Virchows Arch (1998) 433:419–426

ORIGINAL ARTICLE

Hiroyoshi Ota · Jun Nakayama · Masanobu Momose
Masayoshi Hayama · Taiji Akamatsu
Tsutomu Katsuyama · David Y. Graham
Robert M. Genta

Helicobacter pylori infection produces reversible glycosylation changes to gastric mucins

Received: 23 April 1998 / Accepted: 2 July 1998

Abstract The protective ability of gastric mucins may depend largely on their oligosaccharide chains. We evaluated the effects of *H. pylori* infection on the glycosylation of gastric mucins. Gastric biopsy specimens from 20 *H. pylori*-infected patients before and after cure of the *H. pylori* infection and 8 normal uninfected volunteers were examined by immunostaining for simple mucin-type glycoproteins and blood-group-related antigens bearing type 1 chain backbone. The immunoreactivity in different gastric compartments was evaluated. Simple mucin-type glycoproteins and blood-group-related antigens were expressed in surface mucous cells. Simple mucin-type glycoproteins showed antrum-predominant expression in normal volunteers and were found in significantly fewer surface mucous cells in infected patients than in normal volunteers; their expression was restored after eradication of *H. pylori*. Sialyl Lewis^a and Lewis^b were expressed in fewer surface mucous cells after than before eradication. The patterns of glycosylation of gastric mucins vary in different gastric compartments and are reversibly altered by *H. pylori* infection. These alterations may affect the protective functions of gastric mucins.

Key words *Helicobacter pylori* · Gastric mucin · Immunohistochemistry · Gastric mucosa · Glycoprotein

H. Ota · D.Y. Graham · R.M. Genta
Department of Medicine, Veterans Affairs Medical Center and Baylor College of Medicine, Houston, Tex., USA

H. Ota · R.M. Genta
Department of Pathology, Veterans Affairs Medical Center and Baylor College of Medicine, Houston, Tex., USA

D.Y. Graham
Department of Molecular Virology, Veterans Affairs Medical Center and Baylor College of Medicine, Houston, Tex., USA

R.M. Genta
Department of Microbiology and Immunology, Veterans Affairs Medical Center and Baylor College of Medicine, Houston, Tex., USA

H. Ota (✉) · J. Nakayama · M. Momose · M. Hayama
T. Katsuyama
Department of Laboratory Medicine, Shinshu University School of Medicine, Matsumoto, Nagano 390, Japan
Tel.: +81-263-37-2805, Fax: +81-263-34-5316

T. Akamatsu
Second Department of Internal Medicine, Shinshu University School of Medicine, Matsumoto, Nagano 390, Japan

Introduction

Gastric mucins form a continuous gel layer covering gastric mucosa and are believed to play an important part in the protection of gastric mucosa from noxious agents [1]. *Helicobacter pylori*, the major aetiological agent of chronic gastritis and peptic ulcer disease, is found both in the mucous gel layer of the stomach [21, 22] and attached to the gastric surface mucous cells [21, 22]. One of the possible mechanisms through which *H. pylori* may injure the gastric mucosa may be by impairing the effectiveness of the protective function of the mucins [23]. Studies on the effects of *H. pylori* infection on gastric mucins have concentrated on the rheological properties of the gastric mucins [15, 23] and on the quantitative changes in mucus production by gastric mucous cells [10, 22]. Recently less expression of surface mucous cell-type mucin and aberrant expression of gland mucous cell-type mucin in the gastric surface mucous cells of *H. pylori*-infected patients has been reported [3].The protective function of the gastric mucous gel may be related to its physical characteristics, which, in turn, depend largely on the oligosaccharide content of the mucins [25, 29]. Biosynthesis of the oligosaccharide chains of the mucins is initiated by formation of the core structure, followed by branching and elongation of the core structure following addition of sugar residues, which lead to the formation of backbone and peripheral structures [25] (Figs. 1, 2). Blood group antigens are present as the structures in the peripheral regions [25] (Fig. 2). Alterations in the glycosylation process would be likely to have profound effects on the structure and function of mucins and might

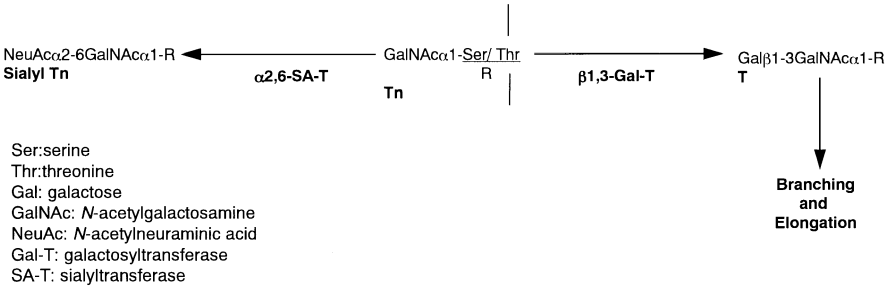


Fig. 1 Initial step of biosynthetic pathways for the formation of mucus glycoproteins (*O*-mucins)

