). These data suggest that expression of F1C fimbriae provides a unique binding capacity of uropathogenic E. coli to galactosylceramide and globotriaosylceramide containing phytosphingosine and hydroxy fatty acids, which may facilitate binding to uroepithelium in vivo for the establishment of infection. Bacterial binding has previously been shown to be a key virulence trait of uropathogenic E. coli (7, 10).

The F1C fimbriae were recently reported to bind a wide variety of glycosphingolipids, i.e. glucosylceramide, galactosylceramide, lactosylceramide, globotriaosylceramide, lactotriaosylceramide, gangliotetraosylceramide, neolactotetraosylceramide, and gangliotetraosylceramide, with most efficient binding to gangliotetraosylceramide (39). We never detected binding to gangliotetraosylceramide, while occasional binding to lactotriaosylceramide, gangliotriaosylceramide, lactotetraosylceramide, and neolactotetraosylceramide was observed when high concentrations of glycosphingolipids were used on the thin-layer chromatograms. The reason for this discrepancy is unclear. Khan et al. (39) mainly used commercially obtained glycosphingolipids isolated from erythrocytes and brain, whose ceramide composition predominantly consists of sphingosine, dihydrosphingosine, and non-hydroxy fatty acids (40). The use of glycosphingolipids lacking the optimal ceramide composition might explain why the high affinity binding to certain glycosphingolipids was overlooked.
Epithelial cells located in the organs of the urinary tract utilize different mechanisms to detect and respond to bacterial infections. Bladder epithelial cells are highly responsive to infections. Bladder epithelial cells are highly responsive to bacterial lipopolysaccharide, the major constituent of bacterial lipopolysaccharide, the major constituent of bacterial lipopolysaccharide, the major constituent of bacterial lipopolysaccharide. Instead, renal epithelial cells must rely on a mechanism based on microbial adhesion for initiating the proinflammatory response. FIC-fimbriated E. coli significantly induces IL-8 production in renal epithelial cells to levels that previously have been reported for adhesion mediated by the type 1 and P fimbriae (2). Considering the fimbriated bacteria non-responsive phenotype, our data suggest that the IL-8 response observed in renal epithelial cells is entirely due to attachment via the F1C fimbriae. Compared with P fimbriae-mediated binding that recognize several Galα4Gal-containing glycosphingolipids present in rat, canine, and human kidneys, the binding pattern of FIC fimbriated bacteria is more restricted. However, the distribution of binding-active compounds within the uropathogen suggests that the F1C fimbriae may facilitate for bacteria to ascend to the kidney, and that immunization with the FimH adhesin of type 1 fimbriae once there, to establish pyelonephritis. It was recently reported that the FimH adhesin of type 1 fimbriae protects mice from urinary tract infections (41). Thus, proteins from the F1C fimbriae may be used as a novel vaccine candidate to confer protection against pyelonephritis caused by non-P fimbriated E. coli strains.

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Identification of Target Tissue Glycosphingolipid Receptors for Uropathogenic, F1C-fimbriated *Escherichia coli* and Its Role in Mucosal Inflammation

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