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Potential Role of Molecular Mimicry between *Helicobacter pylori* Lipopolysaccharide and Host Lewis Blood Group Antigens in Autoimmunity

BEN J. APPELMELK,[1]* INA SIMOONS-SMIT,[1] RICCARDO NEGRINI,[2] ANTHONY P. MORAN,[3] GERALD O. ASPINALL,[4] JOHN G. FORTE,[5] THEODORA DE VRIES,[6] HU QUAN,[1] THEO VERBOOM,[1] JANNEKE J. MAASKANT,[1] PAOLO GHIARA,[7] ERNST J. KUIPERS,[8] ELISABETH BLOEMENA,[9] THEA M. TADEMA,[9] REID R. TOWNSEND,[10] KAMALA TYAGARAJAN,[5] JIM M. CROTHERS, JR.,[5] MARIO A. MONTEIRO,[4] ANTONELLA SAVIO,[11] AND JOHANNES DE GRAAFF[1]

Departments of Medical Microbiology,[1] Medical Chemistry,[6] Gastroenterology,[8] and Pathology,[9] Vrije Universiteit Medical School and Academic Hospital, 1081 BT Amsterdam, The Netherlands; Biotechnology Laboratory, Spedali Civili,[2] and S. Orsola-Fatebenefratelli Hospital,[11] Brescia, and Immunobiological Research Institute, Siena,[7] Italy; Department of Microbiology, University College, Galway, Ireland[3]; Department of Chemistry, York University, North York, Ontario, Canada[4]; and Department of Molecular and Cell Biology, University of California, Berkeley,[5] and Department of Pharmaceutical Chemistry, University of California, San Francisco,[10] California

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***Helicobacter pylori* is involved in gastritis, gastric and duodenal ulcers, gastric adenocarcinoma, and mucosa-associated lymphoid tissue lymphoma. Earlier studies already suggested a role for autoimmune phenomena in *H. pylori*-linked disease. We now report that lipopolysaccharides (LPS) of *H. pylori* express Lewis y, Lewis x, and H type I blood group structures similar to those commonly occurring in gastric mucosa. Immunization of mice and rabbits with *H. pylori* cells or purified LPS induced an anti-Lewis x or y or anti-H type I response, yielding antibodies that bound human and murine gastric glandular tissue, granulocytes, adenocarcinoma, and mucosa-associated lymphoid tissue lymphoma cells. Experimental oral infections in mice or natural infection in humans yielded anti-Lewis antibodies also. The β chain of gastric H^+,K^+-ATPase, the parietal cell proton pump involved in acid secretion, contained Lewis y epitopes; gastric mucin contained Lewis x and y antigenic determinants. Growth in mice of a hybridoma that secretes *H. pylori*-induced anti-Lewis y monoclonal antibodies resulted in histopathological evidence of gastritis, which indicates a direct pathogenic role for anti-Lewis antibodies. In conclusion, our observations demonstrate that molecular mimicry between *H. pylori* LPS and the host, based on Lewis antigens, and provide understanding of an autoimmune mechanism for *H. pylori*-associated type B gastritis.**

Helicobacter pylori plays a causative role in the pathogenesis of gastritis, gastric atrophy, and peptic and duodenal ulcer (20). Infection by this bacterium is also associated with an increased risk of gastric adenocarcinoma, and *H. pylori* is now classified as a type I human carcinogen (16); furthermore, a causative relationship between the presence of *H. pylori* and the occurrence of mucosa-associated lymphoid tissue (MALT) lymphoma was suggested (33). The cytotoxin of *H. pylori* may play a central role in the pathogenesis of gastritis and gastric ulcer, but other factors are also involved. It was shown by us that sera from *H. pylori*-infected patients or from mice immunized with the bacterium contain autoantibodies that react with gastric mucosa of mice and humans (28–30), and murine monoclonal antibodies (MAbs) directed against *H. pylori* that also react with gastric tissue (28–30) have been prepared from *H. pylori*-vaccinated mice. Absorption of murine and human sera with *H. pylori* cells removed the autoreactive antibodies (28). Moreover, growth in mice of a hybridoma which was secreting one of the *H. pylori*-reactive MAbs, and which was autoreactive, caused histopathological changes similar to gastritis (28). These (28–30) and other (11) data strongly suggest that *H. pylori* induces autoantibodies that play a crucial role in the pathogenesis of gastritis and gastric atrophy. Autoimmunity also may play a role in the pathogenesis of *H. pylori*-linked carcinoma (43) and MALT lymphoma (14, 17). However, the nature of the bacterial antigens that induce autoimmunity are currently unknown, as are the host targets recognized. Recently, it has been shown that the O-side chain of the lipopolysaccharide (LPS) of *H. pylori* NCTC 11637 is structurally similar to the Lewis x blood group antigen (3, 4) present also in gastric mucosa (19). The LPS of another strain (MO19) is structurally similar to Lewis y (2), and that of a third (P466) contains Lewis x and Lewis y (2) (Fig. 1).

The present paper investigates the potential role of *H. pylori* LPS in *H. pylori*-linked autoimmunity. We report that Lewis x and/or y structures are present in LPS of clinical isolates of *H. pylori*. Immunizations and experimental and natural infections with *H. pylori* yield anti-Lewis x and/or y antibodies that react with gastric tissue and have a potential pathogenic role. We also identify host autoimmune targets.

* Corresponding author. Mailing address: Department of Medical Microbiology, Vrije Universiteit, Medical School, van der Boechorstraat 7, 1081 BT Amsterdam, The Netherlands. Phone: 31 20 4448297. Fax: 31 20 4448318. Electronic mail address: BJ.Appelmelk.mm@med.vu.nl.

MATERIALS AND METHODS

***H. pylori* strains.** Strain NCTC 11637 was obtained from the National Collection of Type Cultures (Public Health Laboratory Service, London, England);

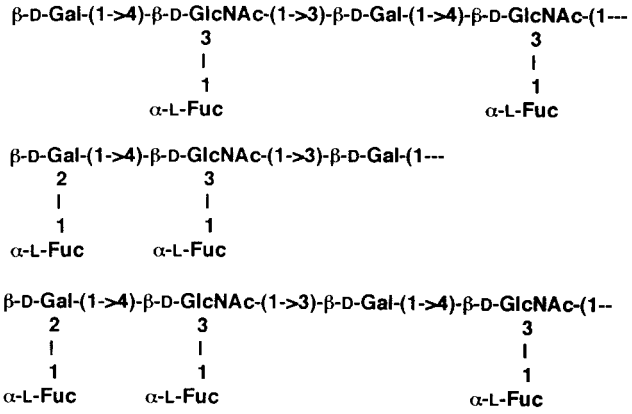

FIG. 1. Structure of the LPS O-antigen of *H. pylori*. Top, strain NCTC 11637 with a fucosylated poly(*N*-acetyllactosamine) chain terminated by a dimeric Lewis x unit. Middle, strain MO19 with an attenuated chain terminated by a single Lewis y unit. Bottom, strain P466 with a fucosylated poly(*N*-acetyllactosamine) chain terminated by a Lewis y-Lewis x (trifucosyl) unit. Fuc, fucose; Gal, galactose; GlcNAc, *N*-acetyl-glucosamine.