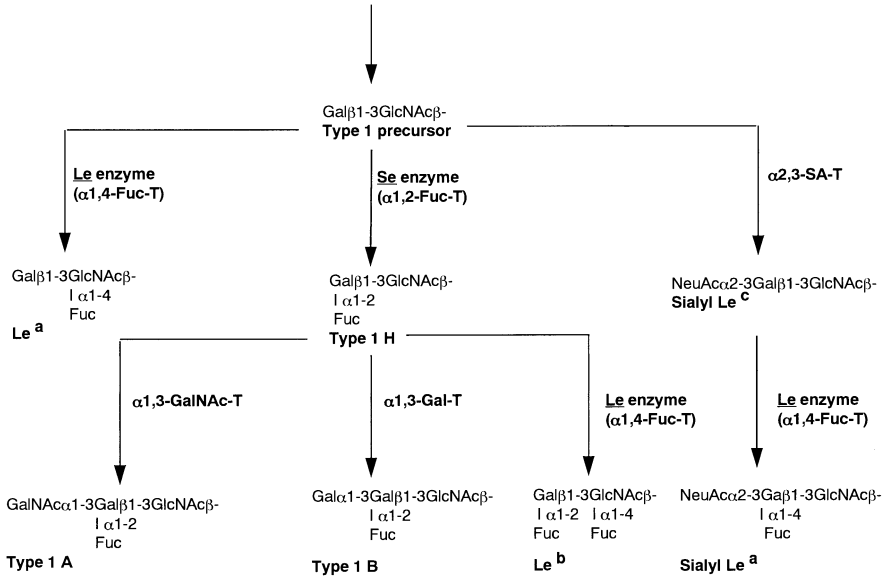


Fig. 2 Biosynthetic pathways for the formation of blood group related antigens bearing type 1 chain back bone (*Fuc* fucose, *Gal* galactose, *GalNAc N*-acetylgalactosamine, *NeuAc N*-acetylneuraminic acid, *Fuc-T* fucosyltransferase, *Gal-T* galactosyltransferase, *GalNAc-T N*-acetyl galactosaminyltransferase, *SA-T* sialyltransferase)

affect their ability to maintain an effective protective barrier in the stomach.

We set up the hypothesis that the effects of *H. pylori* on the protective function of gastric mucus are mediated by alterations induced by the infection in the glycosylation pattern of carbohydrates moieties in gastric mucins. To test this hypothesis we studied the expression of oligosaccharide antigens in the gastric surface mucous cells of noninfected normal volunteers and *H. pylori*-infected patients, both before and after successful treatment. We used monoclonal antibodies against simple mucin-type glycoproteins (Tn, sialyl Tn and Tomsen-Friedenreich antigens) and blood-group-related antigens bearing type 1 chain backbone, Galβ1-3GlcNAc-R (type 1 chain precursor, Lewis^a, sialyl Lewis^a and Lewis^b).

Patients and Methods

H. pylori-infected patients who underwent endoscopic examination at the University Hospital of Shinshu University School of Medicine between 1995 and 1996 gave informed consent to enrollment in this study. At the initial endoscopy, during which the diagnosis of *H. pylori* infection was established, two biopsy specimens were obtained from one site in the antral greater curvature close to the pylorus and from one site in the greater curvature in the region of the upper middle corpus. One specimen from the antrum and one from the corpus were used for the culture of *H. pylori*. The remaining biopsies were used for histological examination.

Each patient was treated with a 2-week course of oral administration of lansoprazole (30 mg/day), amoxicillin (1,500 mg/day), and clarithromycin (600 mg/day). A second endoscopy was performed 4 weeks after completion of the antimicrobial treatment, and an identical biopsy protocol was followed.

Twenty successfully treated patients (6 with gastric ulcer, 8 with duodenal ulcer, 3 with gastroduodenal ulcer and 3 with chronic active gastritis) entered on this study and will be referred to as the *H. pylori*-infected patients. All these 20 patients were positive for *H. pylori* according to both histological examination and culture before treatment and became negative according to both histological examination and culture after treatment. We selected the cases that were negative for intestinal metaplasia on histological examination.

In addition, we studied 8 normal volunteers from the population of non-infected subjects available at the Houston VAMC. These previously described subjects [17] had neither subjective nor objective evidence of gastrointestinal disease, had negative serology for anti-*H. pylori* antibodies, negative urea breath tests, and no visible organisms in any of the biopsy specimens examined. From each of the normal volunteers we studied one section from the antral greater curvature close to the pylorus and one from the greater curvature in the region of the upper middle corpus.

Table 1 shows details of age, sex and diseases for patients and normal volunteers.

Biopsy specimens for histological examination were fixed in 20% buffered formalin solution immediately after they were obtained and then dehydrated in 100% ethyl alcohol, cleared in xylene, and embedded in paraffin. Haematoxylin and eosin staining was used for histological examination.

Serial paraffin sections (3 μm thick) were stained with indirect immunoperoxidase staining for *H. pylori* [21], for simple mucin-type carbohydrate antigens [Tn, sialyl Tn and Tomsen-Frieden-

Table 1 Clinical data of normal volunteers and *H. pylori*-infected patients

	Normal volunteers	*H. pylori*-infected patients	
	Secretors	Secretors	Nonsecretors
Gastric ulcer		2	4
Duodenal ulcer		7	1
Gastroduodenal ulcer		2	1
Chronic active gastritis		3	0
Male	2	12	5
Female	6	2	1
Total	8	14	6
Mean age (± SEM)	42.1±3.5	34.4±2.7	41.0±7.3

Table 2 Sources of antibodies used in this study

Antibody	Clone	Source	Final dilution
Tn	HB Tn1	DAKO (Carpenteria, Calif.)	×100
Sialyl-Tn	HB STn1	DAKO	×100
Thomsen-Friedenreich	HB T1	DAKO	×100
Type 1 chain precursor	K21	SIGNET (Dedham, Mass.)	×100
Lewis[a]	T174	SIGNET	×100
Sialyl Lewis[a]	1116-ns-19-9	SIGNET	×100
Lewis[b]	T218	SIGNET	×100
H. pylori	(rabbit poly clonal)	DAKO	×50
Blood group A (type 1 & 2 chain)	81FR2.2	DAKO	×100
Blood group B (type 1 & 2 chain)	3E7	DAKO	×100
Blood group H (type 2 chain)	92FR-A2	DAKO	×100
Horseradish peroxidase-labelled anti-mouse immunoglobulin		DAKO	×100
Horseradish peroxidase-labelled anti-rabbit immunoglobulin		DAKO	×100

Table 3 Staining scores for simple mucin-type glycoproteins and blood-group-related antigens bearing type 1 chain back bone in gastric biopsy specimens (*STn* sialyl Tn, *T* Thomsen-Friedenreich antigen, *Pre1* type 1 chain precursor, *Le*[a] Lewis[a], *SLe*[a] sialyl Lewis[a], *Le*[b] Lewis[b])

	Tn	STn	T	Pre1	Le[a]	SLe[a]	Le[b]
Antrum							
Secretors							
Normal volunteer (*n*=8 biopsy)	100[a] / 3[b] (3)	100/1.5(1-2)	25/0(0-0.5)	13/0(0)	13/0(0)	13/0(0)	88/2(1-3)
H. pylori gastritis (*n*=14 biopsy)							
Pretreatment	21/ 0 (0)[##]	29/0(0-1)[##]	21.4/0(0)	21/0(0)	43/0(0-3)	29/0(0-1)	100/3(3)
Posttreatment	100 / 2 (2-3)**	100/2(1-2)**	29/0(0-2)	7/0(0)	43/0(0-3)	0/0(0)	100/2(2-3)*
Non-Secretors							
H. pylori gastritis (*n*=6 biopsy)							
Pretreatment	17 / 0 (0)	50/0.5(0-1)	83/1(1)	83/2.5(1-3)	100/3(3)	100/2(1-2)	100/3(3)
Posttreatment	100 / 3 (3)*	100/2(1-3)*	100/1.5(1-2)	83/1(1)	100/3(3)	0/0(0)*	100/3(3)
Corpus							
Secretors							
Normal volunteer (*n*=8 biopsy)	63/1(0-1.5)	63/1(0-1)	13/0(0)	13/0(0)	13/0(0)	13/0(0)	100/2.5(1.5-3)
H. pylori gastritis (*n*=14 biopsy)							
Pretreatment	0/0(0)[##]	0/0(0)[##]	14/0(0)	29/0(0-3)	29/0(0-3)	29/0(0-3)	100/3(3)
Posttreatment	14/0(0)	43/0(0-1)*	29/0(0-3)	29/0(0-3)	29/0(0-3)	21/0(0)	100/2.5(2-3)*
Non-Secretors							
H. pylori gastritis (*n*=6 biopsy)							
Pretreatment	0/0(0)	0/0(0)	17/0(0)	100/3(3)	100/3(3)	100/3(3)	100/3(3)
Posttreatment	17/0(0)	33/0(0-1)*	50/0.5(0-1)	100/3(3)	100/3(3)	83/1(1-2)**	100/3(2-3)*

Scores for staining were analysed by the Mann-Whitney U-test (normal volunteers vs pretreatment: [#] $P<0.05$, [##] $P<0.01$) and by Wilcoxon signed-rank test (pretreatment vs. posttreatment: * $P<0.05$; ** $P<0.01$).

[a] Frequency (%) of positive biopsy specimens
[b] Median score with the interquartile range in parentheses

reich (T) antigen] (Table 2, Fig. 1), or for blood-group-related antigens bearing type 1 chain backbone (type 1 chain precursor, Lewis^a, sialyl Lewis^a and Lewis^b; Table 2, Fig. 2). Briefly, endogenous peroxidase was blocked in all slides by incubating with 3% hydrogen peroxide for 30 min. Tissue sections were then prepared for pretreatment with 0.2% trypsin (Sigma, St. Louis, Mo.) for immunostaining for *H. pylori* [21] or prepared for antigen retrieval in a steamer (Black & Decker HS90, Shelton, Conn.) for 30 min in 0.01 mol/l citrate buffer (pH 6.0) for immunostaining for simple mucin-type carbohydrate antigens or for blood-group-related antigens bearing type 1 chain backbone. Preliminary experiments revealed that steamer retrieval enhanced the immunoreactivity of tissue sections for monoclonal antibodies used in this study, especially for monoclonal antibodies against simple mucin-type carbohydrate antigens. After pretreatment with trypsin or antigen retrieval in a steamer, the slides were incubated with primary antibodies for 2 h. Slides were then icubated with horseradish peroxidase-labelled second antibody for 30 min. Visualization of immunostaining was performed with diaminobenzidine as a substrate, and the tissue sections were counterstained with haematoxylin, dehydrated and mounted. Specifications of the antibodies employed in this study are listed in Table 2.

Negative controls were obtained by omitting the primary antibody. Red blood cells and endothelial cells in the biopsy specimens were used as internal positive controls for blood group A, B and H substances. Slides prepared from colon adenocarcinoma tissue were used as positive controls for Tn, sialy Tn, T, Lewis^a, sialyl Lewis^a and Lewis^b.

The degree of staining of the surface mucous cells with specific carbohydrate antigens was scored semiquantitatively as 0 (negative), 1 (less than one-third of the surface mucous cells), 2 (more than one-third, but less than two-thirds of the surface mucous cells) and 3 (more than two-thirds of the surface mucous cells). The immunoreactivity in the supranuclear region (Golgi region) was also evaluated, but not graded. Grading of immunoreactivity was performed by a single observer, who was unaware of the subjects' *H. pylori* status and clinical group, and of the antibodies used. To validate the grading method, all specimens were graded twice, on two separate occasions. There was no significant intra-observer variation.

The subjects' secretor status for blood group substance was determined by staining the gastric surface mucous cells in the tissue sections with monoclonal antibodies against blood group A, B, and H antigens (Table 2), as previously reported [9, 16]. In secretors the surface mucous cells express ABH blood group substances; in nonsecretors these substances are not expressed.

The Mann-Whitney U test was used to compare the staining scores for carbohydrate antigens of normal volunteers in the antrum and corpus and to compare the staining scores for carbohydrate antigens of normal volunteers and those of patients. Because of ethnic differences in blood-group-related antigens bearing type 1 chain backbone [11, 12], only the staining scores of simple mucin-type antigens were compared when we assessed the staining scores of the carbohydrate antigens in normal volunteers and patients. The Wilcoxon signed-rank test was used to compare the scores before and after treatment. Staining scores are nonparametric and are thus presented as median rather than mean values. Nonsignificant results are summarized in Table 3.

Results

All 8 normal volunteers were secretors. Among the patients with *H. pylori*, 14 were secretors and 6 were nonsecretors (Table 1).

Before treatment of *H. pylori*, every biopsy specimens from the *H. pylori*-infected patients showed chronic active gastritis in which there were various degree of neurophil infiltration in addition to mononuclear cell infiltration. After treatment of *H. pylori*, neurophils disappeared completely, but mononuclear cell infiltration still persisted in biopsy speicmens, albeit with lower density.

Tn, sialyl Tn and T antigens showed diffuse cytoplasmic staining in the surface mucous cells both in the antrum and in the corpus (Figs. 3). Tn antigen was also detected in the supranuclear regions of the surface mucous cells, mucous neck cells, and pyloric gland cells (Fig. 3). The staining scores of Tn and sialyl Tn antigen were significantly higher in the antrum than in the corpus ($P<0.01$) (Table 3, Fig. 3).

Type 1 chain precursor, Lewis^a and sialyl Lewis^a were demonstrated as a diffuse cytoplasmic staining in the surface mucous cells in 1 of 8 biopsy specimens both in the antrum and in the corpus. In this case, which was positive for both Lewis^a and sialyl Lewis^a, Lewis^b was negative. Lewis^b was demonstrated as a diffuse cytoplasmic staining in the surface mucous cells in 7 of 8 biopsy specimens from both the antrum and the corpus.

The localization of simple mucin-type carbohydrate antigens and blood-group related antigens bearing type 1 chain backbone in *H. pylori*-infected subjects was the same as in the normal volunteers (Figs. 4–6).

The staining scores for all three simple mucin-type carbohydrate antigens did not differ significantly between secretor patients and nonsecretor patients (Table 3).

Before treatment the staining scores for both Tn and sialyl Tn antigens were significantly lower than those found in normal volunteers either in the antrum or in the corpus ($P<0.01$; Table 3, Fig. 4A).

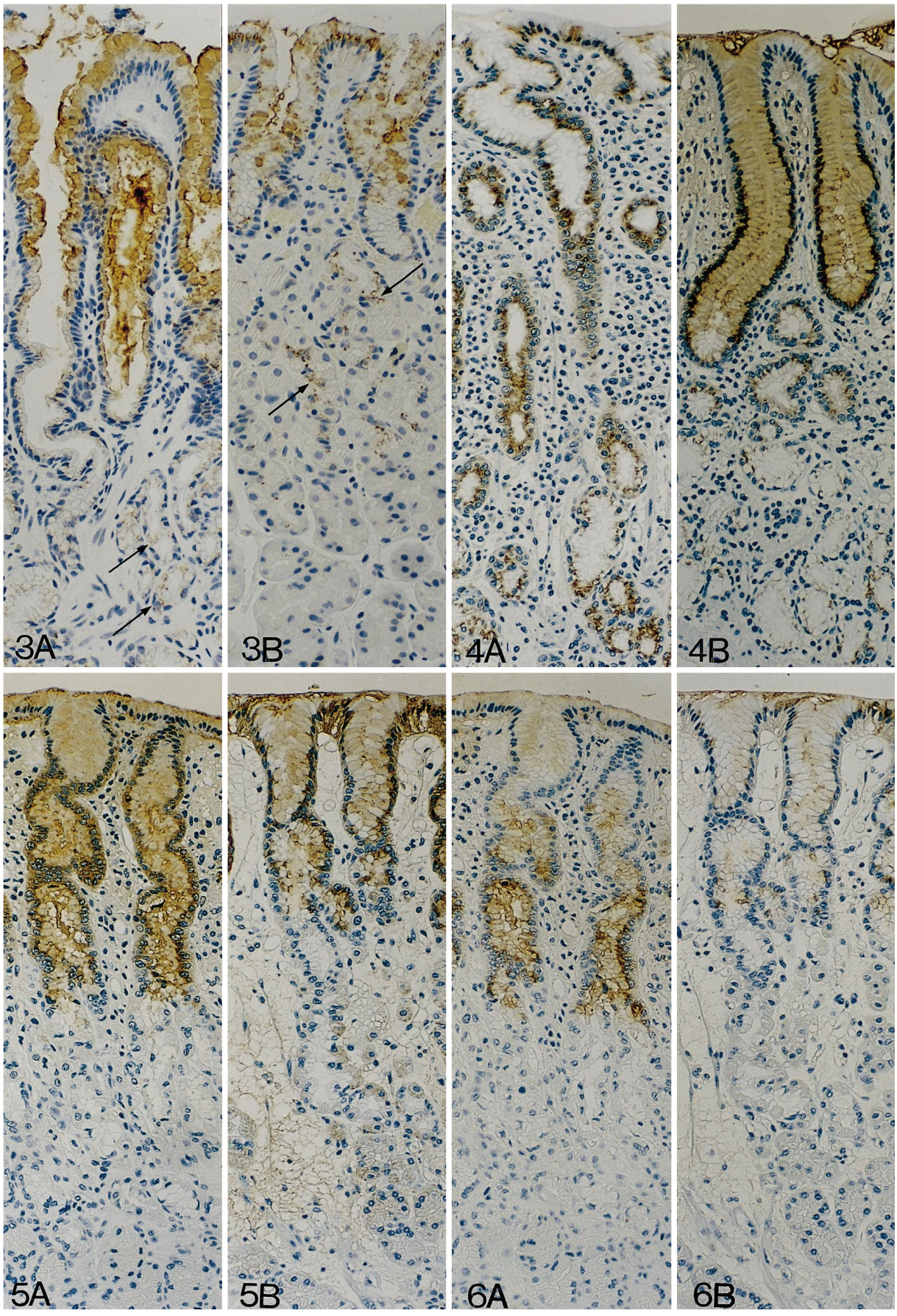
After treatment, the staining scores for Tn and sialyl Tn antigens were significantly increased and restored toward the normal pattern in the antrum, in both secretors

Fig. 3 Expression of Tn antigen in the gastric mucosa **A** in the antrum and **B** in the corpus of a normal volunteer. Gastric surface mucous cells show immunoreactivity for Tn antigen, and its degree of expression is higher in the antrum (**A**) than in the corpus (**B**). Tn is also localized at the supranuclear region (Golgi region) of the pyloric gland cells and mucous neck cells (*arrows*). Immunostaining with HB Tn-1, original magnification ×50 ▶

Fig. 4 Expression of Tn antigen in the pyloric mucosa of a patient infected with *H. pylori* **A** before and **B** after treatment. Before treatment (**A**) Tn is localized at the supranuclear region (Golgi region) of the surface mucous cells and pyloric gland cells. After treatment (**B**) mucus of surface mucous cells expresses immunoreactivity for Tn antigen. Immunostaining with HB Tn-1, original magnification ×50

Fig. 5 Expression of Lewis^a antigen in the fundic mucosa of a *H. pylori*-infected patient **A** before and **B** after treatment. The surface mucous cells express strong immunoreactivity for Lewis^a antigen irrespective of treatment. Immunostaining with T174, original magnification ×50

Fig. 6 Expression of sialyl Lewis^a antigen in the fundic mucosa of a *H. pylori*-infected patient **A** before and **B** after treatment. Before treatment (**A**, serial section from same block as depicted in Fig. 5A) the surface mucous cells express strong immunoreactivity for sialyl Lewis^a antigen. After treatment (**B**, serial section from same block as depicted in Fig. 5B) the surface mucous cells lose immunoreactivity for sialyl Lewis^a antigen. Immunostaining with 1116-ns-19-9, original magnification ×50


3A
3B
4A
4B
5A
5B
6A
6B

($P<0.01$) and nonsecretors ($P<0.05$; Table 3, Fig. 4B); in the corpus only the staining score for sialyl Tn antigen showed a significant increase and recovery toward the normal pattern in both secretors ($P<0.05$) and nonsecretors ($P<0.05$) (Table 3).

Specimens positive for type 1 chain precursor were also positive for Lewis^a, and specimens positive for sialyl Lewis^a were also positive for Lewis^a in all biopsy specimens before and after treatment in both secretors and nonsecretors. The staining scores for all these three blood-group-related antigens bearing type 1 chain backbone were signigficantly higher in nonsecretor patients before and after treatment (Table 3). Lewis^b was positive in all specimens in both secretors and nonsecretors (Table 3).

After treatment the staining scores for type I chain precusor and Lewis^a had not changed significantly (Table 3, Fig. 5), but the staining scores for sialyl Lewis^a had decreased significantly in nonsecretors (Table 3, Fig. 6) and the staining scores for Lewis^b had decreased significantly in both secretors and nonsecretors (Table 3).

Discussion

The histochemical data obtained in this study indicate that the expression of carbohydrate antigens in the gastric surface mucous cells is altered by infection with *H. pylori*. Our results also show that the pattern of expression of carbohydrate antigens in the gastric surface mucous cells differs between the antrum and the corpus.

Tn and sialyl Tn antigens showed antrum-predominant expression in normal volunteers. This finding is consistent with the report that in pig gastric mucins the average length of oligosaccharide chains is shorter in the antrum than in the corpus, and the weight ratios of carbohydrate to protein are lower in the antrum than in the corpus [18]. These findings suggest that mucous glycoproteins may be less highly glycosylated in the antrum than in the corpus. Considering that oligosaccharide chains play important roles in the protective functions of the gastric mucins [25, 29], it is likely that the mucous gel layer covering the gastric mucosa of corpus and antrum may have different levels of protective ability, or may have different functions.

After treatment for *H. pylori*, the decreased expression of Tn and sialyl Tn antigens in gastric mucosa of *H. pylori*-infected patients increased to almost normal levels. This could explain the inconsistent findings when the possible influence of mucosal inflammation on the expression of these antigens in apparently normal gastric mucosa and chronic gastritis was not taken into account [4, 5, 13]. The lower degree of expression of Tn and sialyl Tn antigens in *H. pylori*-infected patients may be explained either by increased glycosylation, leading to the masking of precursor antigens (Fig. 1), or by suppression of the biosynthesis of Tn antigen, leading to underglycosylation of the mucin apoprotein. The presence of type 1 chain carbohydrates in *H. pylori*-infected patients supports the increased glycosylation. Since oligosaccharide chains contribute to the viscosity of mucins [20, 24], an increase in glycosylation of gastric apomucins would coincide with the increased viscosity of gastric mucin in *H. pylori*-infected stomach, as reported previously [8, 15]. This could also explain why *H. pylori* has been shown to have different effects on mucin viscosity in vitro [23] and in vivo [15]. To clarify the change in the expression of Tn antigen, it will be necessary to perform biochemical analysis of oligosaccharide chains of gastric mucins and to measure the activity of αGalNAc-transferase, which forms Tn antigen [3] (Fig. 1).

We confirmed the increased expression of Lewis^b in the *H. pylori*-infected gastric mucosa [3], a phenomenon consistent with a possible role for Lewis^b in *H. pylori* colonization [2]. Although Lewis^b has been reported to be negative in nonsecretor stomachs in Caucasian subjects [6], in our study Lewis^b was expressed in the foveolar epithelium of Japanese nonsecretors. The similar ethnic differences in the expression of blood group antigens have been reported in the salivary gland cells. In a study by Tanegashima et al. [26], expression of the type 1 blood group antigens was present in salivary mucous cells from Japanese nonsecretors, but absent from the corresponding cells obtained from German nonsecretors [26]. The expression of Lewis^b in Japanese nonsecretors may be related to the fact that Japanese nonsecretors are reported to be homozygous for a missense allele-secretor gene, which encodes α(1,2)-fucosyl-transferases with 2–3% of the activity of the wild type [12]. In contrast, the Caucasian nonsecretors are homozygous for an enzyme-inactivating nonsense allele-secretor gene [11]. Thus, mutant secretor gene (*se*) in the Japanese could synthesize type 1 group H substance, which is an acceptor for Le enzyme. Since Lewis^b is one of the putative receptors for *H. pylori* expressed on gastric surface mucous cells [2], ethnic groups with the missense allele of the secretor gene might be more susceptible to *H. pylori* infection than those with the nonsense allele.

The expression of sialyl Lewis^a was significantly increased in the *H. pylori*-infected gastric mucosa. The biosynthesis of sialyl Lewis^a requires the addition of sialic acid by α2,3-sialyltransferase to the type 1 chain precursor to synthesize sialyl Lewis^c and the subsequent addition of fucose by *Le* gene-encoded α1,4-fucosyltransferase (Le enzyme) to sialyl Lewis^c [30] (Fig. 2). However, Lewis^a is synthesized by the addition of fucose to type 1 precursor by Le enzyme [30] (Fig. 2). The pattern of the expression of Lewis^a did not change following successful treatment of *H. pylori* infection; this suggests that the activity of *Le* gene-encoded α1,4-fucosyltransferase is probably stable. We speculate that the increased expression of sialyl Lewis^a in the *H. pylori*-infected mucosa may be related to an increase in the activity of α2,3-sialyltransferase. This hypothesis is supported by the biochemical finding that the expression of sialyl Lewis^a in both normal and carcinomatous tissue of stomach and colon is not completely regulated by *Le* gene-encoded α1,4-fucosyltransferase [7]. In addition, in a colon

cancer cell line, TNFα has been reported to increase the activity of α2,3-sialyltransferase [14], and TNFα has also been reported to be increased in *H. pylori*-infected gastric mucosa [31]. Thus, TNFα may also increase the activity of α2,3-sialyltransferase in gastric surface mucous cells of *H. pylori*-infected gastric mucosa. Similar mechanisms may be involved in the regulation of expression of simple mucin-type glycoproteins.

Analysis of the expression of Lewis^a and sialyl Lewis^a in the gastric mucosa of normal appearance has yielded inconsistent results. Some authors reported the Lewis^a and sialyl Lewis^a were present only in nonsecretor patients [9, 16], while others found the expression of these antigens not only in nonsecretors but also in secretors [19, 27, 28]. To explain the latter findings Sakamoto et al. speculated that secretors who were heterozygous for dimorphic secretor genes (*Se*/*se*), had a lower activity of the α1,2-fucosyltransferases in the gastric mucosa [28]. In our study, Lewis^a and sialyl Lewis^a antigens were detected in both secretors and nonsecretors. In our secretor subjects who expressed Lewis^a and sialyl Lewis^a, type 1 chain precursor antigen was also detected. This pattern of co-expression of type 1 chain precursor antigen with Lewis^a and sialyl Lewis^a is similar to the pattern seen in nonsecretors. This finding supports the hypothesis that α1,2-fucosyltransferases has lower activity in heterozygous secretors (*Se*/*se*).

In summary, *H. pylori* infection causes distinct alterations in the pattern of glycosylation of gastric mucus glycoproteins which might change the protective function of gastric surface mucous gel layer against noxious agents. These alterations are completely reversed when of the infection is cured. Further investigation of the activity of glycosyltransferase may provide useful insights into the regulatory mechanisms of gastric mucous glycoproteins; in addition, it may contribute to expanding our understanding of the pathogenesis of *H. pylori* infection.

References

1. Allen A, Hutton DA, Leonard AJ, Person JP, Seller LA (1986) The role of mucins in the protection of the gastroduodenal mucosa. Scand J Gastroenterol [Suppl] 125:71–77
2. Boren T, Falk P, Roth KA, Larson G, Normark S (1993) Attachment of *Helicobacter pylori* to human gastric epithelium mediated by blood group antigens. Science 262:1892–1895
3. Byrd JC, Yan P, Sternberg L, Yunker CK, Scheiman JM, Bresalier RS (1997) Abberant expression of gland-type gastric mucin in the surface epithelium of Helicobacter pylori-infected patients. Gastroenterology 113:455–464
4. Carneiro F, Santos L, David L, Dabelsteen E, Clausen H, Sobrinho-Simoes M (1994) T (Thomsen-Friedenreich) antigen and other simple mucin-type carbohydrate antigens in precursor lesions of gastric carcinoma. Histopathology 24:105–13
5. David L, Nesland JM, Clausen H, Carneiro F, Sobrinho-Simoes M (1992) Simple mucin-type carbohydrate antigens (Tn, sialosyl-Tn and T) in gastric mucosa, carcinomas and metastases. APMIS Suppl 27:162–72
6. Davidson JS, Triadafilopoulos G (1992) Blood group-related antigen expression in normal and metaplastic human upper gastrointestinal mucosa. Gastroenterology 103:1552–1561
7. Dohi T, Hashiguchi M, Yamamoto S, Morita H, Oshima M (1994) Fucosyltransferase-producing sialyl Lewis^a and sialyl Lewis^x carbohydarate antigen in benign and malignant gastrointestinal mucosa. Cancer 73:1552–1561
8. Graham DY, Anard BS (1995) Gastric mucus viscosity and *Helicobacter pylori*. Gut 37:590
9. Hirohashi S, Shimosato Y, Ino Y, Tome Y, Watanabe M, Hirota T, Itabashi M (1984) Distribution on blood group antigens and CA 19–9 in gastric cancers and non-neoplastic gastric mucosa. Gann 75:540–547
10. Kawano S, Tsujii M, Nagano K, Ogihara T, Tanimura H, Hayashi N, Ito T, Sato N, Kamada T, Tamura K, Tanaka M (1990) Different effect of *Helicobacter pylori* on the human gastric antral and body mucosal intracellular mucin. Scand J Gastroenterol 25:997–1003
11. Kelly RJ, Rouquie RS, Giorgi D, Lennon GG, Lowe JB (1995) Sequence and expression of a candidate for the human secretor blood group α1,2-fucosyltransferase gene (FUT2). Homozygosity for an enzyme-inactivating nonsense mutation commonly correlates with the non-secretor phenotype. J Biol Chem 270:4640–4649
12. Kudo T, Iwasaki H, Nishihara S, Ando T, Narimatsu I, Narimatsu H (1996) Molecular genetic analysis of the human Lewis histo-blood group system. II. Secretor gene inactivation by a novel single missense mutation A385 T in Japanese nonsecretor individuals. J Biol Chem 271:9830–9837
13. Kushima R, Jancic S, Hattori T (1993) Association between expression of sialosyl-Tn antigen and intestinalization of gastric carcinomas. Int J Cancer 55:904–908
14. Majuri ML, Niemela R, Tiisala S, Renkonen O, Renkonen R (1995) Expression and function of α2,3-sialyl and α1,3/1,4-fucosyltransferases in colon adenocarcinoma cell lines: role in synthesis of E-selectin counter-receptors. Int J Cancer 63: 551–559
15. Markesich D, Anand B, Lew G, Graham D (1995) *Helicobacter pylori* infection dose not reduce the viscosity of human gastric mucus gel. Gut 36:327–329
16. Murata K, Egami H, Shibata Y, Sakamoto K, Misumi A, Ogawa M (1992) Expression of blood group-related antigens, ABH, Lewis^a, Lewis^b, Lewis^x, Lewis^y, CA19-9, and CSLEX1 in early cancer, intestinal metaplasia, and uninvolved mucosa of the stomach. Am J Clin Pathol 98:67–75
17. Nakajima S, Krishnan B, Ota H, Segura AM, Hattori T, Graham DY, Genta RM (1997) Mast cell involvement in gastritis with or without *Helicobacter pylori* infection. Gastroenterology 113:746–754
18. Ohara S, Ishihara K, Hotta K (1993) Regional differences in pig gastric mucins. Comp Biochem Physiol [B] 106:153–158
19. Sakamoto S, Watanabe T, Tokumaru T, Takagi H, Nakazato H, Lloyd KO (1989) Expression of Lewis^a, Lewis^b, Lewis^x, Lewis^y, siayl-Lewis^a, and sialyl-Lewis^x blood group antigens in human gastric carcinoma and in normal gastric tissue. Cancer Res 49:745–752
20. Sellers LA, Allen A (1988) Mucus glycoprotein gels. Role of glycoprotein polymeric structure and carbohydrate side-chains in gel-formation. Carbohydr Res 178:93–110
21. Shimizu T, Akamatsu T, Ota H, Katsuyama T (1996) Immunohistochemical detection of *Helicobacter pylori* in the surface mucous gel layer and its clinicopathological significance. *Helicobacter* 1:197–206
22. Shimizu T, Akamatsu T, Sugiyama A, Ota H, Katsuyama T (1996) *Helicobacter pylori* and the surface mucous gel layer of the human stomach. *Helicobacter* 1:207–218
23. Slomiany BL, Slomiany A (1992) Mechanism of Helicobacter pylori pathogenesis: focus on mucus. J Clin Gastroenterol 14 [Suppl 1]:S114–121
24. Slomiany BL, Sarosiek J, Slomiany A (1987) Role of carbohydrates in the viscosity and permeability of gastric mucin to hydrogen ion. Biochem Biophys Res Commun 142:783–790
25. Strous GJ, Dekker J (1992) Mucin-type glycoproteins. Crit Rev Biochem Mol Biol 27:57–92

26. Tanegashima A, Nishi K, Fukunaga T, Rand S, