

Simultaneous Expression of Type 1 and Type 2 Lewis Blood Group Antigens by *Helicobacter pylori* Lipopolysaccharides

MOLECULAR MIMICRY BETWEEN *H. PYLORI* LIPOPOLYSACCHARIDES AND HUMAN GASTRIC EPITHELIAL CELL SURFACE GLYCOFORMS*

(Received for publication, January 15, 1998, and in revised form, February 27, 1998)

Mario A. Monteiro,^{a,b,c,d} Kenneth H. N. Chan,^b David A. Rasko,^{a,c,e} Diane E. Taylor,^{a,c,e,f} P. Y. Zheng,^g Ben J. Appelmelk,^g Hans-Peter Wirth,^{h,i} Manqiao Yang,^{h,i} Martin J. Blaser,^{h,j} Sean O. Hynes,^k Anthony P. Moran,^k and Malcolm B. Perry^{a,b,c}

From the ^aCanadian Bacterial Diseases Network, ^bInstitute for Biological Sciences, National Research Council, Ottawa, K1A 0R6 Ontario, Canada, the ^cDepartment of Medical Microbiology and Immunology, University of Alberta, Edmonton, T6G 2H7 Alberta, Canada, ^dDepartment of Medical Microbiology, Vrije Universiteit Amsterdam, Amsterdam, The Netherlands, the ^eDivision of Gastroenterology, Zurich University School of Medicine, Zurich, Switzerland, the ^fDepartment of Medicine, Vanderbilt University and Veterans Affairs Medical Center, Nashville, Tennessee, and the ^gDepartment of Microbiology, National University of Ireland, Galway, Ireland

Previous structural investigations performed on the lipopolysaccharides (LPSs) from the human gastric pathogen *Helicobacter pylori* have revealed that these cell surface glycan molecules express type 2 partially fucosylated, glucosylated, or galactosylated *N*-acetylglucosamine O antigen chains (O-chains) of various lengths, which may or may not be terminated at the nonreducing end by Lewis X (Le^x) and/or Le^y blood group epitopes in mimicry of human cell surface glycoconjugates and glycolipids. Subsequently, serological experiments with commercially available Lewis-specific monoclonal antibodies also have recognized the presence of Le^x and Le^y blood group antigens in *H. pylori* but, in addition, have indicated the presence of type 1 chain Le^a, Le^b, and Le^d (H-type 1) blood group epitopes in some *H. pylori* strains. To confirm their presence, structural studies and additional serological experiments were undertaken on *H. pylori* strains suspected of carrying type 1 chain epitopes. These investigations revealed that the O-chain region of *H. pylori* strain UA948 carried both Le^a (type 1) and Le^x (type 2) blood group determinants. The O-chain from *H. pylori* UA955 LPS expressed the terminal Lewis disaccharide (type 1 chain) and Le^x and Le^y antigens (type 2). The O-chain of *H. pylori* J223 LPS carried the type 1 chain precursor Le^c, the H-1 epitope (Le^d, type 1 chain) and an elongated nonfucosylated type 2 *N*-acetylglucosamine chain (i antigen). Thus, O-chains from *H. pylori* LPSs can also express fucosylated type 1 sequences, and the LPS from a single *H. pylori* strain may carry O-chains with type 1 and 2 Lewis blood groups simultaneously. That monoclonal antibodies putatively specific for the Le^b determinant can detect gly-

can substructures (Le disaccharide, Le^c, and Le^d) of Le^b indicates their nonspecificity. The expression of both type 1 and 2 Lewis antigens by *H. pylori* LPSs mimics the cell surface glycomolecules present in both the gastric superficial (which expresses mainly type 1 determinants) and the superficial and glandular epithelium regions (both of which express predominantly type 2 determinants). Therefore, each *H. pylori* strain may have a different niche within the gastric mucosa, and each individual LPS blood group antigen may have a dissimilar role in *H. pylori* adaptation.

During the past decade, much attention has been directed toward the Gram-negative bacterium *Helicobacter pylori* and its roles in gastritis, peptic ulcer disease, and gastric malignancies in humans (1). One class of molecules produced by Gram-negative enteric bacteria are the cell surface lipopolysaccharides (LPSs¹; O-chain → core → lipid A), which often play important roles in bacteria-host interactions (2). In 1994, the first detailed chemical structure of *H. pylori* LPS showed that the O-chain region of *H. pylori* type strain (NCTC 11637) was composed of an elongated partially fucosylated type 2 *N*-acetylglucosamine (LacNAc) polysaccharide covalently attached at the reducing end by a core oligosaccharide and terminated at the nonreducing end by mono-, di-, or trimeric Lewis X (Le^x) (β-D-Gal-(1→4)[α-L-Fuc-(1→3)]β-D-GlcNAc-(1→) blood-group epitopes in mimicry of normal human cell surface glycoconjugates and of glycan antigens found in adenocarcinoma tumors (Refs. 3 and 4; see Fig. 1 for the molecular structure of O-chain and core regions from *H. pylori* type strain LPS). Since then, structural studies on other *H. pylori* strains have revealed the presence of the type 2 Le^y (α-L-Fuc-(1→2)-β-D-Gal-(1→4)[α-L-Fuc-(1→3)]β-D-GlcNAc-(1→) determinant in the O-chains of strains P466 (5), MO19 (5), O:3, and O:6 (6). This Le^y epitope may terminate an elongated partially fucosylated type 2 LacNAc O-chain polysaccharide as in the cases of strains P466 (5) and O:3 (6) or may be directly connected to the

* The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

^c Supported by the Canadian Bacterial Diseases Network (Centers of Excellence).

^d To whom correspondence should be addressed: Inst. for Biological Sciences, National Research Council, 100 Sussex Dr., Ottawa, Ontario, K1A 0R6, Canada. Tel.: 613-990-0832; Fax: 613-952-9092; E-mail: Mario.Monteiro@nrc.ca.

^f An Alberta Heritage for Medical Research Scientist; supported by the National Cancer Institute of Canada (funds from the Terry Fox Run).

^j Supported in part by the Medical Research Service of the Department of Veterans Affairs.

¹ The abbreviations used are: LPS, lipopolysaccharide; Fuc, fucose; DD-Hep, D-glycero-D-manno-heptose; LD-Hep, L-glycero-D-manno-heptose; MS, mass spectrometry; FAB-MS, fast atom bombardment-mass spectrometry; Le^x, Lewis X (similarly for other Lewis epitopes); LacNAc, *N*-acetylglucosamine; mAb, monoclonal antibody; ELISA, enzyme-linked immunosorbent assay; O-chain, O antigen chain; Hex, hexose; Hep, heptose. HexNAc, *N*-acetyl-hexosamine.

FIG. 1. The complete structure of O-chain and core regions of the LPS from *H. pylori* type strain NCTC 11637 (4). The O-chain region is an elongated (~10 repeating units) partially fucosylated type 2 *N*-acetylglucosamine polysaccharide terminated by the Le^x determinant. The O-chain is covalently linked to the core oligosaccharide through a side chain DD-Hep. *Kdo*, 3-deoxy-manno-D-octulosonic acid.

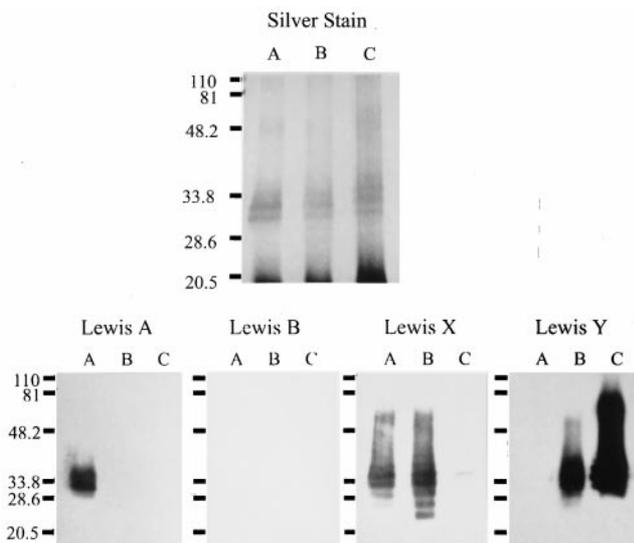
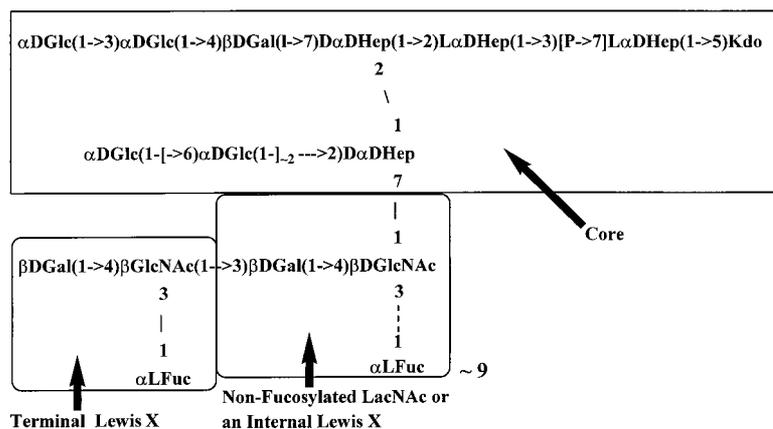


FIG. 2. SDS-polyacrylamide gel electrophoresis and immunoblots of LPSs from *H. pylori* strains UA948, UA955, and UA1182 with Lewis blood group mAbs. Lane A, strain UA948; lane B, strain UA955; lane C, strain UA1182. Positive interactions can be seen between UA948 and Le^a and Le^x mAbs, between UA955 and Le^x and Le^y mAbs, and between UA1182 (control) and Le^y mAb.

remaining LPS molecule as in strains MO19 (5) and O:6 (6). Recently, the LPS of *H. pylori* strain UA861 was also found to contain a type 2 LacNAc O-chain polysaccharide, but the lateral fucose was replaced by a glucose unit, and this LPS was terminated by a LacNAc (β -D-Gal-(1 \rightarrow 4)- β -D-GlcNAc-(1 \rightarrow) epitope and did not express terminal Le^x or Le^y determinants (7). A preliminary account describing the presence of a galactosylated *N*-acetylglucosamine O-chain in *H. pylori* strain 471 LPS has also been reported (8). This molecular mimicry displayed by *H. pylori* LPSs with human cell surface molecules, which also express Le^x and Le^y blood group structures, is now the basis for the hypothesis that there might be an autoimmune component in *H. pylori* pathogenesis (9).