P466 and MO19 were obtained from T. Boren, University of Umeå, Umeå, Sweden; clinical isolates (strains 104, 474, 487, 609, 700, and 1152) were obtained from patients with gastroduodenal diseases at the University Hospital, Vrije Universiteit, Amsterdam, The Netherlands; strain N was isolated from a patient suffering from atrophic gastritis at the Spedali Civili, Brescia, Italy.

Purified naturally occurring antigens. LPS from strain NCTC 11637 (=serotype O:1 [25]) was prepared as described previously by phenol extraction and subsequent enzymatic treatments (26). LPSs from *H. pylori* serotypes O:2 to O:6 and strains P466 and MO19 were provided by J. L. Penner (University of Toronto, Toronto, Canada). The LPSs were extracted by the phenol water technique (26), and subsequently, the extracts were digested with lysozyme plus DNase, RNase, and proteinase K and reextracted with phenol. The purity of the LPS preparations was confirmed by routine sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) with silver staining for protein and LPS (39). *Schistosoma mansoni* circulating cathodic antigen (CCA), a glycoprotein containing polymeric ($n = 25$) Lewis x, was purified by affinity chromatography on an anti-CCA MAb described previously (40). Hog and rabbit microsomes, H^+,K^+-ATPase constituting >90% of the protein present, were prepared by sucrose gradient purification (9). Human microsomes were prepared from gastric cardia obtained after esophagectomy performed on a patient with blood group A; mouse microsomes were prepared from C57BL/6 mice after CO_2 euthanasia. Human and mouse microsomes sedimenting to 33 and 30% sucrose, respectively (9), were analyzed by SDS-PAGE. Rabbit H^+,K^+-ATPase β chain was prepared by lectin affinity chromatography (32). Hog and human intrinsic factor were isolated from mucosal tissue and gastric juice, respectively, by high-pressure liquid chromatography and affinity chromatography (15); purity was confirmed by the observation of a single band in SDS-PAGE with silver staining. Crude mucin was obtained by scraping, following resection, of a fragment of 5 cm^2 of gastric body mucosa of a gastric carcinoma patient, followed by dilution in phosphate-buffered saline (PBS), dialyzation in a 300-kDa cutoff membrane, and filtration through a 0.2-μm-pore-size membrane. Tests with *H. pylori*-specific MAbs showed that the mucin was not contaminated with *H. pylori* antigens. Bovine and human serum albumin (BSA and HSA, respectively) were purchased from Sigma Chemical Co. (St. Louis, Mo.).

Synthetic Lewis antigens used to define antibody specificities. Synthetic Lewis antigens that were obtained from Isosep AB (Tullinge, Sweden) are shown in Table 1. Unless otherwise stated, oligosaccharides were prepared from human milk. Lewis y was prepared synthetically. *p*-Aminophenylethyl and acetylphenylenediamine acted as spacers between the oligosaccharides and albumin. About 20 to 25 oligosaccharide chains are linked to one molecule of HSA or BSA.

Murine MAbs. Bra.4F1 (anti-Lewis x), 12.4 (anti-Lewis y), 19-O Le (anti-H type II), 7 Le (anti-Lewis a), 2-25 Le (anti-Lewis b), and 3-3A (anti-blood group A) were obtained from Bioprobe BV, Amstelveen, The Netherlands. CS Lex (anti-sialyl Lewis x) was obtained from W. van Dijk, Vrije Universiteit. MAb 54-1-F6A is specific for CCA (40). MAbs CB-4, CB-10, 1E52, 4D2, A540, A522, A1, and A18 (see also Table 4) react with both *H. pylori* and host and belong to a previously described panel of 30 *H. pylori*-induced MAbs (28–30). MAb 2G11 is specific for H^+,K^+-ATPase β chain (8).

Polyclonal sera. For production of antisera to whole bacterial cells, chinchilla rabbits (two for each strain) were injected intravenously on days 0, 5, 8, 14, 22, and 32 with increasing volumes (0.25, 0.50, 0.75, 1, 2, and 2 ml, respectively) of formalin-killed cells (10^9 CFU/ml) of *H. pylori* 104, 487, 474, 609, 700, and 1152 and NCTC 11637. Antisera were collected at day 40. Antiserum to *Escherichia coli* O111:B4 was prepared in a similar manner. Preimmune sera were also collected and used as controls.

New Zealand Black mice were injected intraperitoneally at days 0, 14, 28, and 42 with a mixture of 0.25 ml of bacteria (10^9 CFU of strains 104 and 700) and 0.25 ml of complete Freund adjuvant (day 0) or incomplete Freund adjuvant (other days). Sera were collected at day 56. For the production of antiserum to purified LPS, Swiss mice were injected intraperitoneally (days 0, 14, 28, and 42) with 10 μg of purified LPS (strain NCTC 11637), emulsified with complete Freund adjuvant (day 0) or incomplete Freund adjuvant (other days). Murine antiserum to *E. coli* O111:B4 cells was similarly prepared in BALB/c mice. Sera from nonimmunized BALB/c and Swiss mice were also collected. To investigate the presence of anti-Lewis x antibodies in experimentally infected mice, sera from a mouse model for *H. pylori* infection that mimics human disease were collected and analyzed (24). Briefly, bacteria (fresh clinical isolates or mouse-adapted strains) were orally inoculated into male C57BL/6 mice, after which gastric colonization became established. We investigated sera from mice that had been infected with either a cytotoxin-producing strain ($n = 3$) or a noncytotoxic strain ($n = 3$). Preimmune sera were collected, mice were terminated after 8 weeks, and their sera were investigated.

Patient sera. The presence of anti-Lewis x and y antibodies in sera from three patient groups was investigated. One group of sera was obtained from patients who were positive ($n = 18$) or negative ($n = 10$) for *H. pylori* infection by serology (30). Another group of sera was from patients who were positive ($n = 12$) or negative ($n = 10$) for *H. pylori* infection by culture and histology. Sera were also obtained from a group of 90 dyspeptic patients.

