Redefinition of the Carbohydrate Binding Specificity of Helicobacter pylori BabA Adhesin

Revised for publication, June 3, 2012, and in revised form, July 6, 2012. Published, JBC Papers in Press, July 20, 2012, DOI 10.1074/jbc.M112.387654

John Benktander, Jonas Ångström, Michael E. Breimer, and Susann Teneberg

From the Institute of Biomedicine, Department of Medical Biochemistry and Cell Biology, P. O. Box 440, University of Gothenburg, S-405 30 Göteborg, Sweden and Department of Surgery, Sahlgrenska University Hospital, S-41 345 Göteborg, Sweden

Background: The BabA adhesin mediates binding of Helicobacter pylori to the gastric epithelium.

Results: Binding of BabA to blood group O and A determinants on type 4 core chains was demonstrated.

Conclusion: The BabA binds to blood group determinants on both type 1 and type 4 core chains.

Significance: Characterization of the binding specificities of BabA is important for understanding the interactions between H. pylori and target cells.

Certain Helicobacter pylori strains adhere to the human gastric epithelium using the blood group antigen-binding adhesin (BabA). All BabA-expressing H. pylori strains bind to the blood group O determinants on type 1 core chains, i.e. to the Lewis b antigen (Fucα2Galβ3(Fucα4)GlcNAc; Leb) and the H type 1 determinant (Fucα2Galβ3GlcNAc). Recently, BabA strains have been categorized into those recognizing only Leb and H type 1 determinants (designated specialist strains) and those that also bind to A and B type 1 determinants (designated generalist strains). Here, the structural requirements for carbohydrate recognition by generalist and specialist BabA were further explored by binding of these types of strains to a panel of different glycosphingolipids. Three glycosphingolipids recognized by both specialist and generalist BabA were isolated from the small intestine of a blood group O pig and characterized by mass spectrometry and proton NMR as H type 1 pentaglycosylceramide (Fucα2Galβ3GlcNAcβ3Galβ4Glcβ1Cer), Globo H hexaglycosylceramide (Fucα2Galβ3GlcNAcβ3Galα4Galβ4Glcβ1Cer), and a mixture of three complex glycosphingolipids (Fucα2Galβ4GlcNAcβ6(Fucα2Galβ3GlcNAcβ3Galβ3GlcNAcβ3Galβ4Glcβ1Cer), Fucα2Galβ3GlcNAcβ6(Fucα2Galβ3GlcNAcβ3Galβ3GlcNAcβ3Galβ4Glcβ1Cer), and Fucα2Galβ4(Fucα3)GlcNAcβ6(Fucα2Galβ3GlcNAcβ3Galβ3GlcNAcβ3Galβ4Glcβ1Cer). In addition to the binding of both strains to the Globo H hexaglycosylceramide, i.e., a blood group O determinant on a type 4 core chain, the generalist strain bound to the Globo A heptaglycosylceramide (GalNAcα3(Fucα2)Galβ3GlcNAcβ3Galα4Galβ4Glcβ1Cer), i.e., a blood group A determinant on a type 4 core chain. The binding of BabA to the two sets of iso-receptors is due to conformational similarities of the terminal disaccharides of H type 1 and Globo H and of the terminal trisaccharides of A type 1 and Globo A.

Attachment of microbes to cell surface receptors on the target tissue is considered an essential step in the initiation, establishment, and maintenance of infection. In recent years, a large number of studies have aimed at the identification of potential microbial host receptors, the majority of which appear to be glycoconjugates (1–3). Glycoconjugates exhibit a characteristic and specific pattern of expression, which is dependent on the animal species, age, individual, and cell type (4). Thus, the recognition of a specific carbohydrate receptor on the host cell surface determines at least in part the host, tissue, and age specificities of microbial infections.

Adherence of the gastric pathogen Helicobacter pylori to human gastric epithelial cells is required for prolonged persistence in the stomach. Initial studies of potential target cell receptors for H. pylori demonstrated the binding of certain strains of this bacterium to the Lewis b blood group antigen (Fucα2Galβ3(Fucα4)GlcNAc; Leb)3 (5), and subsequently the H. pylori Leb- binding adhesin, blood group antigen-binding adhesin (BabA) was identified (6). H. pylori strains expressing BabA together with the vacuolating cytotoxin VacA and the cytotoxin-associated antigen CagA (triple positive strains) are associated with severe gastric diseases such as peptic ulcer and gastric adenocarcinoma (7, 8).

Subsequent studies demonstrated that the BabA adhesin has adapted to the fucosylated blood group antigens most prevalent in the local population (9). In Europe and the United States where blood group A, B, and O phenotypes all are common, the H. pylori strains (designated generalist strains) bind to blood group A, B, and O type 1 determinants. However, in populations such as the indigenous South American native population, which only has the blood group O phenotype, the H. pylori adhesin has adapted to the fucosylated blood group antigen. Additional studies demonstrated that the BabA adhesin was also present in strains lacking the vacuolating cytotoxin VacA and the cytotoxin-associated antigen CagA (6).

The glycosphingolipid nomenclature follows the recommendations by the IUPAC-IUB Commission on Biochemical Nomenclature (Chester, M. A. (1998) IUPAC-IUB Joint Commission on Biochemical Nomenclature (JCBN). Nomenclature of glycolipids—recommendations 1997. Eur. J. Biochem. 257, 293–298). It is assumed that Gal, Glc, GlcNAc, GalNAc, NeuAc, and NeuGc are of the ε configuration; Fuc is of the α configuration; and all sugars are present in the pyranose form.

The abbreviations used are: Leb, Lewis b antigen; BabA, blood group antigen-binding adhesin; ESI, electrospray ionization; Hex, hexose; HexNAc, N-acetyllactosamine; Cer, ceramide; Lea, Lewis y antigen; Leb, Lewis a antigen; NeuGc, N-glycolylneuraminic acid.

* This study was supported by Swedish Research Council Grant 12628, the Swedish Cancer Foundation, and governmental grants (to the Sahlgrenska University Hospital).

† To whom correspondence should be addressed. Tel.: 46-31-786-34-92; Fax: 46-31-413-190; E-mail: Susann.Teneberg@medkem.gu.se.

This is an Open Access article under the CC BY license.

© 2012 by The American Society for Biochemistry and Molecular Biology, Inc. Published in the U.S.A.
strains (designated specialist strains) bind only to the blood group O type 1 determinants (Leb and the H type 1). Thus, the carbohydrate binding site of BabA of generalist strains can accommodate an extension of the blood group O determinant with an α3-linked GalNAc or Gal (creating the blood group A and B determinants, respectively), whereas this extension is not tolerated by the BabA of specialist strains. Consequently, the BabA adhesins from these strains have differences in the architecture of their carbohydrate binding sites.