Following the discovery of Le^x and Le^y blood group epitopes in *H. pylori* LPSs by chemical analysis, several studies also have detected the presence of these Lewis blood group antigens in many *H. pylori* strains by employing commercially available monoclonal antibodies (mAbs) (10–13). Moreover, some of these investigations also have described the presence of type 1 Lewis blood group antigens, such as Le^a (β -D-Gal-(1 \rightarrow 3)-[α -L-Fuc-(1 \rightarrow 4)]- β -D-GlcNAc-(1 \rightarrow), Le^b (α -L-Fuc-(1 \rightarrow 2)- β -D-Gal-(1 \rightarrow 3)-[α -L-Fuc-(1 \rightarrow 4)]- β -D-GlcNAc-(1 \rightarrow), and H-type 1 (Le^d) (α -L-Fuc-(1 \rightarrow 2)- β -D-Gal-(1 \rightarrow 3)- β -D-GlcNAc-(1 \rightarrow), in some *H. pylori* strains (11, 12). Chemically based structural studies

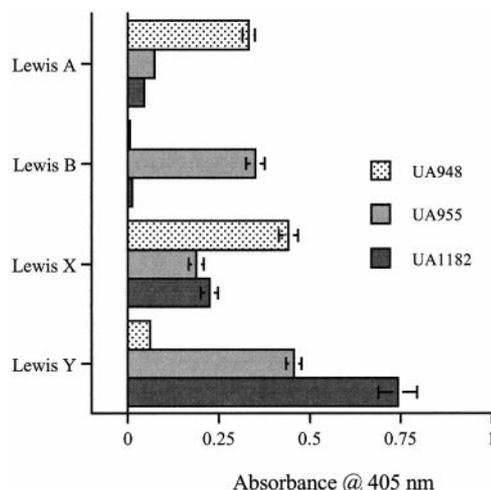


FIG. 3. *H. pylori* whole cell ELISA of strains UA948, UA955, and UA1182. These data suggest that *H. pylori* UA948 expresses both Le^a and Le^x antigens; *H. pylori* UA955 expresses the Le^b, Le^x, and Le^y antigens; and *H. pylori* UA1182 expresses Le^x and Le^y antigens. Only absorbance values over 0.1 atomic unit were considered positive (12).

then were initiated on *H. pylori* strains suspected of carrying type 1 Lewis antigens (as shown by serology) to confirm the presence of these epitopes in the LPS. These *H. pylori* strains are strain UA948, which reacted with both Le^x and Le^a mAbs; UA955, which was recognized by Le^b, Le^y, and Le^x mAbs; and J223, which reacted with a Le^b mAb (12). The results from the structural and serological investigations on the LPSs from these *H. pylori* strains are reported in this paper.

EXPERIMENTAL PROCEDURES

Origin and Cell Production of *H. pylori* Strains—Clinical isolates of *H. pylori* UA948, UA955, and UA1182 were obtained from dyspeptic patients at the University of Alberta Hospital by methods described previously (14). These *H. pylori* strains were frozen immediately after isolation (-70°C). Cultures were subsequently thawed and plated onto brain heart infusion agar (1.2%, w/v) plates supplemented with 0.5% (w/v) yeast extract and 0.5% (v/v) fetal bovine sera (Hyclone, Logan, UT). These *H. pylori* strains were allowed to grow for 3 days under microaerobic conditions at 37°C , subcultured into BHI broth, and allowed to grow for an additional 3 days under the same conditions with agitation. *H. pylori* strain J223 was isolated from a duodenal ulcer patient at the Nashville Veterans Affairs Medical Center, and cells were grown as described (12). *H. pylori* strains UA948, UA955, UA1182, and J223 all were determined to be *cagA*⁺.

Isolation of Lipopolysaccharides—The LPSs were isolated by the hot phenol-water extraction procedure (15). The water-soluble LPSs were purified by gel permeation chromatography on a column of Bio-Gel P-2 (1 m \times 1 cm) with water as eluent. In all cases, only one carbohydrate-positive fraction was obtained which eluted in the high M_r range (16). These intact *H. pylori* LPSs then were used for chemical, spectroscopic, and serological analyses.

Sugar Composition and Methylation Linkage Analyses—Sugar composition analysis was performed by the alditol acetate method (17). The hydrolysis was done in 4 M trifluoroacetic acid at 100 °C for 4 h followed by reduction in H₂O with NaBD₄ and subsequent acetylation with acetic anhydride and with residual sodium acetate as the catalyst. Alditol acetate derivatives were analyzed by gas-liquid chromatography mass spectrometry using a Hewlett-Packard chromatograph equipped with a 30-m DB-17 capillary column (210 °C (30 min) → 240 °C at 2 °C/min), and MS in the electron impact mode was recorded using a Varian Saturn II mass spectrometer. Enantiomeric configurations of the individual sugars were determined by the formation of the respective 2-(*S*)- and 2-(*R*)-butyl chiral glycosides (18). Methylation linkage analysis was carried out by the NaOH/Me₂SO/CH₃I procedure (19) and with characterization of permethylated alditol acetate derivatives by gas-liquid chromatography mass spectrometry in the electron impact mode (DB-17 column, isothermally at 190 °C for 60 min).

Fast Atom Bombardment-Mass Spectrometry (FAB-MS)—A fraction of the methylated sample was used for positive ion FAB-MS, which was performed on a Jeol JMS-AX505H mass spectrometer with glycerol (1): thioglycerol (3) as the matrix. A 6-kV xenon beam was used to produce pseudomolecular ions, which were then accelerated to 3 kV, and their mass was analyzed. Product ion scan (B/E) and precursor ion scan (B²/E) were performed on metastable ions created in the first free field with a source pressure of 5 × 10⁻⁵ torr. The interpretations of positive ion mass spectra of the permethylated LPS derivatives were as described previously by Dell *et al.* (20).

NMR Spectroscopy—¹H NMR spectra of the water-soluble intact LPSs were recorded on a Bruker AMX 500 spectrometer at 300 K using standard Bruker software. Prior to performing the NMR experiments,

TABLE I

Sugar linkage analyses of the intact LPSs of H. pylori UA948, UA955, and J223 showing approximate molar ratios

These ratios are approximate values, and any variances with ratios from composition analysis represent either minor structural differences between the samples used for each analyses due to molecular heterogeneity or are simply a shortfall of the permethylated alditol acetate derivatives in the linkage analysis procedures (an inherited analytical feature of this type of analysis). In addition, composition analysis by the alditol acetate method will detect GlcNAc from the lipid A GlcN units, but these units will not be observed in the methylation linkage analysis procedure, thus leading to some discrepancy between these two ratios.

Linkage type	UA948 LPS	UA955 LPS	J223 LPS
Lewis blood group units (O-Chain)			
Fucp-(1→	1.5	4	1
Galp-(1→	1	1	1
→2)-Galp-(1→	0	1	1
→3)-Galp-(1→	3	4	5
→3)-GlcNac-(1→	0	0	1.5
→4)-GlcNac-(1→	2	1	4
→3,4)-GlcNac-(1→	1	3	0
Core units			
GlcP-(1→	1	1	1
→4)-Galp-(1→	1	1	1
→3)-GlcP-(1→	1	1	1
→7)-DD-Hepp-(1→	1	1	1
→2,7)-DD-Hepp-(1→	1	1	1
→2)-LD-Hepp-(1→	1	1	1