TABLE 1. Neoglycoconjugates used in this study and the Lewis antigens they express[a]

Neoglycoconjugate	Antigen
Galβ1-3GlcNAcβ1-3Galβ1-4(Glc)-APD-HSA 4 Fucα1	Lewis a
Fucα1-2Galβ1-3GlcNAcβ1-3Galβ1-4(Glc)-APD-HSA 4 Fucα1	Lewis b
Galβ1-4GlcNAcβ1-3Galβ1-4(Glc)-APD-HSA 3 Fucα1	Lewis x
Fucα1-2Galβ1-4GlcNAcβ1-O-APE-HSA 3 Fucα1	Lewis y
Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-O-APE-HSA 3 3 3 Fucα1 Fucα1 Fucα1	Trimeric Lewis x
Fucα1-2Galβ1-3GlcNAcβ1-3Galβ1-4(Glc)-APD-HSA	H type I
Fucα1-2Galβ1-4GlcNAcβ-O-APE-BSA	H type II

[a] Gal, galactose; GlcNAc, *N*-acetyl-glucosamine; Fuc, fucose; APD, acetylphenylenediamine; APE, *p*-aminophenylethyl.

TABLE 2. Reactivity[a] of MAbs to Lewis antigens with *H. pylori*

Strain and origin	OD for MAb and specificity[b]			
	Bra.4F1 (Lewis x)	54-1-F6A (Lewis x)	12.4 (Lewis y)	4D2[c] (H type I)
104 (chronic gastritis)	—	—	—	—
474 (chronic gastritis)	1.8	>	>	—
487 (duodenal ulcer)	0.5	—	—	>
609 (asymptomatic)	>	>	>	—
700 (gastric ulcer)	2.7	>	>	—
1152 (adenocarcinoma)	0.6	2.7	1.9	>
NCTC 11637 (gastritis)	—	>	0.4	2.7

[a] Reactivity was measured by ELISA.
[b] —, OD < 0.3; >, OD above 2.7; isotype-matched, negative controls yield OD values of <0.3.
[c] For details on the specificity of MAb 4D2, see Table 4.

Enzyme-linked immunosorbent assay (ELISA). For direct binding assays, bacterial cells (10^6/ml), microsomes (5 μg/ml), LPS, intrinsic factor, CCA, and synthetic Lewis antigens (all at 1 μg/ml), suspended in 100 μl of PBS, were added to wells of microtiter plates and incubated overnight at room temperature (RT). Plates were washed with PBS containing 0.05% Tween 80 (PBST). Subsequently, MAbs (100 ng/ml) or human sera diluted in PBST were added and incubated for 2 h at RT. Before testing in ELISA, murine and rabbit sera were diluted (for dilutions, see Results), incubated in PBST containing 0.1% HSA and 0.1% BSA (PBST-HSA-BSA) for 1 h at 37°C, and centrifuged (5 min, 14,000 × *g*), and the supernatants were subsequently tested. This procedure eliminated high nonspecific background binding to PBS-, BSA-, and HSA-coated control wells. Plates were washed three times with PBST; conjugates (horseradish peroxidase-labeled goat anti-mouse immunoglobulin G [IgG] and IgM, anti-human Igs [IgG plus IgM plus IgA; American Qualex, La Mirada, Calif.]; anti-rabbit IgG [heavy plus light chain; Nordic, Tilburg, The Netherlands]) were added, diluted 1:1,000 in PBST with 0.5% goat serum, and incubated for 2 h at 37°C. Plates were washed, color was developed with H_2O_2 and orthophenylene diamine in citrate-phosphate buffer (pH 5.5) for 30 min at RT, and the optical density (OD) was read at 492 nm after stopping the reaction with 50 μl of sulfuric acid. The following controls were used: binding of MAbs and sera to PBS-, BSA-, and HSA-coated wells; binding of conjugate to antigen-coated wells.

The presence of anti-Lewis x antibodies in human sera was also investigated by their ability to inhibit the binding of a Lewis x MAb (CB-10) to *H. pylori* N. Plates were coated with 100 μl of 10^5 cells of *H. pylori* N per ml and postcoated with PBS containing 0.5% BSA and 50% fetal calf serum (FCS) (PBS-BSA-FCS). Samples (50 μl) of human sera diluted in PBS-BSA-FCS were added and incubated for 1 h at RT. Subsequently, 50 ng of biotinylated CB-10 in 50 μl of PBS-BSA-FCS was added and incubated for 30 min at RT. Streptavidin-biotin-horseradish peroxidase complex was added (1:1,000 in PBS-BSA), and the ELISA was completed as described above. In this assay system, the noninhibited control value of CB-10 binding to *H. pylori* was adjusted to 2.8. The presence of anti-Lewis x antibodies in human serum causes inhibition, i.e., a lowered OD is observed.

SDS-PAGE and immunoblot. Microsomes were electrophoresed in a 9% polyacrylamide gel (21); electroblotted; incubated with MAb 1E52 (anti-Lewis y), CB-10 (anti-Lewis x), or 2G11 (anti-H^+,K^+-ATPase β chain); and immunostained by conventional procedures.

FACS analysis. The binding of an *H. pylori*-induced anti-Lewis x MAb (CB-10) with human polymorphonuclear lymphocytes (PMN) expressing CD15 (=Lewis x) was compared with the binding of a "conventional" anti-Lewis x MAb (Bra.4F1) by means of fluorescence-activated cell sorter (FACS) analysis. PMN (10^6), obtained by dextran sedimentation, were incubated (30 min, 4°C) with ascites (diluted 1:20 in PBS-BSA). Cells were washed and incubated (30 min, 4°C) with phycoerythrin-labeled rabbit anti-mouse Ig (Dako, Glostrup, Denmark) diluted 1:250 in PBS-BSA. Following incubation, cells were washed and fluorescence was analyzed on a FACScan (Becton Dickinson, San Jose, Calif.).

Immunohistochemistry. Paraffin-embedded human gastric mucosa (corpus and antrum) was sectioned (4 μm), deparaffinized, blocked for endogenous peroxidase activity with 0.3% hydrogen peroxide in methanol (30 min, RT), washed in PBS (30 min, RT), blocked with rabbit serum diluted 1:50 in PBS containing 0.1% BSA, incubated (1 h, RT) with MAbs (1 μg/ml) in PBS containing 0.1% BSA, washed with PBS (30 min, RT), incubated (30 min, RT) with peroxidase-labeled rabbit anti-mouse conjugate (Dako) diluted 1:25 in PBS containing 0.1% BSA, and stained (3 min, RT) with diaminobenzidine (0.05%)–hydrogen peroxide (0.02%); the reaction was stopped in running tap water. Sections were counterstained with hematoxylin. We compared Bra.4F1 with CB-10 (conventional and *H. pylori*-derived anti-Lewis x MAbs, respectively; see also Table 4) and 12.4 with CB-4 (conventional and *H. pylori*-derived anti-Lewis y MAbs, respectively).

Measurement of fucosyltransferase (FT) activity. To investigate whether the occurrence of Lewis x and y antigens was concomitant with the presence of enzymes involved in their synthesis, FT activity was measured. A total of 2.3×10^9 bacterial cells of strain 609, which expresses Lewis x and Lewis y (see Table 2), were sonicated (four times for 30 s each, 4°C), and α3-FT activity was measured, with Fucα1-2Galβ1-4GlcNAcβ1-$(CH_2)_8$-$COOCH_3$ as an acceptor (10). In this assay, the transfer of radioactively labeled fucose from GDP-fucose to the acceptor is measured. Strains MO19 (expressing Lewis y), P466 (expressing both Lewis x and y) (2–4), and 609 were likewise investigated for α2-FT activity with Galβ1-phenyl, a universal acceptor for this type of FT (34).