In the present study, the structural requirements for carbohydrate recognition by BabA of generalist and specialist H. pylori strains were further explored. Radiolabeled H. pylori strains were examined for binding to a panel of different glycosphingolipids from various sources separated on thin-layer plates, and glycosphingolipids recognized by wild type specialist and/or generalist H. pylori, but not by a deletion mutant strain lacking the BabA adhesin, were isolated and characterized by mass spectrometry and proton NMR. Comparative binding studies demonstrated that the BabA adhesin in addition to blood group determinants on type 1 core chains recognizes blood group O and A determinants on type 4 core chains with binding to Globo H (i.e. H type 4) by both strains and Globo A (i.e. A type 4) by the generalist strain. Inspection of minimum energy models revealed topographical similarities in the spatial orientation of the terminal disaccharide (Fucα1Galβ3) of the Globo H and H5 type 1 glycosphingolipids, accounting for the BabA cross-reactivity.

EXPERIMENTAL PROCEDURES

H. pylori Strains, Culture Conditions, and Labeling—The generalist H. pylori strain J99 and the construction of the J99/babA::cam were described by Mahdavi et al. (10). The specialist H. pylori strain S831 was described (9).

For chromatogram binding experiments, the bacteria were grown in a microaerophilic atmosphere at 37 °C for 48 h on Brucella medium (Difco) containing 10% fetal calf serum (Harlan Sera-Lab, Loughborough, UK) inactivated at 56 °C and BBL IsoVitaleX Enrichment (BD Biosciences). The mutant strain J99/BabA− was cultured on the same medium supplemented with chloramphenicol (20 μg/ml). Bacteria were radiolabeled by the addition of 50 μCi [35S]methionine (Amersham Biosciences) diluted in 0.5 ml of phosphate-buffered saline (PBS), pH 7.3 to the culture plates. After incubation for 12–72 h at 37 °C under microaerophilic conditions, the bacteria were harvested, centrifuged three times, and thereafter suspended to 1 × 10⁸ cfu/ml in PBS. The specific activities of the suspensions were ~1 cpm/100 H. pylori organisms.

Chromatogram Binding Assays—Reference glycosphingolipids were isolated and characterized by mass spectrometry and proton NMR as described (11).

Thin-layer chromatography was performed on glass- or aluminum-backed silica gel 60 HPTLC plates (Merck). Mixtures of glycosphingolipids (40 μg) or pure compounds (40 ng–4 μg) were separated using chloroform/methanol/water (60:35:8 by volume) as the solvent system. Chemical detection was accomplished by anisaldehyde (12).

Binding of [35S]-labeled H. pylori to glycosphingolipids on thin-layer chromatograms was done as reported previously (13). Dried chromatograms were dipped for 1 min in diethyl ether/n-hexane (1:5 by volume) containing 0.5% (w/v) polyisobutylmethacrylate (Aldrich). After drying, the chromatograms were soaked in PBS containing 2% bovine serum albumin (w/v), 0.1% NaN₃ (w/v), and 0.1% Tween 20 (by volume) for 2 h at room temperature. The chromatograms were subsequently covered with radiolabeled bacteria diluted in PBS (2–5 × 10⁶ cpm/ml). Incubation was done for 2 h at room temperature followed by repeated washings with PBS. The chromatograms were thereafter exposed to XAR-5 x-ray films (Eastman Kodak Co.) for 12 h.

Chromatogram binding assays with mouse monoclonal antibodies directed against the Globo H determinant (MBr1, Enzo Life Sciences), the Leb determinant (BG-6/T218, Signet/Covance), the H type 1 determinant (17-206, Abcam), and the H type 2 determinant (A583, DakoCytomation Norden A/S) were done as described (13) using [125I]-labeled monoclonal antisera and non-acid glycosphingolipid fractions were obtained by chromatography on a DEAE-cellulose column. To separate the non-acid glycolipids from alkali-stable phospholipids, this fraction was acetylated and separated on a second silicic acid column. Acid and non-acid glycosphingolipid fractions were obtained by chromatography on a DEAE-cellulose column. To separate the non-acid glycolipids from alkali-stable phospholipids, this fraction was acetylated and separated on a second silicic acid column followed by deacetylation and dialysis. Final purifications were done by chromatographies on DEAE-cellulose and silicic acid columns.

The non-acid glycosphingolipid fractions were separated by repeated silicic acid chromatography, and final separation was achieved by HPLC or by chromatography on Iatrobeads (Iatrobeads 6RS-8060, Iatron Laboratories, Tokyo, Japan) columns and elution with chloroform/methanol/water (65:25:4 by volume) followed by chloroform/methanol/water (60:35:8 by volume) and finally chloroform/methanol/water (40:40:12 by volume). Throughout the separation procedures, aliquots of the fractions obtained were analyzed by thin-layer chromatography, and fractions that were colored green by anisaldehyde were tested for binding of H. pylori using the chromatogram binding assay. The fractions were pooled according to the mobility on thin-layer chromatograms and their H. pylori binding activity.

Endoglycosidase Digestion and LC-ESI/MS—Endoglycosidase II from Rhodococcus spp. (14) (Takara Bio Europe S.A., Gennevilliers, France) was used for hydrolysis of glycosphingolipids. Briefly, 50 mg of glycosphingolipids were suspended in 100 ml of 0.05 M sodium acetate buffer, pH 5.0 containing 120 mg of sodium cholate and sonicated briefly. Thereafter, 1 milliliter of endoglycosidase II was added, and the mixture was incubated at 37 °C for 48 h. The reaction was stopped by addition of chloroform/methanol/water to the final proportions 8:4:3 (by volume). The oligosaccharide-containing upper phase thus obtained was separated from deter-
H. pylori BabA-binding Glycosphingolipids

gent on a Sep-Pak QMA cartridge (Waters, Milford, MA). The eluant containing the oligosaccharides was dried under nitrogen and under vacuum.

The glycosphingolipid-derived oligosaccharides were analyzed by LC/MS and MS/MS as described (15). In brief, the oligosaccharides were separated on a column (200 × 0.180 mm) packed in house with 5-mm porous graphite particles (Hypercarb, Thermo Scientific) and eluted with an acetonitrile gradient (A, 10 mM ammonium bicarbonate; B, 10 mM ammonium bicarbonate in 80% acetonitrile). The saccharides were analyzed in the negative ion mode on an LTQ linear quadrupole ion trap mass spectrometer (Thermo Electron, San José, CA).

LC-ESI/MS and ESI/MS/MS of Native Glycosphingolipids—The glycosphingolipids (dissolved in methanol/acetonitrile, 75:25 by volume) were separated on a 200 × 0.150-mm column packed in house with 5-mm polyamine II particles (YMC Europe GmbH, Dinslaken, Germany) and eluted with a water gradient (A, 100% ammonium bicarbonate; B, 10 mM ammonium bicarbonate). Samples were analyzed on an LTQ linear quadrupole ion trap mass spectrometer by LC-ESI/MS at 30 °C. Samples were dissolved by LC-ESI/MS and MS/MS as described (15). In brief, the glycosphingolipids of the intestinal epithelium of a blood group O pig (Fig. 1, lane 2, reference B type 1 heptaglycosylceramide (Galcβ2Galβ3Fucα3Galβ4GlcNAcβ3Galβ4Glcβ1Cer), 2 μg; lane 4, reference Le<sub>β</sub> hexaglycosylceramide (Fucα2Galβ3Fucα4GlcNAcβ3Galβ4Glcβ1Cer), 4 μg; lane 5, reference B type 1 heptaglycosylceramide (Galcβ2Galβ3Fucα4GlcNAcβ3Galβ4Glcβ1Cer), 2 μg).