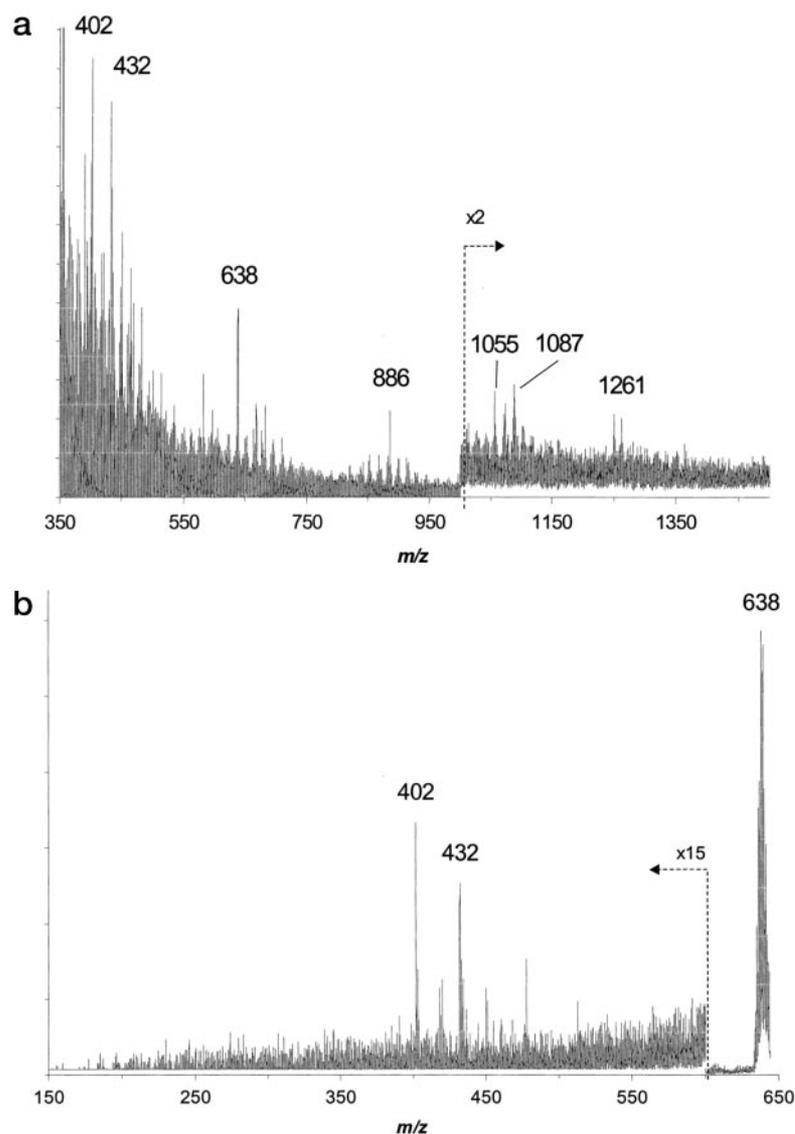


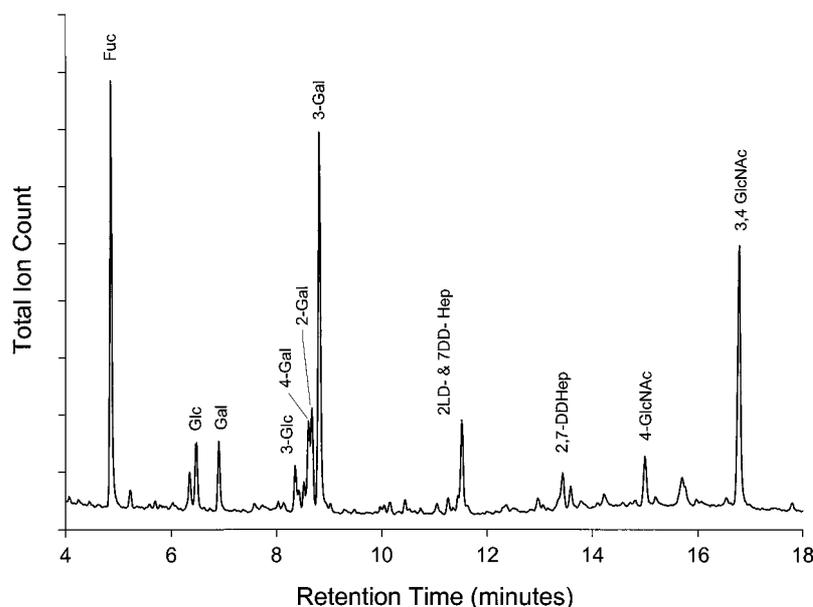
FIG. 4. FAB-MS spectra of *H. pylori* UA948 methylated intact LPS. *a*, the complete FAB-MS spectrum of UA948. *b*, the product ion MS/MS of *m/z* 638.

TABLE II
Interpretation of the ions from the FAB-MS spectrum of methylated intact *H. pylori* UA948 LPS

The secondary ions shown originate from β -elimination of the residue at the O-3 position of the GlcNAc at the reducing end. The shall indicates that either structure is possible.

Primary ions	Secondary ions	Proposed structure
<i>m/z</i> 638	<i>m/z</i> 402 (638 - 236)	Fuc-→4-GlcNAc ⁺ 3 ↑ Gal-1 (Le ^a , type 1 chain)
638	432 (638 - 206)	Gal-1→4-GlcNAc ⁺ 3 ↑ Fuc-1 (Le ^x , type 2 chain)
886		Gal-1→4/3-GlcNAc-1→Hep ⁺ 3/4 ↑ Fuc-1 (Le ^{a/x} →Hep)
1087	1055 (1087 - 32)	Gal-1→4/3-GlcNAc-1→3-Gal-1→4-GlcNAc ⁺ 3/4 ↑ Fuc-1 (Le ^{a/x} →LacNAc)
1261	1055 (1261 - 206)	Gal-1→4/3-GlcNAc-1→3-Gal-1→4-GlcNAc ⁺ 3/4 ↑ Fuc-1 3 ↑ Fuc-1 (Le ^{a/x} →Le ^x)

FIG. 5. Gas-liquid chromatogram of the permethylated alditol acetates from *H. pylori* UA955 intact LPS. The major components are terminal Fuc, 3-linked Gal, and 3,4-linked GlcNAc.



the samples were lyophilized three times with D₂O (99.9%). The HOD peak was used as the internal reference at δ_H 4.786.

Serological Procedures—For ELISA experiments on *H. pylori* strains UA948, UA955, and UA1182, cells were harvested from cultures by centrifugation (5000 × *g* for 5 min) and washed once with coating buffer (0.01 M sodium carbonate, pH 9.5). The whole cell protein concentration was adjusted to 10 μ g/ml and 100 μ l of solution and added to each well of a microtiter plate. The microtiter plate was incubated overnight at 4 °C, and then nonadherent cells were removed by washing three times with phosphate-buffered saline (pH 7.4) containing 0.05% (w/v) bovine serum albumin, 0.05% (v/v) Tween 20, and 0.004% (w/v) Thimersol (wash buffer). The reaction wells were blocked with blocking buffer (phosphate-buffered saline, pH 7.4, containing 2.5% (w/v) bovine serum albumin, 5% (v/v) fetal bovine serum, 0.05% (v/v) Tween 20, and 0.004% (w/v) Thimersol overnight at 4 °C. The microtiter plates were washed three times with wash buffer and then incubated with the primary antibodies against Lewis antigens. The antibodies used were anti-Le^a (mAb BG-5, clone T174), anti-Le^b (mAb BG-6, clone T218), anti-Le^x

(mAb BG-7, clone P12), anti-Le^y (mAb BG-8, clone F3). All mAbs were obtained from Signet Laboratories Inc. (Dedham, MA). The primary antibodies were diluted 1:100 in phosphate-buffered saline containing 0.5% (w/v) bovine serum albumin, 1% (v/v) fetal bovine serum, 0.05% (v/v) Tween 20, and 0.004% (w/v) Thimersol for 2 h at 37 °C and washed as described above. The secondary antibody (1:2000 dilution of anti-mouse IgG plus IgM conjugated to horseradish peroxidase (Biocan, catalog number 115 035 063, Mississauga, Ontario, Canada) was added to the wells and incubated for 1.5 h at 37 °C. The microtiter plate was developed at room temperature with 1 mM 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (Sigma, catalog number A-1888), H₂O₂ (0.03%) in 0.01 M citrate buffer (pH 4.2) for 20–30 min with agitation (150 rpm). The reaction was stopped with 4 mM sodium azide, and the absorbance was recorded at 405 nm using a Titretrek (Helsinki, Finland) Multiscan MC microtiter plate reader.

For the acrylamide gel electrophoresis and immunoblot studies, proteinase K-treated whole cells extracts of *H. pylori* strains UA948, UA955, and UA1182 were prepared as described previously by Hitch-

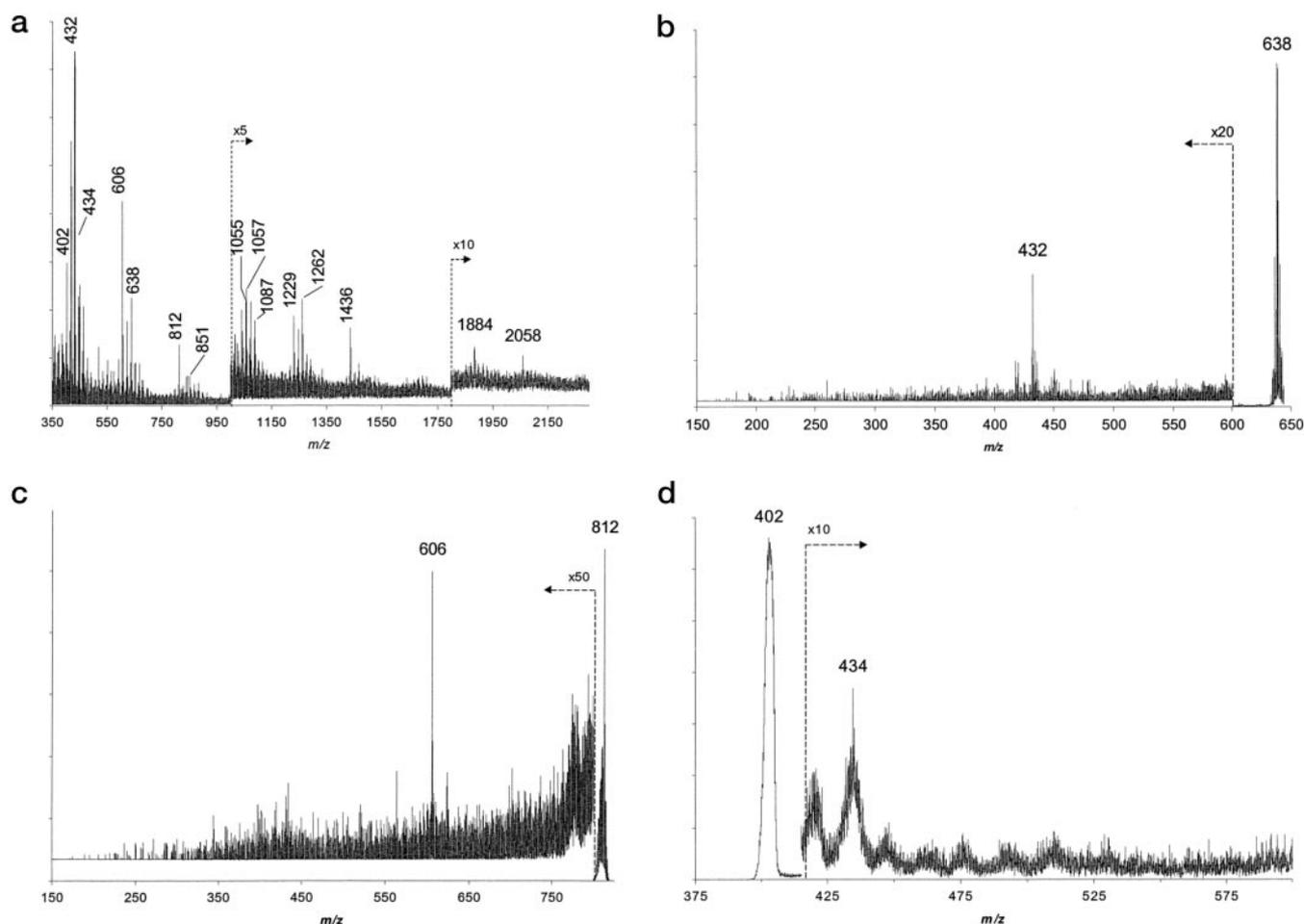


FIG. 6. FAB-MS spectra of *H. pylori* UA955 methylated intact LPS. *a*, the complete FAB-MS spectrum of UA955. *b*, the product ion MS/MS of m/z 638. *c*, the product ion MS/MS spectrum of m/z 812. *d*, the precursor ion MS/MS spectrum of m/z 402.