RESULTS

Occurrence of Lewis x and y and H type I in *H. pylori*. The reactivity of MAbs specific for Lewis x (Bra.4F1 and 54-1-F6A), Lewis y (12.4), and H type I (4D2) with *H. pylori* clinical isolates is shown in Table 2. Apart from strain 104, all strains reacted with one or more of the MAbs. Strain NCTC 11637 reacted with one anti-Lewis x MAb (54-1-F6A) but not with another (CB-10) and did not express Lewis y, which is in agreement with the established chemical structure of its LPS (3, 4). Strains 474, 609, 700, and 1152 expressed both Lewis x and Lewis y. Strains NCTC 11637, 487, and 1152 expressed H type I, be it individually (strain 487) or in combination. Thus, Lewis x and y and H type I are common antigens in *H. pylori.*

Presence of FTs in *H. pylori*. A total of 1.92 nmol of fucose per min was incorporated into the α3-FT acceptor per 10^9 sonicated cells of *H. pylori* 609. The presence of α3-FT activity is in agreement with the expression of Lewis x by strain 609 (Table 2). No α2-FT was detected in strains 609, P466, and MO19, which is not in agreement with the established presence of Lewis y in these strains (Table 2) (2–4).

Immune response to *H. pylori* LPS. Rabbit antisera to seven *H. pylori* strains reacted with synthetic Lewis x and y and H type I and with purified LPS of strain NCTC 11637 (Table 3).

TABLE 3. Reactivity[a] of rabbit antisera to *H. pylori* with synthetic Lewis antigens and *H. pylori* LPS in ELISA

Antiserum to strain	Serotype (Table 2)	Reactivity with antigen[b]							
		Trimeric Le^x	Le^x	Le^y	Le^a	Le^b	H-I	H-II	LPS
ATCC 11637	x, H type I	1.6	—	0.4	—	—	—	—	>
104	NT[c]	2.7	1.0	0.5	—	—	—	—	2.6
487	H type I	0.7	—	—	—	0.5	0.7	—	2.4
1152	x, y, H type I	2.1	—	0.6	—	—	—	—	>
474	x, y	2.4 (1.1)[d]	—	2.5 (0.5)[d]	—	—	—	—	> (1.7)[d]
609	x, y	2.7	—	—	—	—	—	—	1.8
700	x, y	2.4	0.6	1.2	—	—	—	—	>

[a] See Table 2. —, OD < 0.3; >, OD above 2.7.
[b] Le^x, Le^y, Le^a, and Le^b, Lewis x, y, a, and b, respectively; H-I and -II, H types I and II.
[c] NT, not tested.
[d] OD value of preimmune serum of strain 474.

TABLE 4. Reactivity[a] of *H. pylori*-induced autoreactive MAbs with synthetic Lewis antigens

MAb	Reactivity with synthetic antigen[b]				
	Trimeric Le^x	Le^y	Le^a	Le^b	H type I
A522	1.0	—	—	—	—
A1	2.7	—	—	—	—
A18	>	—	—	—	—
CB-10	>	—	—	—	—
A540	—	>	—	—	—
CB-4	—	1.4	—	—	—
1E52	—	>	—	—	—
4D2	—	—	—	—	1.3

[a] See Table 2. —, OD < 0.3; >, OD above 2.7.
[b] See Table 3 for antigen abbreviations.

Control sera (preimmune sera and serum to *E. coli*) did not react with these synthetic antigens and NCTC 11637 LPS. Sera did not bind to PBS-, HSA-, or BSA-coated wells. Thus, *H. pylori* LPS is immunogenic in rabbits.

Mouse antisera to strains 104 and 700 reacted strongly with trimeric Lewis x. A 1:250 dilution of antiserum gave OD values from 1.1 to >2.6. Mouse antiserum to purified *H. pylori* NCTC 11637 LPS reacted with trimeric Lewis x (Table 1) (OD = 0.9 at 1:250). Reactivity of antisera to PBS-treated wells and to BSA- or HSA-coated wells was low (OD below 0.5). Control sera (antiserum to *E. coli* O111 and nonimmune mouse sera) did not react with trimeric Lewis x. Sera from experimentally infected mice were tested for reactivity with trimeric Lewis x. At a dilution of 1:250, serum from one of the six mice tested reacted (OD = 2.6) while none of the preimmune sera did. Previously, we have described a panel of 30 *H. pylori*-reactive MAbs, obtained after immunization of mice with *H. pylori*, of which eight were autoreactive, i.e., they reacted with human and murine gastric tissue (28–30). We tested this panel for binding with Lewis antigens. The eight autoreactive MAbs reacted: four of them (A522, A1, A18, and CB-10) proved to be specific for Lewis x, three (A540, CB-4, and 1E52) were specific for Lewis y, and one (4D2) was specific for H type I (Table 4). None of the nonautoreactive MAbs tested bound to Lewis antigens or *H. pylori* LPS. Thus, *H. pylori* LPS is also immunogenic in mice, and MAbs to Lewis antigens may be prepared from *H. pylori*-immunized mice.

Human antibody response. Sera from *H. pylori* patients (n = 18) and noninfected controls (n = 10) (defined by serology) were tested for binding with the currently available purified LPS from *H. pylori* NCTC 11637, O1 to O6, P466, and MO19. The highest OD reached on any of the LPSs is shown in Fig. 2a. Patient sera reacted with ODs ranging from 0.2 to >2.6, while control sera reacted much less (0.2 to 0.4). A second group of *H. pylori* patients (n = 12) and controls (n = 10), defined by culture and histology, yielded similar data (not shown). These data show that *H. pylori* LPS is immunogenic in humans. The reactivity of one patient serum with three different LPSs is shown in Fig. 2b. The serum reacts with LPS of strains NCTC (expressing Lewis x) and P466 (Lewis x and Lewis y) but not with LPS of strain MO19 (Lewis y). Several sera (data not shown) reacted with LPS of strains NCTC 11637 and P466 and also with LPS of strain MO19. To further define the epitope specificity of the human antibody response to *H. pylori* LPS, patient sera were tested for binding with Lewis x and CCA, which contains polymeric Lewis x. An example is given in Fig. 2c. The patient serum reacted well with CCA but much less with trimeric Lewis x or monomeric Lewis x. We also investigated the presence of anti-Lewis x antibodies in the sera by another method, i.e., by the ability of the sera to inhibit the binding of a Lewis x MAb (biotinylated CB-10) to *H. pylori*. At first, for comparison, we tested a group of sera from 90 dyspeptic patients both in inhibition and for reactivity with NCTC 11637 LPS, which expresses Lewis x. Figure 2d shows that the ability of sera to inhibit correlated with their reactivity with the LPS ($r = -0.82$, $P < 0.0001$). Three patient serum samples, highly reactive with LPS and indicated with asterisks in Fig. 2a, strongly inhibited the binding of biotinylated CB-10. The noninhibited OD was 2.8; the three serum samples reduced this value to 0.1. We conclude that *H. pylori* LPS elicits antibodies to Lewis x in humans.

Comparison of Lewis x MAbs induced by *H. pylori* and conventional anti-Lewis x MAbs. In FACS analysis of binding with human PMN, the *H. pylori*-derived anti-Lewis x MAb CB-10 reacted comparably with Bra.4F1, a conventional Lewis x-specific MAb (Fig. 3). Likewise, in immunohistochemistry, the reactivity of *H. pylori*-induced anti-Lewis x and y (CB-10 and CB-4, respectively) MAbs was comparable with that of the conventional anti-Lewis x and y MAbs (Bra.4F1 and 12.4, respectively) (Fig. 4). Anti-Lewis x and y MAbs react with the glandular epithelium, but anti-Lewis x MAbs are reactive with a more restricted area. Furthermore, cells from one of three patients with gastric MALT lymphoma reacted with CB-4. MAbs CB-4 and CB-10 also reacted with gastric adenocarcinoma cells of one of three patients tested (not shown). We conclude that *H. pylori*-induced antibodies to Lewis antigens bind to host cells.

Identification of autoimmune targets. Previously, we have shown that human and murine *H. pylori*-induced antibodies are directed against gastric foveolar secretions and intracellular mucin of foveolar and neck cells, chief cells, and canaliculi of parietal cells (28–30), but the molecular species remained unidentified and we do not know if one or more species are involved. We tested a variety of anti-Lewis MAbs for binding with gastric mucin, with intrinsic factor, and with gastric H^+,K^+-ATPases. Both intrinsic factor and the β chain of gastric H^+,K^+-ATPase are glycoproteins synthesized by the gastric parietal cells and are known targets in gastric autoimmunity (15, 22, 38). The results shown in Table 5 indicate that there is serological evidence for the presence of Lewis x and y in gastric mucin (also a glycoprotein) and for Lewis y and H type I in pig and rabbit H^+,K^+-ATPase, respectively. By immunoblotting, it was shown that human and mouse H^+,K^+-ATPases express Lewis y and Lewis x plus y, respectively (Fig. 5). The evident heterogeneity in molecular weight is due to heterogeneity in glycosylation, and deglycosylation results in a single narrow band. In conclusion, gastric mucin and gastric H^+,K^+-ATPase β chain are potential targets of *H. pylori*-induced autoantibodies.

Pathogenic role of *H. pylori* LPS-induced anti-Lewis antibodies. In a previous study, we have described that growth during 2 weeks in mice of an *H. pylori*-induced hybridoma (CB-4) that secretes autoantibodies causes gastric histopathological changes, similar to those occurring during gastritis, e.g., infiltration with inflammatory cells (28). However, this did not occur upon growth in vivo of a control hybridoma. Thus, a causative role for *H. pylori*-induced autoantibodies in the disease process was demonstrated. In this study, we determined that the MAb tested in vivo (CB-4) is specific for Lewis y (Table 4).

DISCUSSION

In this paper, we have shown that Lewis x and y and H type I (for structures, see Table 1), which are common epitopes on

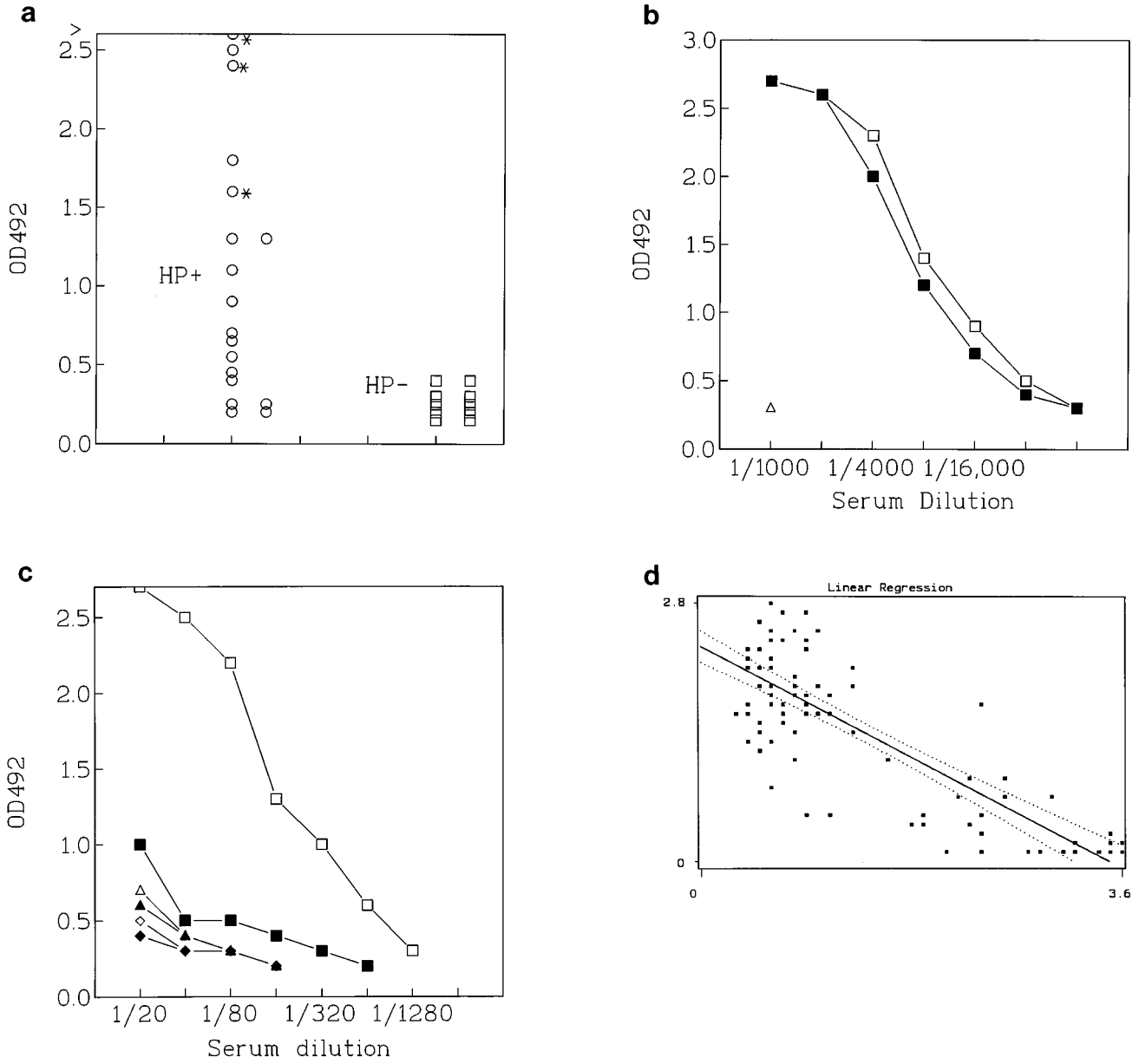

FIG. 2. (a) Reactivity of sera from *H. pylori*-positive patients ($n = 18$) and noninfected controls ($n = 10$) with LPS of *H. pylori*. The three serum samples marked with asterisks strongly inhibited the binding of anti-Lewis x MAb CB-10. (b) Binding of patient serum to LPS of *H. pylori* NCTC 11637 (□), P466 (■), and MO19 (△). (c) Binding of patient and control sera (open and closed symbols, respectively) to polymeric Lewis x (=CCA [□ and ■]), trimeric Lewis x (△ and ▲), and monomeric Lewis x (◇ and ◆). (d) Regression analysis of binding of sera to *H. pylori*-LPS (horizontal axis) versus ability of sera to inhibit binding of anti-Lewis x MAb with *H. pylori*. The 95% confidence limits for the regression line are shown as dotted curves.

H. pylori LPS, induce autoantibodies in animals and humans. We also have identified a potential target of the autoimmune response, i.e., gastric H^+,K^+-ATPase, the proton pump. Finally, we have shown that *H. pylori*-induced anti-Lewis y antibodies may cause gastric immunohistopathological changes.

Lewis x, structurally present in *H. pylori* LPS (2–4), was also detectable by serology (Table 2) (7, 35). Lewis y is present structurally (2, 4) and can be detected by serology (Table 2); H type I is as yet detectable by serology only. Striking is the difference in reactivity of two anti-Lewis x MAbs, CB-10 and 54-1-F6A (Table 2), a MAb obtained after immunization with *Schistosoma* spp. Our data demonstrate cross-reactivity between *H. pylori* LPS and *Schistosoma* CCA, which is based on the presence of polymeric Lewis x (3, 4, 40). α3-FT activity was detected in strain 609; the presence of α3-FT has been reported before (7). In contrast, no α2-FT activity was detected in Lewis y-containing strains despite use of Galβ-1-phenyl, the most general acceptor for α2-FT. Thus, *H. pylori* α2-FT behaves unlike the most well known α2-FT enzymes (34).

We now have developed a serotyping system for *H. pylori* based on the occurrence of Lewis epitopes; more than 85% of strains obtained from various parts of the world and from various disease entities expressed Lewis antigens (36). Thus, Lewis antigens are common antigens on *H. pylori*.

Rabbit antisera to six *H. pylori* strains reacted strongly with Lewis x; antiserum to strain 487 reacted with H type I and

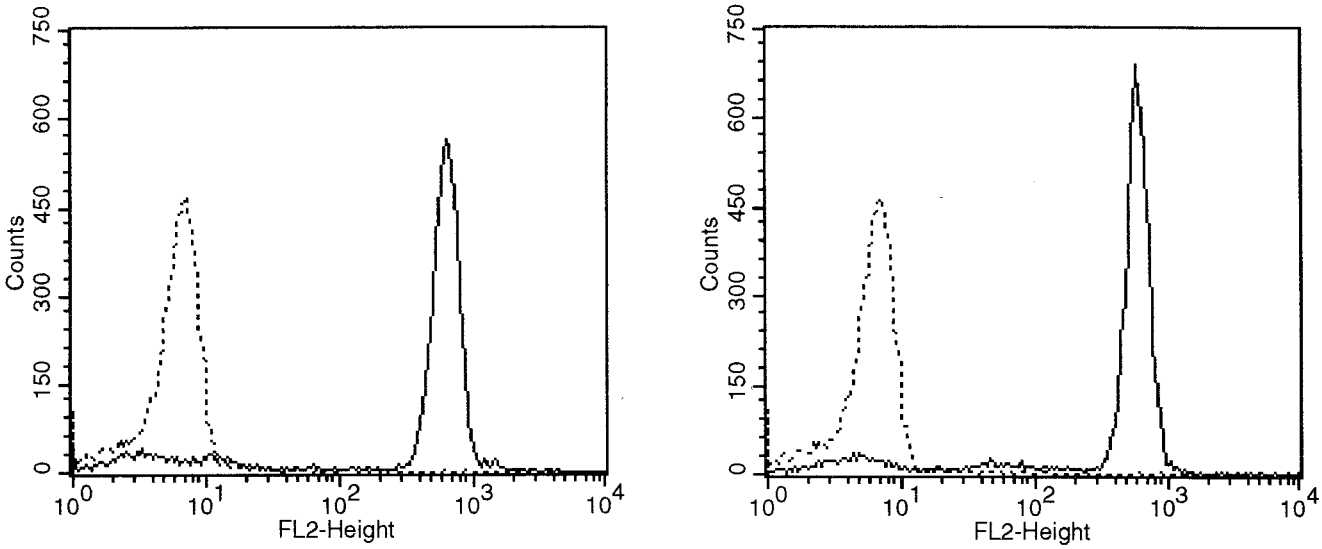

FIG. 3. Comparison of conventional anti-Lewis x MAb Bra.4F1 (left) with CB-10 (right), an *H. pylori*-induced anti-Lewis x MAb, in their binding to human PMN by FACS analysis. The peak of the dashed line at the left of each panel is the negative control.

Lewis b. These antisera all reacted with LPS of NCTC 11637, which indicates the presence of common epitopes in *H. pylori* LPS (25). Mice vaccinated parenterally or infected orally with *H. pylori* reacted with trimeric Lewis x. Vaccination of mice with *H. pylori* yielded eight MAbs that bound with Lewis antigens (Table 4) and *H. pylori* LPS and cross-reacted with murine and human gastric mucosa. MAbs ($n = 22$) to other *H. pylori* antigens did not bind to gastric mucosa, and we conclude that only LPS was responsible for the *H. pylori*-induced gastric cross-reactivity in the mouse, reported by us before (28–30). Compared with control sera, sera from *H. pylori* patients reacted much more strongly with *H. pylori* LPS (Fig. 2a). Serum from a patient from whom a strain expressing both Lewis x and y was isolated reacted strongly with LPS of strain NCTC 11637 (Lewis x) but not with LPS of strain MO19 (Lewis y) (Fig. 2b). Why in this case no anti-Lewis y response can be detected is currently not understood. The binding of patient sera to schistosomal CCA (=polymeric Lewis x) was much lower compared with the LPS response (compare Fig. 2b and c), and binding to trimeric and monomeric Lewis x was virtually zero. Possibly, a major fraction of the human serum response is directed to non-Lewis epitopes present in the LPS core region (2–4). We also obtained evidence for an anti-Lewis x response in humans by measuring the ability of human sera to inhibit the binding of a biotinylated anti-Lewis x MAb (CB-10) with *H. pylori* LPS, and several sera that reacted well with LPS of strain NCTC 11637 (Lewis x) were strongly inhibitory (Fig. 2a). The ability of sera to inhibit was concomitant with their inhibitory power (Fig. 2d). In summary, infected patients react to *H. pylori* LPS with an anti-Lewis x response as measured by binding to purified, Lewis x-containing LPS and to CCA and by inhibition of an anti-Lewis x MAb.

H. pylori-induced anti-Lewis x bound well to PMN (Fig. 3), cells known to express Lewis x (CD15). The glandular region of human gastric epithelium was recognized by anti-Lewis x and anti-Lewis y MAbs (Fig. 4). Finally, in one of three cases tested, human gastric adenocarcinoma cells and MALT lymphoma cells reacted with *H. pylori*-induced anti-Lewis MAbs. In previous studies, we have shown (28–30) that sera from mice immunized with *H. pylori* and sera from infected patients bind also to the glandular region (parietal cells, mucus-producing cells) and that this binding disappeared after absorption with *H. pylori* cells (<10% coccoid forms present). We hypothesized that *H. pylori* LPS-induced antibodies reactive with gastric Lewis antigens are involved in this autoimmune reaction. The gastric parietal cell is a known target for autoimmune antibodies; in particular, the H^+,K^+-ATPase gastric proton pump, a heterodimeric enzyme (α and β chains) that acidifies the stomach and is localized in the parietal canaliculi, may be recognized by autoantibodies (18). Correlation between patient serum antibody titers to *H. pylori* and to H^+,K^+-ATPase has been reported previously (23). More-detailed studies showed that the β subunit (22, 38), including its oligosaccharide chain (13, 44), is involved in autoimmunity. Recent structural work has shown that the glycan part is composed of galactose, fucose, and *N*-acetylglucosamine (44) in the form of polylactosamine structures, i.e., similar to Lewis antigens (6). Taken all together, we hypothesized that the glycan chain of the gastric H^+,K^+-ATPase β chain is one of the targets of *H. pylori* LPS-induced anti-Lewis autoimmunity. Supportive data (Table 5 and Fig. 5) show the presence of Lewis y in the β chain of gastric H^+,K^+-ATPases of pigs, rabbits, mice, and humans. The pig and mouse β units also expressed H type I and Lewis x, respectively. A second well-recognized parietal cell glycoprotein autoantigen, intrinsic factor, did not express Lewis antigens. A crude gastric mucin preparation expressed Lewis x and y antigens (Table 5), but this observation requires validation with fully purified material.

In the past (28), we have tested the effect of high concentrations of autoreactive MAbs in vivo in the murine host by allowing growth of an *H. pylori*-derived autoreactive hybridoma. Results of these experiments showed immunohistopathological aberrations, similar to gastritis in the form of inflammatory cell infiltrates and enhanced degenerative and regenerative processes (28). In the present study, we prove that the epitope specificity of that particular MAb (CB-4) is Lewis y (Table 4). Thus, Lewis y antibodies themselves have detrimental effects in vivo.

How the antibodies to Lewis antigens initiate gastric damage remains an open question. Anti-Lewis x and y antibodies might bind to the gastric H^+,K^+-ATPases and block pump function. Proton pump defects (achlorhydria) associated with *H. pylori*

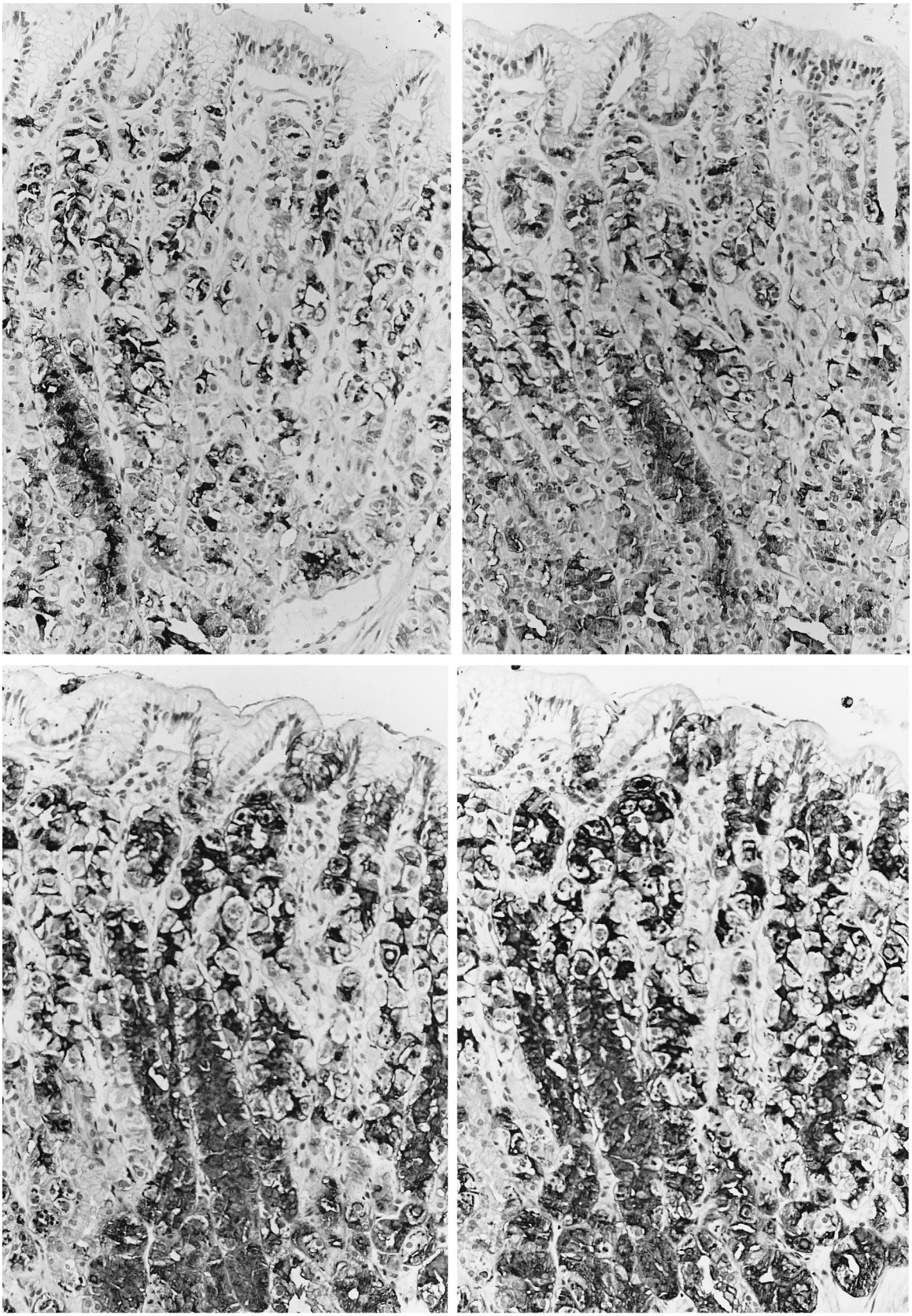

FIG. 4. Similarity in gastric immunohistochemical staining by *H. pylori*-induced anti-Lewis MAbs (right) and anti-Lewis MAbs induced by human cells (left). Upper micrographs, anti-Lewis x; left, Bra.4F1; right, CB-10. Lower micrographs, anti-Lewis y; left, 12.4, right, CB-4. The gastric lumen is visible at the top of the photographs.

TABLE 5. Presence[a] of Lewis antigens in gastric H^+,K^+-ATPase, intrinsic factor, and gastric mucin

MAb (specificity)[b]	Reactivity with antigen					
	H^+,K^+-ATPase (pig)	H^+,K^+-ATPase (rabbit)	H^+,K^+-ATPase β unit (rabbit)	Intrinsic factor (hog)	Intrinsic factor (human)	Gastric mucin
CB-10 (Le^x)	—	—	—	—	—	—
54-1-F6A (Le^x)	—	—	—	—	—	1.6
12.4 (Le^y)	0.9	0.9	0.7	—	—	2.6
1E52 (Le^y)	>	1.1	1.1	—	—	2.6
7 Le (Le^a)	—	—	—	—	—	0.6
2-25 Le (Le^b)	—	—	—	—	—	2.6
4D2 (H type I)	2.1	—	—	—	—	0.4
2G11 (H^+,K^+-ATPase)	>	>	>	ND	ND	ND
CS Lex (sialyl Le^x)	—	—	—	—	—	—
19-O Le (H type II)	>	1.4	0.4	—	—	>
3-3A (blood group A)	ND	ND	ND	ND	ND	—

[a] Expressed as reactivity in ELISA; see Table 2. —, OD < 0.3; >, OD above 2.7; ND, not done.
[b] See Table 3 for specificity abbreviations.

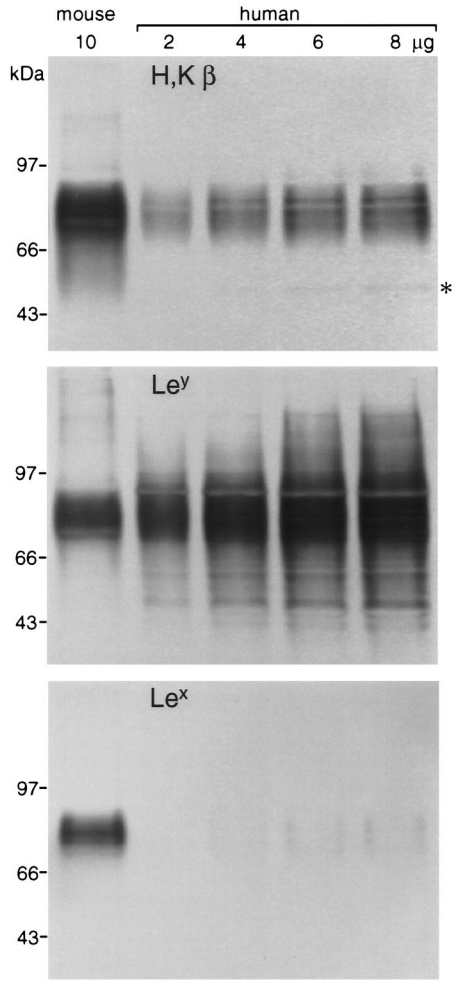

FIG. 5. Presence of Lewis y in the β chain of human and murine gastric H^+,K^+-ATPase as detected by immunoblot following SDS-PAGE. Numbers indicate the amount of protein per lane. Top panel, MAb 2G11 specific for β chain; middle and lower panels, 1E52 and CB-10, *H. pylori*-induced anti-Lewis y and x, respectively. The maturely glycosylated β subunit appears as a very broad band centered at 75 kDa; the faint band at 52 kDa (asterisk) is the high-mannose, immature subunit (9). Because of the heterogeneity of glycosylation, the β chain appears as a broad band.

have been reported elsewhere. Human autoantibodies that block pump function have been described previously (5). Another host target recognized by anti-Lewis x antibodies might be PMN (Fig. 3). It has been reported that binding of anti-Lewis x MAbs to PMN causes increased adherence of the cells (37). Adherent, "angry" PMN may cause local tissue damage and inflammation. It has been reported elsewhere that *S. mansoni* infection in humans yields anti-Lewis x antibodies that, together with complement, cause lysis of human PMN or HL-60 cell line cells (31, 41). Complement might also be involved in gastric damage induced by antibodies to Lewis antigens. Antibodies to *H. pylori* might also be involved in the pathogenesis of *H. pylori*-associated malignancies. *H. pylori*-reactive autoantibodies have been shown to cause cell division of adenocarcinoma cells in vitro (43). This may be true for MALT lymphoma cells, too (14). An alternative mechanism by which *H. pylori* might be involved in the pathogenesis of MALT lymphoma is the ability of Lewis x itself to cause B-cell proliferation (42).

Thus, the molecular mimicry that exists between *H. pylori* LPS and host glycoconjugates may give rise to autoimmune phenomena. In this respect, the case of *H. pylori* is not unique. Autoimmunity due to molecular mimicry between bacteria and host has been documented, as has the involvement of glycoproteins and glycolipids (12). An example relevant to the present study is *Campylobacter jejuni*. The molecular structure of *C. jejuni* LPS mimics that of gangliosides (1) and elicits antibodies that react with gangliosides, a process which is believed to be central to the pathogenesis of the autoimmune neurodegenerative Guillain-Barré syndrome.

To conclude, upon infection with *H. pylori* an anti-Lewis x and/or y antibody response develops, one that is directly involved in gastritis and possibly later in parietal cell damage. Within this concept, type B gastritis also and possibly *H. pylori*-associated malignancies have an autoimmune component. Our findings are also relevant to the design of *H. pylori* vaccines to avoid an autoimmune response. Thus, molecular mimicry between *H. pylori* LPS and human Lewis x and y antigens may play a pivotal role in *H. pylori*-linked disease.

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F6A and CCA, and T. Boren (Umeå) for strains MO19 and P466. H. Kroes (Amsterdam) kindly performed the FACS experiment. J. L. Penner (Toronto) kindly provided LPS of serotypes O2 to O6. A. Safi and J.-L. Gueant (Nancy) provided human and hog intrinsic factor. Artwork and micrographs were skillfully done by H. Verbeek and J. van Veldhuisen. We thank C. M. J. E. Vandenbroucke-Grauls, A. J. van Winkelhoff, D. H. van den Eijnden, D. H. Joziasse, and S. Pena for critical reading.

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