Proton NMR Spectroscopy—<sup>1</sup>H NMR spectra were acquired on a Varian 600-MHz spectrometer at 30 °C. Samples were dissolved in dimethyl sulfoxide/D<sub>2</sub>O (98:2 by volume) after deuterium exchange. Two-dimensional double quantum-filtered correlated spectroscopy (COSY) spectra were recorded using the standard pulse sequence (16).

Molecular Modeling—Minimum energy models of different glycosphingolipids were constructed using the CHARMM force field within the Discovery Studio molecular modeling package (Accelrys, Inc., San Diego, CA) and literature values as starting points for the glycosidic torsion angles (17, 18).

RESULTS

Binding of H. pylori to Glycosphingolipid Mixtures—Screening for BabA-mediated binding of H. pylori was done by binding of the generalist H. pylori strain J99, the specialist strain S831, and the deletion mutant strain J99/BabA— to non-acid glycosphingolipid fractions from various sources to expose the bacteria to a large number of potentially binding-active carbohydrate structures. Thus, the binding of the bacteria to non-acid glycosphingolipid mixtures isolated from the small intestine of different species (human, rat, cat, and pig (19–23)), erythrocytes of different species (human, cat, rabbit, dog, horse, chicken, and sheep (24)), human cancers (lung, kidney, colon, liver, and gastric cancers (25)), and human stomach (26) was tested. Thereby, three glycosphingolipids recognized by both the generalist and specialist H. pylori strain were detected in the non-acid glycosphingolipid fraction from the small intestinal epithelium of a blood group O pig (Fig. 1, B and C, lane 1). The binding-active compounds migrated in the penta-, hexa-, and octa-/nonaglycosylceramide regions, respectively. No binding of the deletion mutant strain J99/BabA— to the porcine intestinal glycosphingolipids was obtained (data not shown), indicating that the binding of the wild type bacteria to these compounds was mediated by BabA.

Isolation of the H. pylori-binding Glycosphingolipids from Porcine Intestine—A total non-acid fraction from blood group O porcine small intestinal epithelium (160 mg) was separated by repeated silica gel chromatography and Iatrobead column chromatography, and the subfractions obtained were tested for H. pylori binding activity. After pooling of binding-active fractions, three subfractions containing H. pylori-binding glycosphingolipids were obtained. One of these fraction (designated fraction P-I (0.2 mg)) migrated in the pentaglycosylceramide region, whereas the fraction designated fraction P-II (0.2 mg) demonstrated the Globo H hexaglycosylceramide (Galα2Galβ3Fucα3Galβ4GlcNAcβ3Galβ4Glcβ1Cer, 4 μg; lane 2, reference B type 1 heptaglycosylceramide (Galcβ2Galβ3Fucα4GlcNAcβ3Galβ4Glcβ1Cer), 2 μg).

Characterization of the H. pylori-binding Fraction P-I from Porcine Intestine—LC-ESI/MS, proton NMR, and antibody binding demonstrated that fraction P-I was a mixture of the H type 1 pentaglycosylceramide (Fucα2Galβ3GlclNAcβ3Galβ4Glcβ1Cer) and the B5 pentaglycosylceramide (Galα3Galβ4GlclNAcβ3Galβ4Glcβ1Cer) (data not shown).

Characterization of the H. pylori-binding Fraction P-II from Porcine Intestine—Characterization of the BabA binding fraction P-II demonstrated the Globo H hexaglycosylceramide (Fucα2Galβ3GlclNAcβ3Galβ4Glcβ1Cer) as the major compound. This conclusion was based on the following properties. (i) ESI/MS of the native fraction P-II gave a major [M − 2H<sup>+</sup>]<sup>2+</sup> ion at m/z 784, corresponding to a molecular ion at m/z 1568, demonstrating a glycosphingolipid with one Fuc, one HexNAc, and four Hex residues and phytosphingosine with hydroxy 16:0 fatty acid (data not shown). The series of C, Y, and Z ions obtained by MS<sup>2</sup> of the [M − 2H<sup>+</sup>]<sup>2+</sup> ion at m/z 784

FIGURE 1. Binding of a generalist and a specialist H. pylori strain to non-acid glycosphingolipids of the small intestinal epithelium of a blood group O pig. The glycosphingolipids were separated on aluminum-backed silica gel plates using chloroform/methanol/water (60:35:8 by volume) as the solvent system. The chromatogram in A was stained with anisaldehyde. Duplicate chromatograms were incubated with the <sup>35</sup>S-labeled H. pylori generalist strain J99 (B) and the H. pylori specialist strain S831 (C) followed by autoradiography for 12 h as described under “Experimental Procedures.” Lane 1, non-acid glycosphingolipids of the intestinal epithelium of a blood group O pig, 40 μg; lane 2, reference H type 2 pentaglycosylceramide (Fucα2Galβ3GlclNAcβ3Galβ4Glcβ1Cer), 4 μg; lane 3, reference Le<sub>β</sub> pentaglycosylceramide (Galβ3Fucα4GlclNAcβ3Galβ4Glcβ1Cer), 2 μg; lane 4, reference Le<sub>β</sub> hexaglycosylceramide (Fucα2Galβ3Fucα4GlclNAcβ3Galβ4Glcβ1Cer), 4 μg; lane 5, reference B type 1 heptaglycosylceramide (Galα3Galβ4GlclNAcβ3Galβ4Glcβ1Cer), 2 μg.
MS² of the molecular ion at m/z 1014 (Fig. 3E) also gave a series of C type fragment ions with C₂ at m/z 325, C₃ at m/z 528, and C₄ at m/z 690 along with a C₅ ion at m/z 852, identifying a Fuc-Hex-HexNAc-Hex-Hex-Hex sequence. The 0.2A₅ fragment ion at m/z 792 and the 0.2A₆ fragment ion at m/z 954 indicated that the two hexoses at the reducing end were substituted at C-4, i.e., a Fuc-Hex-HexNAc-Hex-4Hex-4Hex sequence.

(iii) The anomeric region of the proton NMR spectrum of fraction P-II (Fig. 3F) revealed a single dominating species with six carbohydrate residues that is identical to the previously published Globo H glycosphingolipid (29) as evidenced by signals at 4.949 (Fucα2), 4.802 (Galα2), 4.468 (GalNAcβ3), 4.456 (Galβ3), 4.247 (Galβ4), and 4.208 ppm (Glcβ1), thus yielding the sequence Fucα2Galβ3GalNAcβ3Galβ4Glcβ1Cer in accordance with the mass spectrometry data above.