cock and Brown (21). For the LPS analysis, a 15% (w/v) acrylamide separating gel containing urea (Life Technologies, Inc.) and a 5% (w/v) polyacrylamide stacking gel were used. Electrophoresis was conducted with a constant current of 35 mA for 1 h. These gels were either silver-stained according to the method of Tsai and Frasch (22) or electroblotted onto a nitrocellulose membrane (Micron Separations Inc. Westboro, MA; pore size, 0.22 μm) according to the methods described by Towbin *et al.* (23). Nitrocellulose membranes with transferred LPSs were probed with the antibodies described previously. Anti-Lewis (Signet Laboratories Inc.) antibodies diluted 1:100 were used as the primary antibody, and goat anti-mouse IgG plus IgM conjugated to horseradish peroxidase diluted 1:2000 was the secondary antibody as described above. Reactions were visualized using an enhanced chemiluminescence kit (Amersham Pharmacia Biotech) according to the manufacturer's specifications, and blots were developed on BioMax film (Eastman Kodak Co.).

The LPS from strain J223 was serotyped in ELISA using procedures that have been previously described (9, 11). For serotyping of *H. pylori* J223, the following mAbs were employed (11): CB-10 and 54.1F6A (both anti-Le^x), 1E52 (anti-Le^y), 7-Le (anti-Le^a), 225-Le (anti-Le^b), 4D2 (anti-H type 1), 3-3A (anti-blood group A), and NAM61-1A2 (anti-i antigen) (24). *H. pylori* J223 LPS was subjected to SDS-polyacrylamide gel electrophoresis, silver-stained, and immunoblotted as described previously (11).

Polyclonal antisera against the core of LPS of four *H. pylori* strains were used whose specificity had been determined in a previous study (25). Antisera were raised in rabbits immunized with formalin-treated heat-killed *H. pylori* strains that expressed rough form LPS lacking O-chain. Following incubation with the relevant primary antibody, the nitrocellulose membranes were washed as described above and incubated with goat anti-mouse IgG-horseradish peroxidase conjugate (Sigma) and goat anti-rabbit IgG-horseradish peroxidase conjugate (Bio-Rad), as appropriate, for 1 h at room temperature. After washing, reactions were visualized with the Bio-Rad premixed en-

zyme substrate kit (2.5 ml of 4-chloro-1-naphthol in diethylene glycol, 25 ml of Tris-buffered saline, and 15 μl of H₂O₂) according to the manufacturer's instructions.

RESULTS

All chemical, spectroscopic, and serological experiments were performed on intact water-soluble LPS to prevent any aberrant results that might arise due to the inadvertent loss of acid-labile glycosyl units (such as fucose and sialic acid) that could take place during the standardized mild acetic acid treatment of LPS that is normally used to liberate the polysaccharide from the insoluble lipid A moiety of the LPS molecule. Contrary to LPSs from typical Gram-negative bacteria, *H. pylori* high M_r smooth-form LPSs are soluble in water. The structural studies were performed on the LPSs isolated from the same *H. pylori* cells with which the serological experiments were carried out.

Characterization of the O-chain Region of *H. pylori* Strain UA948—The criteria of Wirth *et al.* (12), that an absorbance value of <0.1 absorbance units was considered a negative result in the ELISA, whereas higher values were positive, was used in these studies. The serological experiments shown in Figs. 2 and 3 indicated that the LPS from *H. pylori* strain UA948 expressed both Le^a and Le^x antigenic determinants. *H. pylori* strain UA1182, whose LPS expresses a terminal Le^y epitope that terminates an O-chain of approximately eight repeating internal Le^x units, Le^y-[→ Le^x]₈ → core → lipid A², used as a control, was recognized by mAbs to Le^x and Le^y, as expected. The reactions of the Le^x and Le^a mAbs were directed at the O-chain region of UA948 LPS as shown by the SDS-

TABLE III
 Interpretation of the ions from the FAB-MS spectrum of methylated intact *H. pylori* UA955 LPS

The secondary ions shown originate from β -elimination of the residue at the O-3 position of the GlcNAc at the reducing end.

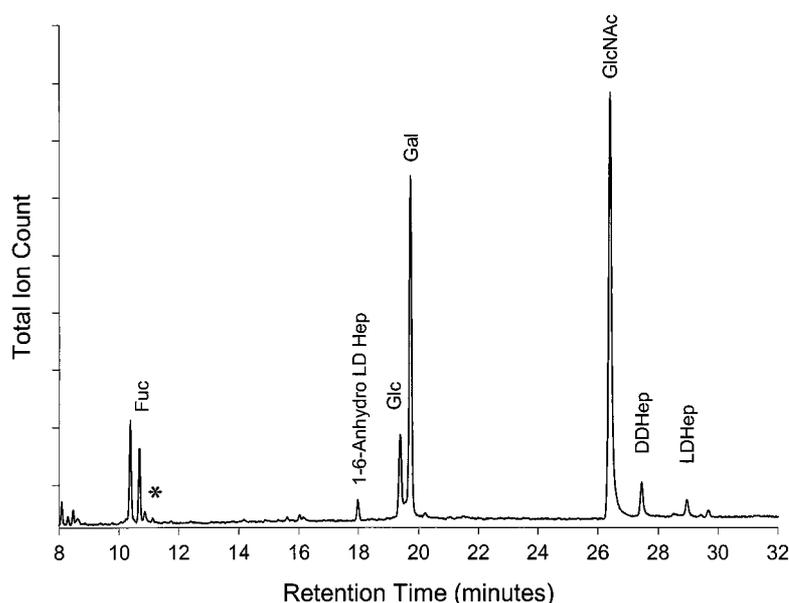
Primary ions	Secondary ions	Proposed structure
<i>m/z</i>	<i>m/z</i>	
434	402 (434 - 32)	Fuc-1→4-GlcNAc ⁺ (Le ^{dis} , type 1 chain)
638	432 (638 - 206)	Gal-1→4-GlcNAc ⁺ (Le ^x , type 2 chain)
		3 ↑
812	(812 - 206)	Fuc-1 Gal-1→4-GlcNAc ⁺ (Le ^y , type 2 chain)
		2 3 ↑ ↑
1057	851 (1057 - 206)	Fuc-1 Fuc-1 Fuc-1→4-GlcNAc-1→3-Gal-1→4-GlcNAc ⁺
		(Fuc→4-GlcNAc→Le ^x , type 1 and 2 regions) 3 ↑
1087	1055 (1087 - 32)	Gal-1→4-GlcNAc-1→3-Gal-1→4-GlcNAc ⁺
		3 ↑ (Le ^x →LacNAc)
1261	1055 (1261 - 206)	Gal-1→4-GlcNAc-1→3-Gal-1→4-GlcNAc ⁺
		3 3 ↑ ↑
1261	1229 (1263 - 32)	(Le ^x →Le ^x) Fuc-1 Fuc-1 Gal-1→4-GlcNAc-1→3-Gal-1→4-GlcNAc ⁺
		2 3 3 ↑ ↑ ↑
1435	1229 (1435 - 206)	Fuc-1 Fuc-1 (Le ^y →Le ^x) Fuc-1 Gal-1→4-GlcNAc-1→3-Gal-1→4-GlcNAc ⁺
		2 3 3 3 ↑ ↑ ↑ ↑
2058		Gal-1→4-GlcNAc-1→3-Gal-1→4-GlcNAc-1→3-Gal-1→4-GlcNAc ⁺
		2 3 3 3 ↑ ↑ ↑ ↑
		Fuc-1 Fuc-1 Fuc-1 Fuc-1 (Le ^y →Le ^x →Le ^x)

polyacrylamide gel electrophoresis and immunoblot profiles (Fig. 2).

Sugar compositional analysis on the water-soluble intact UA948 LPS by the alditol acetate method showed the presence of L-Fuc, D-Glc, D-Gal, D-GlcNAc, D-glycero-D-manno-heptose (DD-Hep), and L-glycero-D-manno-heptose (LD-Hep) in the approximate molar ratio 1:1.4:3.6:3.4:1.5:1.3, respectively. The methylation linkage analysis performed on the intact UA948 LPS (Table I) revealed the presence of the following Lewis blood group-related units, terminal Fuc and Gal, 3-linked Gal, and 4- and 3,4-linked GlcNAc. No 2-linked Gal was detected. Other permethylated alditol acetate derivatives of Glc, Gal, DD-Hep, and LD-Hep were also detected (Table I) and, as shown later, are placed within the core oligosaccharide framework of *H. pylori* LPS architecture (Fig. 1). To deduce the sequence of the glucose units within the chain, FAB-MS was performed on the methylated intact LPS. The FAB-MS spectrum of the methylated UA948 LPS derivative (Fig. 4a) showed primary fragment (A-type) glycosyl oxonium ions, from preferential cleavage at the GlcNAc units (20, 26) and of defined compositions (Table II), at *m/z* 638 (deoxy-Hex, Hex, HexNAc), *m/z* 1087 (deoxy-Hex, Hex₂, HexNAc₂), and at *m/z* 1261 (deoxy-Hex₂, Hex₂, HexNAc₂). The FAB-MS spectrum also showed two possible secondary fragment ions from *m/z* 638, arising from β -elimination of the substituent at O-3 of the branched 3,4-linked GlcNAc unit, those being, *m/z* 402 through loss of terminal Gal-OH (236 atomic mass units), which is a characteristic pattern of the Le^a structure (26), and *m/z* 432 from the elimination of Fuc-OH (206 atomic mass units), which is associated with the presence of the Le^x structure (26) (Table II). To demonstrate that ions *m/z* 638, 432, and 402 were in fact related, MS/MS experiments were performed on these ions.

Product (parent) ion MS/MS on *m/z* 638 (Fig. 4b) showed that both *m/z* 402 and 432 stem from *m/z* 638, indicating that *m/z* 638 represented both terminal Le^x and Le^a epitopes. Also, when *m/z* 402 was subjected to a precursor (daughter) ion MS/MS it showed *m/z* 638 has its origin, thus confirming the presence of the Gal-1→3-[Fuc-1→4]GlcNAc (Le^a) trisaccharide. The higher mass primary ions at *m/z* 1087 and 1261 and the secondary ion at *m/z* 1055 (*m/z* 1087-32 and *m/z* 1261-206) represent a further type 2 chain extension of the terminal Lewis epitope (638 atomic mass units) by a LacNAc unit (449 atomic mass units), →3-Gal-1→4-GlcNAc-1→, and by an additional internal Le^x trisaccharide (623 atomic mass units), respectively (Table II). These data do not differentiate whether the type 2 LacNAc and Le^x chain extensions are attached to the type 1 terminal Le^a or to the type 2 terminal Le^x epitope. No evidence was obtained indicating that a type 1, →3-Gal-1→3-GlcNAc-1→, chain extension existed, nor could prominent *m/z* ions, which would have pointed toward a connection of the Le^{x/a} → LacNAc and Le^{x/a} → Le^x regions to units of the core be detected. However, one dominant A-type primary fragment ion of defined composition and that must include one heptose residue in addition to a terminal Lewis trisaccharide was seen at *m/z* 886 (deoxy-Hex, Hex, Hep, HexNAc) (Fig. 4a). Since there were no terminal heptose and no 2-substituted galactose derivatives detected, this primary ion must originate from cleavage at the heptosyl glycosidic bond to give Gal→[Fuc→]GlcNAc→Hep⁺. Either the terminal Le^a or Le^x antigen may be attached to this heptose core-related unit. The primary fragment ion *m/z* 886 has also been observed in previous FAB-MS experiments with other *H. pylori* strains, that showed the attachment of the first Le^x O-chain repeating unit to the core oligosaccharide (4, 5). The ¹H NMR spectrum of the intact UA948 LPS confirmed the presence of several Fuc resi-

FIG. 7. Gas-liquid chromatogram of the alditol acetate derivatives from *H. pylori* J223 intact LPS. The major components are Fuc, Gal, and GlcNAc.



dues (${}^6\text{CH}_3$, δ 1.15–1.4) and GlcNAc (COCH_3 , δ 2.02 s) units. These structural and serological studies were consistent in showing that the O-chain of the LPS from *H. pylori* UA948 carried type 1 Le^a and type 2 Le^x terminal epitopes, which may be extended by a further LacNAc or internal Le^x units (Table II).

Characterization of the O-chain Region of *H. pylori* Strain UA955—The whole cell ELISA (Fig. 3) study performed on *H. pylori* UA955 suggested the presence of Le^x , Le^y , and Le^b epitopes in the LPS. However, in immunoblots only a positive reaction with Le^x and Le^y mAbs with the O-chain regions could be observed, but not with the Le^b mAb (Fig. 2).

Sugar compositional analysis of the intact UA955 LPS showed the presence of L-Fuc, D-Glc, D-Gal, D-GlcNAc, DD-Hep, and LD-Hep in the approximate ratio of 4:2:7:6:2:1, respectively. Lewis blood group-related derivatives were the major components observed in the linkage analysis of UA955 LPS (Fig. 5 and Table I), those being terminal Fuc, 3-linked Gal, and 3,4-linked GlcNAc. Other detected derivatives of the Lewis blood group lineage were terminal and 2-linked Gal and 4-linked GlcNAc. The FAB-MS spectrum of the methylated UA955 LPS (Fig. 6a) showed the following primary glycosyl oxonium ions (see Table III for interpretation of ions), m/z 434 (deoxy-Hex, HexNAc), 638 (deoxy-Hex, Hex, HexNAc), 812 (deoxy-Hex₂, Hex, HexNAc), 1057 (deoxy-Hex₂, Hex, HexNAc₂), 1087 (deoxy-Hex₂, Hex₂, HexNAc₂), 1261 (deoxy-Hex, Hex₂, HexNAc₂), 1435 (deoxy-Hex₃, Hex₂, HexNAc₂), 1884 (deoxy-Hex₃, Hex₃, HexNAc₃), and 2058 (deoxy-Hex₄, Hex₃, HexNAc₃). Notwithstanding the small quantities of material available, selective MS/MS experiments were performed on three critical m/z ions, those being the primary fragment ions m/z 638 (characteristic of Le^a and Le^x epitopes), m/z 812 (typical of Le^b and Le^y units), and the secondary fragment ion m/z 402, which may arise from m/z 638 (Le^a (638–236)), 812 (Le^b (812–410)), or from m/z 434 (Lewis disaccharide, Fuc-1→4-GlcNAc (434–32)). First, the product ion MS/MS spectrum of m/z 638 (Fig. 6b) showed only one secondary ion at m/z 432 due to β -elimination of Fuc-OH (206) from one Le^x epitope (26), but no m/z 402, which would have implicated Le^a , was observed. Next, the product ion MS/MS spectrum of ion m/z 812 (Fig. 6c) revealed that it lost 206 atomic mass units (Fuc-OH) to yield m/z 606, which indicated that m/z 812 originated from a Le^y determinant and not from Le^b (26). Finally, the precursor ion MS/MS spectrum of m/z 402 (Fig. 6d) showed that this secondary ion emanated from m/z 434 (Fuc-1→4-GlcNAc⁺) (Lewis disaccha-

ride) through β -elimination of methanol (434–32) and, as shown in the product ion MS/MS experiments discussed above, did not originate from m/z 638 nor 812, ruling out the presence of terminal Le^a and Le^b epitopes in UA955 LPS. Also seen in the higher m/z region of the FAB-MS spectrum of UA955 LPS (Fig. 6a) are the primary and their corresponding secondary ions that represent extensions of the O-chain region, which can vary in glycosylation patterns (Table III). Of particular interest is the presence of m/z 1057 and 851 (1057–206), whose composition must include the Fuc-1→4-GlcNAc Lewis disaccharide terminal epitope covalently attached to one internal Le^x repeat. The presence of these two different fucosylated GlcNAc derivatives, Fuc-1→4-GlcNAc and Fuc-1→3-GlcNAc, implies that the same O-chain molecule can express type 1 and type 2 structural regions simultaneously. The longest chain observed in the FAB-MS spectrum of UA955 LPS was a tetrafucosylated $\text{Le}^y \rightarrow \text{Le}^x \rightarrow \text{Le}^x$ sequence similar to that found in *H. pylori* strain P466 (5). The ${}^1\text{H}$ NMR spectrum of the intact UA955 LPS confirmed the presence of several Fuc residues (${}^6\text{CH}_3$, δ 1.2–1.4 $J_{5,6,6'} \sim 6$ Hz) with one of major intensity at δ_{H} 1.2 belonging to the more abundant α -L-Fuc-1→3- β -D-GlcNAc and GlcNAc (COCH_3 , δ 2.02 s) units. These investigations on *H. pylori* UA955 LPS showed that the O-chain region consisted of type 1 and 2 chain sequences. The type 1 chain was exemplified by the α -L-Fuc-1→4- β -D-GlcNAc-1→ (Lewis disaccharide) terminal segment, and Le^x and Le^y determinants represented the type 2 class in this LPS (Table III). No Le^b epitope was detected in the LPS from *H. pylori* UA955.

Characterization of the O-chain Region of *H. pylori* Strain J223—In a previous study (12) *H. pylori* J223 was recognized by a Le^b -specific mAb (mAb BG-6 from Signet Laboratories, clone T218). To confirm the presence of the type 1 Le^b determinant in the LPS of this strain, the following structural experiments were performed.

D-Gal and D-GlcNAc were the major glucose units present in the J223 LPS (Fig. 7), L-Fuc, D-Glc, DD-Hep, and LD-Hep were also present in J223 LPS, the approximate molar ratio being 7.5:8:1:1.5:1.2:1.2, respectively. The Lewis blood group related permethylated alditol acetate derivatives detected by the sugar linkage analysis of J223 LPS (Table I) were terminal Fuc and Gal, 2- and 3-linked Gal, and 3- and 4-linked GlcNAc. The FAB-MS spectrum (Fig. 8a) of the methylated J223 LPS derivative displayed prominent primary glycosyl oxonium ions at m/z 464 (Hex, HexNAc), 638 (deoxy-Hex, Hex, HexNAc), 913

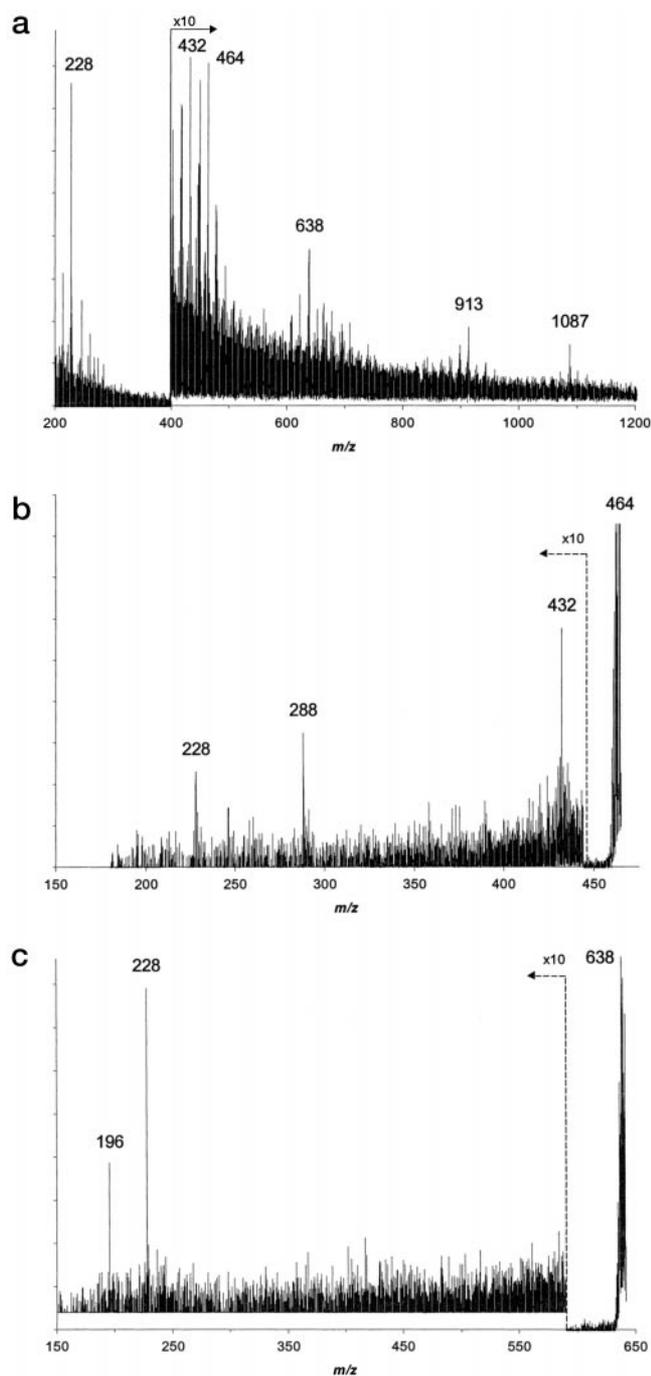


FIG. 8. FAB-MS spectra of *H. pylori* J223 methylated intact LPS. *a*, the complete FAB-MS spectrum J223. *b*, the product ion MS/MS of m/z 464. *c*, the product ion MS/MS spectrum of m/z 638.

(Hex₂, HexNAc₂), and 1087 (deoxy-Hex, Hex₂, HexNAc₂); the interpretation of these ions is shown in Table IV. The product ion MS/MS spectrum of m/z 464 (Hex-1→3- or →4-HexNAc⁺) showed m/z 432 as its major precursor ion (Fig. 8*b*) arising from β-elimination of methanol from O-3 of HexNAc, suggesting the presence of Gal-1→4-GlcNAc (type 2 LacNAc) as a terminal sequence. Another secondary ion arising from m/z 464 was m/z 228 (464-236) from a Gal-1→3-GlcNAc segment (type 1 precursor, Le^c). m/z 638 precursor m/z 228 (638-410), from β-elimination of Fuc→Gal-OH, as the sole secondary ion (Fig. 8*c*) that established the blood group H-type 1 epitope (Le^d) (Fuc-1→2-Gal-1→3-GlcNAc) (26) as a terminal antigen in the J223 LPS. There was no evidence pointing toward the presence of an H-type 2 epitope (*i.e.* m/z 638 → 606). The primary ions

at m/z 913 and 1087 represent extended *N*-acetylglucosamine chains (Table IV). m/z 913 represents two sequential fucose-free LacNAc repeats, which, due to the fact that only slightly more than one unit of 3-linked GlcNAc derivative was detected (Table I), is consistent with the presence of a type i antigen (Gal-1-[→4-GlcNAc-1→3-Gal-1-]_n) (Table IV), which correlates to the significant number of 4-linked GlcNAc units observed (Table I). However, since only a trace of m/z 881 (913-32) was observed in the J223 LPS FAB-MS, the possibility of a type 1 chain extension with a →3-Gal-1→3-GlcNAc-1→ disaccharide cannot be excluded. The absence of a branched 3,4-substituted GlcNAc unit and, consequently, of any terminal Le^x or Le^a blood group epitopes implies that m/z 1087 must be composed of a terminal H-1 unit (Le^d) and one Gal→GlcNAc repeat as shown in Table IV. The ¹H NMR spectrum of the intact J223 LPS showed the characteristic deoxy resonances from the sole Fuc residue (⁶CH₃, δ 1.25 J_{5,6} ~ 6 Hz) and the acetamido signals from the GlcNAc (COCH₃, δ 2.02 s) units.

Purified *H. pylori* J223 LPS was then used in a series of additional serological experiments with mAbs specific for Lewis blood group antigens. In ELISA, J223 LPS reacted strongly ($A_{492} > 2.6$) with mAbs 4D2 (anti H-type 1) and NAM61-1A2 (anti-i antigen). No reactions ($A < 0.3$) were observed between J223 LPS and mAbs specific for Le^x, Le^y, blood group A, or Le^a, nor with Le^b mAb 225-Le. The immunoblot (Fig. 9) shows the recognition of H-type 1 (Le^d) and of the i antigen in J223 LPS. Both mAbs in Fig. 9 recognize the “runs” of the J223 LPS ladder which represent the O-chain region. Collectively, structural and serological investigations performed on the J223 LPS revealed that the O-chain region was composed of an H-type 1 terminal antigen (Le^d) and of a non-fucosylated type 2 LacNAc chain β-D-Gal-1→4-β-D-GlcNAc-1-[→3-β-D-Gal-1→4-β-D-GlcNAc-1-]_n (i antigen); no Le^b antigen was detected.

Core Regions of *H. pylori* Strains UA948, UA955, and J223—In addition to the O-chain Lewis blood group glycosyl units, for all three strains, all chemical analysis revealed the presence of LD-Hep, DD-Hep, D-Glc, and D-Gal derivatives, which were surmised to originate from the core region (Table I; Figs. 5 and 7). The LD-Hep, DD-Hep, D-Glc, and D-Gal permethylated alditol acetate derivatives (Table I) emanating from the core regions of the LPS molecules studied here were of the same type as previously found in the core area of other *H. pylori* strains (4–7). However, in strains UA948, UA955, and J223, the same type of core may carry type 1 and 2 blood group epitopes as members of the O-chain. To add assurance to this apparent similarity between the core regions of *H. pylori* strains, *H. pylori* J223 LPS was subjected to immunodot analysis with antisera against the core of four strains, the type strain NCTC 11637 (4), C-5437, S-24, and K1 (25). *H. pylori* J223 LPS reacted with the four antisera, confirming the presence of similar epitopes as in the core of these *H. pylori* LPSs.

The chemical analyses performed in these studies were not aimed at obtaining information about the residues belonging to the lipid A moiety. However, in the ¹H NMR spectra of the intact LPSs of the strains investigated here, different patterns of resonances emanating from the lipid A fatty acids were observed, implying that there exists some variety in fatty acid substitution between *H. pylori* strains. Two recent investigations have dealt with the structural features of *H. pylori* lipid A molecules (27, 28).

DISCUSSION

This investigation on the chemical composition of LPSs from *H. pylori* has added new insight into the structure of these molecules. The most significant new finding is that *H. pylori* LPSs can express type 1 Lewis blood group determinants,

TABLE IV
Interpretation of the ions from the FAB-MS spectrum of methylated intact *H. pylori* J223 LPS

The secondary ions shown originate from β -elimination of the residue at the O-3 position of the GlcNAc at the reducing end. The \pm indicates that either structure is possible.

Primary ions	Secondary ions	Proposed structure
<i>m/z</i>	<i>m/z</i>	
464	432 (464 - 32)	Gal-1 \rightarrow 4-GlcNAc ⁺ (LacNAc, type 2 chain)
464	228 (464 - 236)(minor)	Gal-1 \rightarrow 3-GlcNAc ⁺ (type 1 precursor, Le ^e)
638	228 (638 - 410)	Fuc-1 \rightarrow 2-Gal-1 \rightarrow 3-GlcNAc ⁺ (H-type 1, Le ^d)
913		Gal-1 \rightarrow 4-GlcNAc-1 \rightarrow 3-Gal-1 \rightarrow 4-GlcNAc ⁺ (i antigen, LacNAc \rightarrow LacNAc (type 2 chain))
1087		Fuc-1 \rightarrow 2-Gal-1 \rightarrow 3-GlcNAc-1 \rightarrow 3-Gal-1 \rightarrow 3/4-GlcNAc ⁺

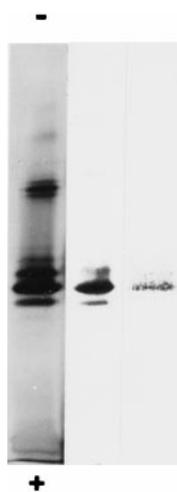


FIG. 9. SDS-polyacrylamide gel electrophoresis and immunoblot of J223 LPS. Left lane, silver stain of J223 LPS; middle lane, immunoblot with anti-H-type 1 (Le^d) mAb; right lane, immunoblot with anti-i antigen mAb.

namely, Le^a, H-1 (Le^d), and the type 1 chain precursor (Le^e). Type 2 epitopes, Le^x and Le^y, have been described to be present in *H. pylori* LPSs in earlier studies (Refs. 3–6; Table V). Chemical analyses in combination with serological experiments (mAbs) showed that the O-chain from *H. pylori* strain UA948 LPS possessed both Le^a (type 1 chain) and Le^x (type 2 chain) antigens (Figs. 2–4; Table II). The O-chain of *H. pylori* strain UA955 LPS (Figs. 2, 3, 5, and 6; Table III) expressed both type 2 chain Le^x and Le^y determinants and a sequence composed of type 1 and 2 chain regions in Fuc-1 \rightarrow 4-GlcNAc-1 \rightarrow Le^x. The LPS of *H. pylori* UA955 also was composed of elongated sundrily fucosylated LacNAc chains (Table III). *H. pylori* strain J223 LPS (Figs. 7–9; Table IV) also showed the ability to express type 1 chains by carrying the type 1 chain precursor Le^e, the H-1 epitope (Le^d), and type 2 chain molecules (LacNAc chain (i antigen)). The core oligosaccharide regions of *H. pylori* UA948, UA955, and J223 appear to have the same structure as those from *H. pylori* strains previously investigated (Fig. 1 and Table I) (4–7), despite their ability to carry a variety of type 1 and 2 Lewis blood group antigens representing the O-chain segment.

The expression of type 1 Lewis blood group antigens suggests that for the biosynthesis of some *H. pylori* LPS molecules there must be glycosyltransferases of the type 1 family, such as a fucosyl transferase(s) that places the α -L-Fuc unit at O-4 of β -D-GlcNAc and a galactosyl transferase that adds β -D-Gal to O-3 of GlcNAc. The LPS from the type strain of *H. mustelae*, the *Helicobacter* gastric pathogen from ferrets, also carries a type 1 histo-blood group epitope, the monofucosyl blood group A type

1 (α -D-GalNAc-1 \rightarrow 3[α -L-Fuc-1 \rightarrow 2]- β -D-Gal-1 \rightarrow 3- β -D-GlcNAc) (29). The concurrent expression of type 1 and 2 chains and of various chains with different glycosylation patterns within the LPS of a single *H. pylori* strain represents a complex biosynthesis of these molecules, which differs from the well known “block-by-block” O-chain repeating unit addition mechanism of *Escherichia* and *Salmonella* LPS biosynthesis (30). Various factors, such as differential enzyme kinetics, regulation, and mutation or clonal variation may control the assembly of *H. pylori* LPS molecules.

The genome of *H. pylori* strain 26695 and other strains contain two copies of the α -1-3-fucosyltransferase and multiple copies may play a role in the expression of the Lewis antigens (31–33). Recently, the presence of a possible α -1-2-fucosyltransferase gene in *H. pylori* has also been described (34). The α -1-4-fucosyltransferase genes to produce the Le^a and Le^b epitopes have not yet been identified. Many strains express Le^x and Le^y, but few seem to express type 1 epitopes (11, 12). This phenomenon might be due to regulation whereby all *H. pylori* strains contain the genes for the assembly of these alternative structures, but in certain instances some transferases are not expressed; alternatively, only certain strains may contain the appropriate genes required to produce the type 1 antigens. Separately grown bacterial culture batches of the same *H. pylori* strain may express different degrees of fucosylation (Fuc-1 \rightarrow 3-GlcNAc and Fuc-1 \rightarrow 2-Gal), suggesting that the transferases responsible for these glycosylations are variably expressed.² Diversity in fucosylation and thus in Le^x and Le^y expression among single colonies derived from the same gastric biopsy suggests that this phenomenon might occur *in vivo* as well (35).

The serological studies that showed recognition of the Le^b epitope in *H. pylori* LPSs with the Le^b-specific mAb BG-6 (clone T218) in strains UA955 and J223 were recognizing only substructures of the Le^b antigen. In *H. pylori* UA955, mAb BG-6 presumably detected the α -L-Fuc-1 \rightarrow 4- β -D-GlcNAc Lewis disaccharide, which is a region of the Le^b determinant (α -L-Fuc-1 \rightarrow 2- β -D-Gal-1 \rightarrow 3[α -L-Fuc-1 \rightarrow 4]- β -D-GlcNAc) (Fig. 10), and in *H. pylori* J223, mAb BG-6 either recognized the H-1 antigen (Le^d) (α -L-Fuc-1 \rightarrow 2- β -D-Gal-1 \rightarrow 3- β -D-GlcNAc) or Le^e (β -D-Gal-1 \rightarrow 3)- β -D-GlcNAc), which are biosynthetic precursors of Le^b (α -L-Fuc-1 \rightarrow 2- β -D-Gal-1 \rightarrow 3[α -L-Fuc-1 \rightarrow 4]- β -D-GlcNAc) (Fig. 10) (see Ref. 36 for a review concerning Lewis biosynthetic pathways and Le^b mAb cross-reactivity). The Fuc-1 \rightarrow 4-GlcNAc terminal unit in *H. pylori* UA955 may either be a biosynthetic precursor of Le^a or Le^b in *H. pylori* LPS biosynthesis, or it represents a dead end product due to premature fucosylation of GlcNAc at O-4, inhibiting further galactosylation of GlcNAc at O-3. The cross-reactivity observed between Le^b mAb BG-6 and

² M. A. Monteiro, unpublished results.

TABLE V
Molecular makeup of *H. pylori* LPSs investigated by structural analysis to date and their classification into glycotypes

Strain	Terminal epitope →	O-chain ^a →	DD-heptan →	Core ^b	Glycotype	Reference
Type strain NCTC 11637, O:1	Lewis X→	Lewis X→		Core	A	4
P466, UA1182 ²	Lewis Y→	Lewis X→		Core	A	5
Serogroup O:3	Lewis X/Y→ ^c	Lewis X→	DD-Heptan→	Core	B	6
MO19	Lewis Y→		DD-Heptan→	Core	C	5
Serogroup O:6	Lewis Y→		DD-Heptan→	Core	C	6
			DD-Heptan→	Core		6
UA861 ^d	LacNAc→	LacNAc→		Core	D	7
		↑				
		α-D-Glc				
471 ^d	Lewis X/Y→	LacNAc→		Core	E	8
		↑				
		D-Gal				
UA948 ^e	Lewis A→			Core	F	This study
	Lewis X→	Lewis X→		Core		This study
UA955	Lewis dis→	Lewis X→		Core	F	This study
	Lewis X/Y→	Lewis X→		Core		This study
J223	Lewis C→	LacNAc→		Core	F	This study
	Lewis D(H-1)→	LacNAc→		Core		This study
	LacNAc→	LacNAc→		Core		This study

^a The Lewis X elongated O-chains also contain randomly placed LacNAc repeats (Lewis X without fucose).

^b All cores share a similar structure (Fig. 1). Some cores include phosphate and contain amino-ethyl phosphate at the inner heptose.

^c A slash indicates that either structure is possible.

^d The α-D-Glc and D-Gal side chains are attached to the O-6 position of the GlcNAc. The UA861 O-chain also expresses one internal Lewis X. The 471 LPS also contains O-3-fucosylated GlcNAc units.

^e The Lewis A terminal epitope might also be attached to an internal Lewis X repeat.

H. pylori J223 LPS seems to be a mAb-specific phenomenon, since Le^b mAb 225-Le did not react with J223 LPS. Imberty *et al.* (37) has reported that blood group determinants have certain conformational dependent “microepitopes” toward which different mAbs have dissimilar reactivities. It is also worth noting that the same Le mAb might have different sensitivity toward the same LPS epitopes when tested under ELISA or immunoblot conditions, as indicated by the reaction of Le^b mAb with UA955 in ELISA (Fig. 3), but not in immunoblot after removal of the proteins by proteinase K (Fig. 2). Isolated *H. pylori* UA955 proteins did not react with the Le^b mAb, suggesting that the observed activity in Fig. 3 is not to the protein. Also, when whole cells of UA955 were tested in immunoblot, the Le^b mAb BG-6 showed no reaction, suggesting that recognition of UA955 LPS by mAb BG-6 is lost under immunoblot conditions. The same type of phenomenon can also be observed with strain UA1182, whose internal Lewis X epitopes are well recognized by the Lewis X mAb in ELISA (Fig. 3), but show only a weak interaction in immunoblot (Fig. 2). We suspect that these two differences in reactivities are caused by conformational changes in the LPS molecules. These studies illustrate the necessity for performing structural rather than serological studies to define *H. pylori* LPS antigens.

Using the structural information for *H. pylori* LPSs available to date (Table V) permits their classification into six different groups (glycotypes A–F; see Table V). (i) Glycotype A contains LPSs with long fucosylated type 2 LacNAc O-chains attached at the reducing end to the core oligosaccharide, as in strains NCTC11637 (4), P466 (5), and UA1182²; (ii) glycotype B contains LPSs with long fucosylated type 2 LacNAc O-chains attached to a heptan domain, as in strain O:3 (6); (iii) glycotype C contains LPSs with short O-chains (one repeat), which are attached to a heptan domain, as in strains MO19 (5) and O:6 (6); (iv) glycotype D contains LPSs with glucosylated type 2 LacNAc O-chains as in strain UA861 (7); (v) glycotype E contains LPSs with galactosylated type 2 LacNAc O-chains as in strain 471 (8); and (as found in this study) (vi) glycotype F contains LPSs, which, in addition to type 2 Lewis determinants, also possess chains composed of type 1 Lewis epitopes, as in strains UA948, UA955, and J223.

The most striking feature of the O-chains of *H. pylori* LPSs is

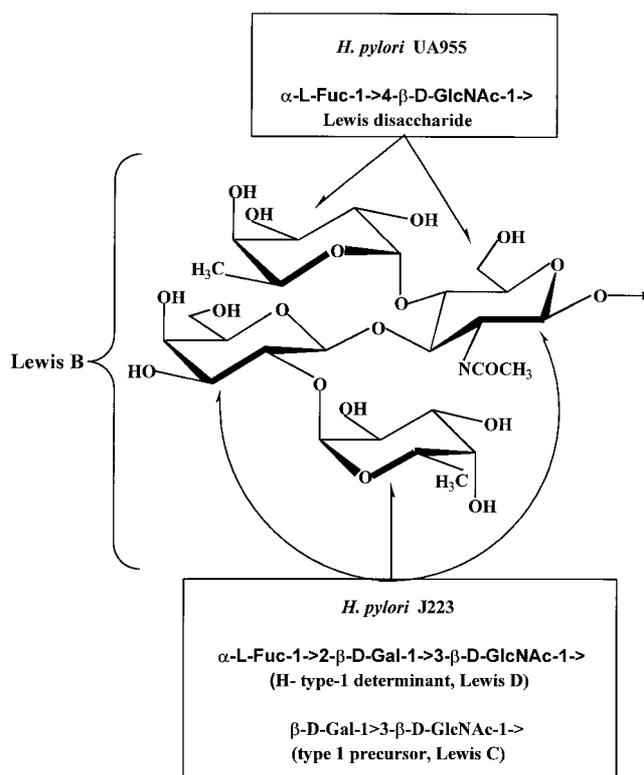


FIG. 10. The Le^b structure. The Lewis disaccharide α-L-Fuc-1→4-β-D-GlcNAc-1→ is present in *H. pylori* UA955, and the H-type 1 (Le^d) α-L-Fuc-1→2-β-D-Gal-1→3-β-D-GlcNAc-1→ and Le^c β-D-Gal-1→3-β-D-GlcNAc-1→ epitopes are part of the LPS from *H. pylori* J223.

their ability to mimic human cell surface glycoconjugates and glycolipids that have Lewis structures. Past structural investigations revealed that *H. pylori* LPSs mimicked type 2 Le^x and Le^y human cell surface glycoforms (Refs. 3–6; Table V) and that this molecular imitation may be an antecedent for autoimmunity (9). Alternatively, host-related microbial Lewis expression could help avoid host immune responses (38). The molecular mimicry between *H. pylori* LPSs and host molecules

has now been extended to include the type 1 determinants Le^a, Le^d, and Le^c. This ability of *H. pylori* to produce various Lewis isoforms permits mimicking of all regions of the gastric epithelium, those being the gastric superficial and glandular epithelium, which display mainly type 2 molecules, and the superficial epithelium, which expresses predominantly type 1 chains (39–41). Consequently, each *H. pylori* strain, depending on the antigens expressed by its LPS, may have a different ecological niche within the gastric mucosa, and ultimately the role of LPS in pathogenesis and adaptation may differ between *H. pylori* strains.

Acknowledgment—We are grateful to D. Blanchard (Nantes, France) for providing mAb NAM61-1A2.

REFERENCES

- Dunn, B. E., Cohen, H., and Blaser, M. J. (1997) *Clin. Microbiol. Rev.* **10**, 720–741
- Rieschel, E. T., Brade, H., Holst, O., Brade, L., Muller-Loennies, S., Mamat, U., Zahringer, U., Beckmann, F., Seydel, U., Brandenburg, K., Ulmer, A. J., Mattern, T., Heine, H., Schletter, J., Loppnow, H., Schonbeck, U., Flad, H.-D., Hauschildt, S., Schade, U. F., Di Padova, F., Kusumoto, S., Schumann, R. R. (1996) *Curr. Top. Microbiol. Immunol.* **216**, 39–81
- Aspinall, G. O., Monteiro, M. A., Pang, H., Walsh, E. J., and Moran, A. P. (1994) *Carbohydr. Lett.* **1**, 156–165
- Aspinall, G. O., Monteiro, M. A., Pang, H., Walsh, E. J., and Moran, A. P. (1996) *Biochemistry* **35**, 2489–2497
- Aspinall, G. O., and Monteiro, M. A. (1996) *Biochemistry* **35**, 2498–2504
- Aspinall, G. O., Monteiro, M. A., Shaver, R. T., Kurjanczyk, L. A., Penner, J. L. (1997) *Eur. J. Biochem.* **248**, 592–601
- Monteiro, M. A., Rasko, D., Taylor, D. E., and Perry, M. B. (1998) *Glycobiology* **8**, 107–112
- Aspinall, G. O., Mainkar, A. S., and Moran, A. P. (1997) *Ir. J. Med. Sci.* **166**, Suppl. 3, 26–27
- Appelmelk, B. J., Simoons-Smit, I. M., Negrini, R., Moran, A. P., Aspinall, G. O., Forte, J. G., Vries, T. D., Quan, H., Verboom, T., Maaskant, J. J., Ghiara, P., Kuipers, E. J., Bloemena, E., Tadema, T. M., Townsend, R. R., Tyagarajan, K., Crothers, J. M., Jr., Monteiro, M. A., Savio, A., and Graaff, J. D. (1996) *Infect. Immun.* **64**, 2031–2040
- Sherburne, R., and Taylor, D. E. (1995) *Infect. Immun.* **63**, 4564–4568
- Simoons-Smit, I. M., Appelmelk, B. J., Verboom, T., Negrini, R., Penner, J. L., Aspinall, G. O., Moran, A. P., Fei, S. F., Bi-Shan, S., Rudnica, W., Savio, A., and Graaff, J. D. (1996) *J. Clin. Microbiol.* **34**, 2196–2200
- Wirth, H.-P., Yang, M., Karita, M., and Blaser, M. J. (1996) *Infect. Immun.* **64**, 4598–4605
- Amano, K., Hayashi, S., Kubota, T., Fujii, N., Yokota, S. (1997) *Clin. Diagn. Lab. Immun.* **4**, 540–545
- Taylor, D. E., Hargreaves, J. A., Ng, L. K., Sherbniuk, R. W., and Jewell, L. D. (1987) *Am. J. Clin. Pathol.* **87**, 49–54
- Westphal, O., and Jann, K. (1965) *Methods Carbohydr. Chem.* **5**, 83–91
- Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., and Smith, F. (1956) *Anal. Chem.* **28**, 350–356
- Sawardeker, J. H., Sloneker, J. H., and Jeanes, A. (1967) *Anal. Chem.* **39**, 1602–1604
- Leotein, K., Lindberg, B., and Lonngren, J. (1978) *Carbohydr. Res.* **62**, 359–362
- Ciucanu, I., and Kerek, F. (1984) *Carbohydr. Res.* **131**, 209–217
- Dell, A., Azadi, P., Thomas-Oates, J. E., Jennings, H. J., Beurret, M., and Michon, F. (1990) *Carbohydr. Res.* **200**, 59–76
- Hitchcock, P. J., and Brown, T. M. (1983) *J. Bacteriol.* **154**, 269–277
- Tsai, C.-M., and Frasch, C. E. (1982) *Ann. Biochem.* **119**, 115–119
- Towbin, H., Staehelin, T., and Gordon, J. (1979) *Proc. Natl. Acad. Sci. U. S. A.* **76**, 4350–4354
- Blanchard, D., Bernard, D., Loirat, M. J., Frioux, Y., Guimbretiere, J., and Guimbretiere, L. (1992) *Rev. Fr. Transfus. Hemobiol.* **35**, 239–254
- Walsh, E. J., and Moran, A. P. (1997) *Ir. J. Med. Sci.* **166**, Suppl. 3, 27
- EGge, H., and Peter-Katalinic, J. (1987) *Mass Spectrom. Rev.* **6**, 331–393
- Suda, Y., Ogawa, T., Kashihara, W., Oikawa, M., Shimoyama, T., Hayashi, T., Tamura, T., and Kusumoto, S. (1997) *J. Biochem. (Tokyo)* **121**, 1129–1133
- Moran, A. P., Lindner, B., and Walsh, E. J. (1997) *J. Bacteriol.* **179**, 6453–6463
- Monteiro, M. A., Zheng, P. Y., Appelmelk, B. J., and Perry, M. B. (1997) *FEMS Microbiol. Lett.* **154**, 103–109
- Whitfield, C. (1995) *Trends Microbiol.* **3**, 178–185
- Martin, S. L., Edbrooke, M. R., Hodgman, T. C., van den Eijnden, D. H., and Bird, M. I. (1997) *J. Biol. Chem.* **272**, 21349–21356
- Ge, Z., Chan, N. W. C., Palcic, M. M., and Taylor, D. E. (1997) *J. Biol. Chem.* **272**, 21357–21363
- Tomb, J.-F., White, O., Kerlavage, A. R., Clayton, R. A., Sutton, G. G., Fleischmann, R. D., Ketchum, K. A., Klenk, H. P., Gill, S., Dougherty, B. A., Nelson, K., Quackenbush, J., Zhou, L., Kirkness, E. F., Peterson, S., Loftus, B., Richardson, D., Dodson, R., Khalak, H. G., Glodek, A., McKenney, K., Fitzgerald, L. M., Lee, N., Adams, M. D., Hickey, E. K., Berg, D. E., Gocayne, J. D., Utterback, T. R., Peterson, J. D., Kelley, J. M., Cotton, M. D., Weldman, J. M., Fujii, C., Bowman, C., Watthey, L., Wallin, E., Hayes, W. S., Borodovsky, M., Karp, P. D., Smith, H. O., Fraser, C. M., and Venter, J. C. (1997) *Nature* **388**, 539–547
- Berg, D. E., Hoffman, P., Appelmelk, B. J., and Kusters, J. G. (1997) *Trends Microbiol.* **12**, 468–474
- Wirth, H.-P., Yang, M., Peek, R. M., Hook-Nikanne, J., and Blaser, M. J. (1997) *Gastroenterology* **112**, 331 (abstr.)
- Henry, S., Oriol, R., and Samuelsson, B. (1995) *Vox Sang.* **69**, 166–182
- Imberty, A., Mollicone, R., Mikros, E., Carrupt, P.-A., Perez, S., and Oriol, R. (1996) *Bioorg. Med. Chem.* **4**, 1979–1988
- Wirth, H.-P., Yang, M., Peek, R. M., Tham, K. T., and Blaser, M. J. (1997) *Gastroenterology* **113**, 1091–1098
- Mollicone, R., Bara, J., Le Pendu, J., and Oriol, R. (1985) *Lab. Invest.* **53**, 219–227
- Davidson, J. S., and Triadafilopoulos, G. (1992) *Gastroenterology* **103**, 1552–1561
- Kobayashi, K., Sakamoto, J., Kito, T., Yoshitaka, Y., Koshikawa, T., Fujita, M., Watanabe, T., and Nakazato, H. (1993) *Amer. J. Gastroenterol.* **88**, 919–924

Simultaneous Expression of Type 1 and Type 2 Lewis Blood Group Antigens by *Helicobacter pylori* Lipopolysaccharides: MOLECULAR MIMICRY BETWEEN H. PYLORI LIPOPOLYSACCHARIDES AND HUMAN GASTRIC EPITHELIAL CELL SURFACE GLYCOFORMS

Mario A. Monteiro, Kenneth H. N. Chan, David A. Rasko, Diane E. Taylor, P. Y. Zheng, Ben J. Appelmelk, Hans-Peter Wirth, Manqiao Yang, Martin J. Blaser, Sean O. Hynes, Anthony P. Moran and Malcolm B. Perry

J. Biol. Chem. 1998, 273:11533-11543.

doi: 10.1074/jbc.273.19.11533

Access the most updated version of this article at <http://www.jbc.org/content/273/19/11533>

Alerts:

- [When this article is cited](#)
- [When a correction for this article is posted](#)

[Click here](#) to choose from all of JBC's e-mail alerts

This article cites 39 references, 11 of which can be accessed free at <http://www.jbc.org/content/273/19/11533.full.html#ref-list-1>