Thus, by mass spectrometry and proton NMR, the BabA-binding hexaglycosylceramide of blood group O pig intestine was identified as the Globo H glycosphingolipid. In the base peak chromatogram from LC-ESI/MS of the oligosaccharides obtained by hydrolysis of fraction P-II with *Rhodococcus* endoglycoceramidase (Fig. 3A), the major molecular ion was found at m/z 852, corresponding to the H type 1 pentaglycosylceramide. Still, proton NMR demonstrated that fraction P-II was a relatively pure Globo H glycosphingolipid. This discrepancy is due to the restricted hydrolytic capacity of the *Rhodococcus* endoglycoceramidase II, which has a relative resistance of hydrolysis for globo series glycosphingolipids (14, 30). The ideal enzyme would have been the ceramide glucanase from *Macrobdella decora* that has a more universal hydrolytic activity toward glycosphingolipids (31). However, the *M. decora* enzyme is no longer available commercially.

**Characterization of the Slow Migrating *H. pylori*-binding Fraction P-III from Porcine Intestine—Antibody binding, mass spectrometry, and proton NMR demonstrated that fraction P-III was a mixture of two branched decaglycosylceramides with terminal H type 1 epitopes (Fucα2Galβ3GlcNAcβ6-(Fucα2Galβ3GlcNAcβ3)Galβ3GlcNAcβ3Galβ4Glcβ1Cer and Fucα2Galβ3GlcNAcβ6(Fucα2Galβ3GlcNAcβ3)Galβ3GlcNAcβ3Galβ4Glcβ1Cer) and a related undecaglycosylceramide with a Fucα3 substitution of the GlcNAc of the 6-branch, yielding an Le³ determinant (Fucα2Galβ4(Fucα3GlcNAcβ6-(Fucα2Galβ3GlcNAcβ3)Galβ3GlcNAcβ3Galβ4Glcβ1Cer). This conclusion is based on the following observations. (i) The glycosphingolipid fraction P-III was stained by both the anti-H type 1 antibody and the anti-H type 2 antibody (Fig. 2, D and E, lane 3).

(ii) ESI/MS of the native fraction P-III gave a major [M−2H⁺]+ ion at m/z 1132, corresponding to a molecular ion at m/z 2264, indicating a decasaccharide with two Fuc, three HexNAc, and five Hex residues combined with sphingosine and non-hydroxy 16:0 fatty acid (data not shown). In addition, there was an [M−2H⁺]+ ion at m/z 1205, corresponding to a molecular ion at m/z 2410, suggesting an undecasaccharide with three Fuc, three HexNAc, and five Hex residues combined with sphingosine and non-hydroxy 16:0 fatty acid.

(iii) LC-ESI/MS of the oligosaccharides obtained by hydrolysis of fraction P-III with *Rhodococcus* endoglycoceramidase II had two [M−2H⁺]+ ions at m/z 864, corresponding to molecu-
ular ions at m/z 1728, demonstrating two decasaccharides, both with two Fuc, three HexNAc, and five Hex residues (supplemental Fig. S2). The minor [M − 2H⁺]²⁻ ion eluted at 23.4–24.5 min, and the major [M − 2H⁺]²⁻ ion eluted at 25.8–26.1 min. The MS² spectra of the minor and major [M − 2H⁺]²⁻ ions both had weak lower mass regions, but in both cases, a terminal Fuc-Hex-HexNAc sequence was indicated by C₂ ions at m/z 325 and/or C₃ ions at m/z 528 or B₄ ions at m/z 510 (Fig. 4, A and B). In addition, there were intense C type ions at m/z 1201, 1404, and 1566.

MS³ of the ion at m/z 1201 at retention time 23.4 min gave a C₃ ion at m/z 528, again demonstrating a terminal Fuc-Hex-HexNAc sequence (Fig. 4C). In contrast, the MS³ spectrum of the ion at m/z 1201 at retention time 26.3 min was dominated.

**FIGURE 3. Characterization of the *H. pylori* BabA-binding fraction P-II from the small intestinal epithelium of a blood group O pig. A, base peak chromatogram from LC-ESI/MS of the oligosaccharides obtained by digestion of the *H. pylori* BabA-binding fraction P-II with *Rhodococcus* endoglycocerebrosidase II. B, mass chromatogram of m/z 852. C, mass chromatogram of m/z 1014. D, MS² spectrum of the [M − H⁻]⁻ ion at m/z 852 (retention time (RT), 26.8 min). The interpretation formula shows the deduced oligosaccharide sequence. E, MS² spectrum of the [M − H⁻]⁻ ion at m/z 1014 (retention time, 25.3 min). The interpretation formula shows the deduced oligosaccharide sequence. F, anomic region of the 600-MHz proton NMR spectrum of fraction P-II (30 °C). The sample was dissolved in dimethyl sulfoxide/D₂O (98:2 by volume) after deuterium exchange.
by an intense $^{0.2}A_3$ ion at $m/z$ 427 and an $^{0.2}A_3 - H_2O$ ion at $m/z$ 409, which together with the $C_3$ ion at $m/z$ 528 identified a terminal Fuc-Hex-HexNAc sequence with 4-substitution of the HexNAc, i.e. a type 2 core chain (Fig. 4D) (15, 27, 28).

Both MS$^3$ spectra (Fig. 4, C and D) had $C_4/Z_4$ ions at $m/z$ 672. These ions are obtained by double glycosidic cleavage at the 3-linked bond of the branched Hex residue and thus comprise the 6-linked carbohydrate chain and the core branching Hex residue (32). The $^{0.3}A_4$ ions at $m/z$ 600 obtained by cross-ring cleavages present in both MS$^3$ spectra further confirm the Fuc-Hex-HexNAc sequence on the 6-branch (32).

Thus, these MS$^2$ and MS$^3$ spectral features suggested that fraction P-III contained two branched decasaccharides, i.e. two Fuc-Hex-HexNAc-(Fuc-Hex-HexNAc)-Hex-HexNAc-Hex saccharides. The terminal Fuc-Hex-HexNAc sequences of the minor compound had type 1 core chains, whereas the terminal Fuc-Hex-HexNAc sequences of the major compound had type 2 core chain on at least one branch.

The LC-ESI/MS base peak chromatogram of the oligosaccharides from fraction P-III (supplemental Fig. S2) also had an $[M - 2H^+]^{2-}$ ion at $m/z$ 937, corresponding to a molecular ion at $m/z$ 1874, indicating an undecasaccharide with three Fuc, three HexNAc, and five Hex residues. In addition, the MS$^2$ and MS$^3$ spectra obtained had weak lower mass regions (Fig. 5). There was a $C_2$ ion at $m/z$ 325 and a $B_3$ ion at $m/z$ 510, indicating a terminal Fuc-Hex-HexNAc sequence. In addition, the $B_3$ ion at $m/z$ 656 demonstrated a terminal Fuc-Hex-(Fuc-)HexNAc sequence. Furthermore, both the $C_4/Z_4$ ion and the $^{0.3}A_4$ cross-ring cleavage ion at $m/z$ 746. Furthermore, both the $C_4/Z_4$ ion and the $^{0.3}A_4$ ion demonstrated that the Fuc-Hex-(Fuc-)HexNAc sequence was carried by the 6-branch (32).

The spectra also had a series of prominent C type fragment ions ($C_3$ at $m/z$ 1347, $C_5$ at $m/z$ 1550, and $C_6$ at $m/z$ 1712). Taken all together, MS$^2$ and MS$^3$ indicated a branched undecasaccharide (Fuc-Hex-(Fuc-)HexNAc-(Fuc-Hex-HexNAc)-Hex-HexNAc-Hex-Hex with a Fuc-Hex-(Fuc-)HexNAc sequence on the 6-branch and an H type 1 epitope on the 3-branch.

**FIGURE 4.** LC-ESI/MS of the decasaccharides obtained by hydrolysis of *H. pylori*-binding fraction P-III with *Rhodococcus* endoglycoceramidase II. A, MS$^2$ spectrum of the $[M - 2H^+]^{2-}$ ion at $m/z$ 864 (retention time (RT), 23 min). B, MS$^2$ spectrum of the $[M - 2H^+]^{2-}$ ion at $m/z$ 864 (retention time, 26.3 min). C, MS$^3$ spectrum of the ion at $m/z$ 1201 (retention time, 23.4 min). D, MS$^3$ spectrum of the ion at $m/z$ 1201 (retention time, 26.3 min). E, Interpretation formula showing the deduced oligosaccharide sequence.
Using this set of reference glycosphingolipids, only the Leb was evaluated. The results are summarized in Table 2. When the specialist strain J99 to a number of reference glycosphingolipids and an H type 1 determinant on the 3-branch (Fuc3Gal2GlcNAC3Gal4GlcB1Cer) and Fuco2Galβ3GlcNACβ6(Fuco2Galβ3GlcNACβ3Galβ3GlcNAC3Gal4GlcB1Cer) that have been isolated previously from rat (33) and pig intestine (23) and characterized in detail by NMR (using DMSO/D2O (98:2) as solvent). In fraction P-III, the glycosphingolipid with mixed type 1/type 2 branches (Fuco2Galβ4GlcNACβ6(Fuco2Galβ3GlcNACβ3Galβ3GlcNAC3Gal3GlcNAcβ3Gal4GlcB1Cer) is the major compound as evidenced by the relative intensities of the Fuco2 signals. The chemical shift data are summarized in Table 1. In addition, a novel glycosphingolipid structure with an Leβ determinant on the 6-branch and an H type 1 determinant on the 3-branch (Fuco2Galβ4(Fuco3)GlcNACβ6(Fuco2Galβ3GlcNACβ3Galβ3GlcNACβ3Galβ4GlcB1Cer) could be characterized as shown in Fig. 6 and Table 1.

Comparative Glycosphingolipid Binding Assays—Thereafter, the binding of the specialist H. pylori strain S831 and the generalist strain J99 to a number of reference glycosphingolipids was evaluated. The results are summarized in Table 2. When using this set of reference glycosphingolipids, only the Leβ hexaglycosyceramide was recognized by the specialist strain S831 (Fig. 7C, lane 1), whereas the generalist H. pylori strain J99 in addition to the Leb hexaglycosyceramide bound to the A type 1 hexaglycosyceramide (GalNAcα3(Fuco2)Galβ3GlcNACβ3Galβ4GlcB1Cer; Fig. 7B, lane 4), the B type 1 hexaglycosyceramide (Galα3(Fuco2)Galβ3GlcNACβ3Galβ4GlcB1Cer; Table 2, Number 9), the A type 1 heptaglycosyceramide (GalNAcα3(Fuco2)Galβ3GlcNACβ3Galβ4GlcB1Cer; Fig. 7B, lane 3), the B type 1 heptaglycosyceramide (Galα3(Fuco2)Galβ3GlcNACβ3Galβ4GlcB1Cer; Fig. 7B, lane 2), the A type 1 octaglycosyceramide (GalNAcα3(Fuco2)Galβ3GlcNACβ3Galβ3GlcNACβ3Galβ4GlcB1Cer; Table 2, Number 17), and the repetitive A type 1 nonaglycosyceramide (GalNAcα3(Fuco2)Galβ3GlcNACβ3Galβ4GlcB1Cer; Fig. 7B, lane 6). Furthermore, the chromatogram binding assay revealed that the A type 4 heptaglycosyceramide (Globo A; GalNAcα3(Fuco2)Galβ3GlcNACβ3Galα4GlcB1Cer; Fig. 7B, lane 5) was also recognized by the generalist strain.

However, no type 2 core counterparts of these compounds were recognized such as e.g. the H type 2 pentaglycosyceramide (Fig. 1, lane 2; Table 2, Number 5), the Leβ hexaglycosyceramide (Number 8), the A type 2 hexaglycosyceramide (Number 12), the B type 2 hexaglycosyceramide (Number 10), the A...
type 2 heptaglycosylceramide (Fig. 8B, lane 7; Number 15), and the A type 2 nonaglycosylceramide (Number 19). Furthermore, the A tetraglycosylceramide (Number 1) and the A type 3 nonaglycosylceramide (Number 20) were also non-binding.

When the generalist and specialist H. pylori strains were compared with respect to their ability to bind to dilutions of the binding-active glycosphingolipids on thin-layer chromatograms, the Leb hexaglycosylceramide was the preferred ligand of both strains, and two strains bound to this compound with similar detection limits (Fig. 8, lanes 1–3). In addition, the generalist strain J99 bound to the GalNAc3-substituted Leb (i.e. the A type 1 heptaglycosylceramide), the Globo A heptaglycosylceramide, and the nonaglycosylceramide with repetitive type 1 blood group A determinants in all cases with detection limits at 40 ng (Fig. 8A).

Molecular Modeling—Inspection of the minimum energy models of the H type 1 pentaglycosylceramide and the Globo H hexaglycosylceramide revealed a substantial topographical similarity, which makes it reasonable that these two compounds may be accommodated within the same carbohydrate binding site of BabA (Fig. 9). In contrast, the terminal disaccharide of the non-binding H type 2 pentaglycosylceramide (right) is rotated relative to the same disaccharide in the H type 1 pentaglycosylceramide (left) and the Globo H hexaglycosylceramide (center) by ~90°, explaining why this compound is non-binding.

Binding of Anti-Globo H to Glycosphingolipids from Human Stomach—Having established that H. pylori recognizes the Globo H glycosphingolipid, we next examined whether this glycosphingolipid is present in the target tissue of H. pylori by binding of monoclonal antibodies directed against the Globo H determinant to non-acid glycosphingolipid fractions from human stomach. Thereby, binding in the hexaglycosylceramide region was observed in the non-acid fractions from the stomach of the two individuals tested (Fig. 10B, lanes 1 and 2). Both human stomach...
| No. | Abbreviation | Structure | H. pylori J99 | H. pylori S831 | H. pylori J99/BabA− | Source (Ref.) |
|-----|--------------|-----------|---------------|---------------|---------------------|--------------|
| 1   | A-4          | GalNAc3(Fucα2)Galβ4Glcβ1Cer | + | + | + | Rat intestine (20) |
| 2   | Leα-5        | Galβ3(Fucα4)GlcNAcβ3Galβ4Glcβ1Cer | − | − | − | Human intestine (42) |
| 3   | Leα-5        | Galβ4(Fucα3)GlcNAcβ3Galβ4Glcβ1Cer | − | − | − | Dog intestine (42) |
| 4   | H5 type 1     | Fucα2Galβ3GlcNAcβ3Galβ4Glcβ1Cer | + | + | + | Porcine intestine (22) |
| 5   | H5 type 2     | Fucα2Galβ4GlcNAcβ3Galβ4Glcβ1Cer | + | + | + | Human erythrocytes (43) |
| 6   | H6 type 4 (Globo H) | Fucα2Galβ3GalNAcβ3Galβ4Glcβ1Cer | + | + | + | Porcine intestine (43) |
| 7   | Leα-6        | Fucα2Galβ3(Fucα6)GlcNAcβ3Galβ4Glcβ1Cer | + | + | + | Human intestine (42) |
| 8   | Leβ-6        | Fucα2Galβ4(Fucα3)GlcNAcβ3Galβ4Glcβ1Cer | + | + | + | Human intestine (42) |
| 9   | B6 type 1     | Galα3(Fucα2)Galβ3GlcNAcβ3Galβ4Glcβ1Cer | + | + | + | Human intestine (42) |
| 10  | B6 type 2     | Galα3(Fucα2)Galβ4GlcNAcβ3Galβ4Glcβ1Cer | + | + | + | Human intestine (42) |
| 11  | A6 type 1     | GalNAcα3(Fucα2)Galβ3GlcNAcβ3Galβ4Glcβ1Cer | + | + | + | Human intestine (42) |
| 12  | A6 type 2     | GalNAcα3(Fucα2)Galβ4GlcNAcβ3Galβ4Glcβ1Cer | + | + | + | Human intestine (42) |
| 13  | B7 type 1     | Galα3(Fucα2)Galβ3(Fucα4)GlcNAcβ3Galβ4Glcβ1Cer | + | + | + | Human intestine (42) |
| 14  | A7 type 1     | GalNAcα3(Fucα2)Galβ3(Fucα4)GlcNAcβ3Galβ4Glcβ1Cer | + | + | + | Human intestine (42) |
| 15  | A7 type 2     | GalNAcα3(Fucα2)Galβ4(Fucα3)GlcNAcβ3Galβ4Glcβ1Cer | + | + | + | Human intestine (42) |
| 16  | A7 type 4 (Globo A) | GalNAcα3(Fucα2)Galβ3GalNAcβ3Galβ4Glcβ1Cer | + | + | + | Human intestine (42) |
| 17  | A8 type 1     | Galα3(Fucα2)Galβ3GlcNAcβ3Galβ3GlcNAcβ3Galβ4Glcβ1Cer | − | − | − | Porcine intestine (22) |
| 18  | A9 type 1     | GalNAcα3(Fucα2)Galβ3GlcNAcβ3(Fucα3)Galβ3GlcNAcβ3Galβ4Glcβ1Cer | − | − | − | Porcine intestine (22) |
| 19  | A9 type 2     | GalNAcα3(Fucα2)Galβ4(Fucα3)GlcNAcβ3Galβ4Glcβ1Cer | + | + | + | Cat intestine (21) |
| 20  | A9 type 3     | GalNAcα3(Fucα2)Galβ3GlcNAcβ3(Fucα3)Galβ4Glcβ1Cer | + | + | + | Cat intestine (21) |
| 21  | Dimeric Leα  | Galβ3(Fucα4)GlcNAcβ3Galβ3(Fucα4)GlcNAcβ3Galβ4Glcβ1Cer | − | − | − | Human erythrocytes (46) |
| 22  | Dimeric Leβ  | Galβ4(Fucα3)GlcNAcβ3Galβ4(Fucα3)GlcNAcβ3Galβ4Glcβ1Cer | − | − | − | Human erythrocytes (46) |
| 23  | 24  | Fucα2Galβ3GlcNAcβ3(Fucα2)Galβ3GlcNAcβ3Galβ4Glcβ1Cer | + | + | + | Pig intestine (46) |
| 25  | 26  | Fucα2Galβ4GlcNAcβ3(Fucα2)Galβ3GlcNAcβ3Galβ4Glcβ1Cer | + | + | + | Pig intestine (46) |

* Binding is defined as follows: + denotes a binding when 1 μg of the glycosphingolipid was applied on the thin-layer chromatogram, whereas − denotes no binding even at 4 μg.

* Present study.

* Glycosphingolipid Number 22 was prepared from sialyl-dimeric Leα (10) by mild acid hydrolysis.

* H. pylori binding to glycosphingolipids Numbers 23–25 was determined using a mixture of the three compounds.
samples also contained the Le\textsuperscript{b} hexaglycosyceramides as indicated by the binding of the anti-Le\textsuperscript{b} antibody (Fig. 10C). The anti-H type 1 antibody cross-reacted with the Globo H glycosphingolipid to some extent. However, no binding of the anti-H type 1 antibody to the non-acid glycosphingolipid fractions from human stomach was obtained, although it bound intensely to the pentaglycosylceramide and to the slow migrating glycosphingolipid of blood group O pig intestine (Fig. 10D).

**DISCUSSION**

The binding of microbes to host target cells is crucial to the delivery of virulence factors, and in the case of *H. pylori*, it was recently shown that BabA-mediated binding of the bacteria to Le\textsuperscript{b} on the epithelium leads to an increased type IV secretion system activity, resulting in the production of proinflammatory cytokines and precancer-related factors (34).

The initial observation that the fucosylated blood group antigens H type 1 and Le\textsuperscript{b} are mediators of *H. pylori* adhesion to human gastric epithelial cells (5) was followed by a division of BabA-producing *H. pylori* strains into specialist and generalist strains, depending on their mode of binding to Le\textsuperscript{b} and related
carbohydrate sequences (9). The BabA of specialist strains binds only to glycoconjugates with an unsubstituted terminal Fucα2Gal sequence as in the H type 1 and Leβ determinants, whereas the generalist BabA tolerates a substitution at 3-position of the Gal with an αGal or αGalNAc as in the A or B type 1 and ALeβ or BLeβ determinants.

Here, we further explored the structural requirements for carbohydrate recognition by BabA of generalist and specialist H. pylori by isolating and characterizing glycosphingolipids recognized by wild type specialist and/or generalist H. pylori but not by the deletion mutant strain lacking the BabA adhesin. The Leβ epitope has only been found in humans, but we initially thought that we had found a porcine Leβ glycosphingolipid when an H. pylori BabA-binding glycosphingolipid co-migrating with the Leβ hexaglycosylceramide was detected in the non-acid fraction of blood group O pig intestine. However, after isolation, this BabA-binding glycosphingolipid was characterized as the Globo H hexaglycosylceramide. Further comparative binding studies using our glycosphingolipid collection confirmed that the BabA adhesin in addition to blood group determinants on type 1 core chains recognizes blood group O and A determinants on type 4 core chains with binding to Globo H by both strains and Globo A by the generalist strain. The terminal disaccharides (Fucα2Galβ3) of the H type 1 pentaglycosylceramide and the Globo H hexaglycosylceramide adopt conformations very similar to each other, and this is also the case for the terminal trisaccharides (GalNAcα3(Fucα2)Galβ3) of the A type 1 and the Globo A heptaglycosylceramides (18). These conformational similarities thus explain the binding of BabA to the two sets of isoreceptors.

The enzymatic machinery involved in the biosynthesis of Globo H has not yet been fully elucidated. In humans, there are two functional fucosyltransferases, designated FUT1 and FUT2, that catalyze addition of an α2-linked fucose to a terminal galactose to form the blood group H epitope (for a review, see Ref. 35). These two fucosyltransferases are encoded by two genes, FUT1 and FUT2. FUT1 acts preferentially on type 2 chains, whereas type 1 and type 3 chains and to some extent type 2 chains are acceptors for FUT2. Using siRNAs targeting FUT1 and FUT2 in breast cancer stem cells, Chang et al. (36) showed that Globo H may be synthesized by both FUT1 and FUT2.

Non-secretor individuals have an increased risk of peptic ulcer disease (37). In these individuals, the precursor of the Leβ sequence, i.e. the H type 1 sequence, is not formed due to lack of a functional FUT2 enzyme. Consequently, non-secretors have low amounts of or no Leβ antigens on their epithelial surfaces. However, the Globo H sequence can still be formed by FUT1 and might thus function as an adhesion factor for BabA-expressing H. pylori in non-secretor individuals.

The slow migrating BabA-binding fraction P-III was characterized as a mixture of three complex glycosphingolipids (Fucα2Galβ4GlcNAcβ6(Fucα2Galβ3GlcNAcβ3)Galβ3GlcNAcβ3Galβ4Glcβ1Cer, Fucα2Galβ3GlcNAcβ6(Fucα2Galβ3GlcNAcβ3)Galβ3GlcNAcβ3Galβ4Glcβ1Cer, and Fucα2Galβ4(Fucα3)GlcNAcβ6(Fucα2Galβ3GlcNAcβ3)Galβ3GlcNAcβ3Galβ4Glcβ1Cer). The undecaglycosylceramide with an Leβ epitope on the 6-branch and an H type 1 epitope on the 3-branch are to our knowledge novel glycosphingolipid structures. The three compounds in fraction P-III all had an H type 1 determinant on at least one branch, and thus, all three could be recognized by both specialist and generalist BabA.

The binding of the generalist H. pylori strain to the nonaglycosylceramide with a repetitive blood group A determinant and an internal type 1 core chain (GalNAcα3(Fucα2)Galβ3-GalNAcα3(Fucα2)Galβ3GlcNAcβ3Galβ4Glcβ1Cer) is a wild card. We have previously found that generalist H. pylori strains bind to the ganglio-Leβ hexaglycosylceramide (Fucα2Galβ3(Fucα4)GalNAcβ4Galβ4Glcβ1Cer) (13). Thus, the binding to this nonaglycosylceramide is most likely due to recognition of the terminal A determinant on the ganglio core by the generalist BabA.

Characterization of the binding specificities of the BabA variants is important for understanding the molecular interactions between H. pylori and the target host cells. The presence of the BabA-binding Globo H glycosphingolipid in the human stomach was here indicated by the binding of monoclonal antibodies directed against the Globo H determinant to human gastric glycosphingolipids. Thus, Globo H may have a role in the BabA-mediated target tissue adherence of H. pylori.

Expression of BabA by H. pylori is associated with severe gastric inflammation and an increased risk of developing peptic ulcer or gastric cancer (38, 39). H. pylori infects more than half of the world’s population, and although the prevalence of infection is decreasing in developed countries, the infection rate is still high in developing countries (40). Furthermore, the treatment options in developing countries are currently inadequate. Targeting BabA might be important for the development of novel treatment strategies against H. pylori.

Acknowledgment—The use of the LTQ linear quadrupole ion trap mass spectrometer (obtained by Swedish Research Council Grant 342-2004-4434 to Gunnar Hansson) and the Varian 600-MHz machine at the Swedish NMR Centre, Hasselblad Laboratory, University of Gothenburg, is gratefully acknowledged.

REFERENCES
1. Karlsson, K. A. (1989) Animal glycosphingolipids as membrane attachment sites for bacteria. Annu. Rev. Biochem. 58, 309–350
2. Esko, J. D. (1999) Essentials in Glyobiology (Varki, A., Cummings, R., Esko, J., Freeze, H., Hart, G., and Marth, J., eds) pp. 429–440, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
3. Pieters, R. J. (2011) Carbohydrate mediated bacterial adhesion. Adv. Exp. Med. Biol. 715, 227–240
4. Stults, C. L., Sweeley, C. C., and Macher, B. A. (1989) Glycosphingolipids: structure, biological source and properties. Methods Enzymol. 179, 167–214
5. Borén, T., Falk, P., Roth, K. A., Larson, G., and Normark, S. (1993) Attachment of Helicobacter pylori to human gastric epithelium mediated by blood group antigens. Science 262, 1892–1895
6. Ilver, D., Arnqvist, A., Ogren, J., Frick, I. M., Kersulyte, D., Incecik, E. T., Berg, D. E., Covacci, A., Engstrand, L., and Borén, T. (1998) Helicobacter pylori adhesin binding fucosylated histo-blood group antigens revealed by retagging. Science 279, 373–377
7. Gerhard, M., Leln, N., Neumayer, N., Borén, T., Rad, R., Schep, W., Mielike, S., Classen, M., and Prinz, C. (1999) Clinical relevance of the Helicobacter pylori gene for blood-group antigen-binding adhesin. Proc.
H. pylori BabA-binding Glycosphingolipids

Breimer, M. E. (2008) Glycolipid studies in small intestine and pancreas of α1,3-galactosyltransferase knockout miniature swine: α1,3GALT-KO animals lack αGal antigens and contain novel blood group H compounds. Transplant. Proc. 40, 543–546

24. Kundu, S. K. (1993) Glycoconjugates. Composition, Structure and Function (Allen, H. J., and Kisailus, E. E., eds) pp. 203–262, Marcel Dekker Inc., New York

25. Hakomori, S. (2001) Tumor-associated carbohydrate antigens defining tumor malignancy: basis for development of anti-cancer vaccines. Adv. Exp. Med. Biol. 491, 369–402

26. Teneberg, S., Leonardsson, I., Karlsson, H., Jovall, P. A., Angstrom, J., Danielsen, D., Naslund, I., Ljung, A., Wadstrom, T., and Karlsson, K. A. (2002) Lactotetraosylceramide, a novel glycosphingolipid receptor for Helicobacter pylori, present in human gastric epithelium. J. Biol. Chem. 277, 19709–19719

27. Chai, W., Piskarev, V., and Lawson, A. M. (2001) Negative-ion electrospray mass spectrometry of neutral underivatized oligosaccharides. Anal. Chem. 73, 651–657

28. Robbe, C., Capon, C., Coddeville, B., and Michalski, J. C. (2004) Diagnostic ions for the rapid analysis by nano-electrospray ionization quadrupole time-of-flight mass spectrometry of O-glycans from human mucins. Rapid Commun. Mass Spectrom. 18, 412–420

29. Holgersson, I., Jovall, P. A., Samuelsson, B. E., and Breimer, M. E. (1990) Structural characterization of non-acid glycosphingolipids in kidneys of single blood group O and A pigs. J. Biochem. 108, 766–777

30. Li, Y. T., Chou, C. W., Li, S. C., Kobayashi, U., Ishibashi, Y. H., and Ito, M. (2009) Preparation of homogenous oligosaccharide chains from glycosphingolipids. Glycoconj. J. 26, 929–933

31. Zhou, B., Li, S. C., Laine, R. A., Huang, R. T., and Li, Y. T. (1989) Isolation and characterization of ceramide glycans from the leech, Macrobodella decora. J. Biol. Chem. 264, 12272–12277

32. Chai, W., Piskarev, V., and Lawson, A. M. (2002) Branching pattern and sequence analysis of underivatized oligosaccharides by combined MS/MS of singly and doubly charged molecular ions in negative-ion electrospray mass spectrometry. J. Am. Soc. Mass Spectrom. 13, 670–679

33. Angström, J., Larsson, T., Hansson, G. C., Karlsson, K. A., and Henry, S. (1989) Purification and characterization of glycolipid-binding glycosphingolipid created by expression of human α-1,3/4-fucosyltransferase in FVB/N mouse stomach. Glycobiology 19, 182–191

34. Imberty, A., Mikros, E., Koca, J., Mollicone, R., Oriol, R., and Pérez, S. (1995) Computer simulation of histo-blood group oligosaccharides: energy maps of all constituting disaccharides and potential energy surfaces of 14 ABH and Lewis carbohydrate antigens. Glycoconjug. J. 12, 331–349

35. Nyholm, P. G., Samuelsson, B. E., Breimer, M., and Pascher, I. (2009) Novel LeX-like Helicobacter pylori-binding glycosphingolipid created by expression of human α1,3/4-fucosyltransferase in FVB/N mouse stomach. Glycobiology 19, 108, 331–349

36. Karlsson, K. A. (1987) Preparation of total non-acid glycolipids for overlay analysis of receptors for bacteria and viruses and for other studies. Methods Enzymol. 138, 212–220

37. Waldi, D. (1962) in Thin-layer Chromatography (Stahl, E., ed.) pp. 496–515, Springer-Verlag, Berlin

38. Björk, S., Breimer, M. E., Hansson, G. C., Karlsson, K. A., and Leffler, H. (1987) Structures of blood group glycosphingolipids of human small intestine. A relation between the expression of fucosylations of epithelial cells and the ABO, Le and Se phenotype of the donor. J. Biol. Chem. 262, 6758–6765

39. Breimer, M. E., Hansson, G. C., Karlsson, K. A., and Leffler, H. (1982) Isolation and partial characterization of blood group A and H active glycosphingolipids of rat small intestine. J. Biol. Chem. 257, 906–912

40. Angström, J., Bäckström, M., Berntsson, A., Karlsson, N., Holmgren, I., Karlsson, K. A., Levens, M., and Teneberg, S. (2000) Novel carbohydrate binding site recognizing blood group A and B determinants in a cholera toxin/heat-labile enterotoxin B-subunit hybrid. J. Biol. Chem. 275, 3231–3238

41. Coddens, A., Disswall, M., Angström, J., Breimer, M. E., Goddeeris, B., Cox, E., and Teneberg, S. (2009) Recognition of blood group ABH type 1 determinants by the FedF adhesin of F18-fimbriated Escherichia coli. J. Biol. Chem. 284, 9713–9726

42. Disswall, M., Angström, J., Schuurman, H. J., Dor, F. J., Rydelberg, L., and

43. Natl. Acad. Sci. U.S.A. 96, 12778–12783

44. Rad, R., Gerhard, M., Lang, R., Schöniger, M., Rösch, T., Schepp, W., Becker, I., Wagner, H., and Prinz, C. (2002) The Helicobacter pylori blood group antigen-binding adhesin facilitates bacterial colonization and augments a non-specific immune response. J. Immunol. 168, 3033–3041
H. pylori BabA-binding Glycosphingolipids

erythrocyte membranes. Biochemistry 24, 3578–3586
42. McKibbin, J. M., Spencer, W. A., Smith, E. L., Mansson, J. E., Karlsson, K. A., Samuelsson, B. E., Li, Y. T., and Li, S. C. (1982) Lewis blood group fucolipids and their isomers from human and canine intestine. J. Biol. Chem. 257, 755–760
43. Stellner, K., Watanabe, K., and Hakomori, S. (1973) Isolation and characterization of glycosphingolipids with blood group H specificity from membranes of human erythrocytes. Biochemistry 12, 656–661
44. Kościelak, J., Plasek, A., Górnia, H., Gardas, A., and Gregor, A. (1973) Structures of fucose-containing glycolipids with H and B blood-group activity and of sialic acid and glucosamine-containing glycolipid of human-erythrocyte membrane. Eur. J. Biochem. 37, 214–225
45. Kościelak, J., Plasek, A., and Gorniak, H. (1970) in Blood and Tissue Antigens (Aminoff, D., ed) pp. 163–176, Academic Press, New York
46. Clausen, H., Levery, S. B., Nudelman, E., Tsuchiya, S., and Hakomori, S. (1985) Repetitive A epitope (type 3 chain A) defined by blood group A1-specific monoclonal antibody TH-1: chemical basis of qualitative A1 and A2 distinction. Proc. Natl. Acad. Sci. U.S.A. 82, 1199–1203
47. Iwamori, M., and Nagai, Y. (1981) Ganglioside composition of rabbit thymus. Biochim. Biophys. Acta 665, 205–213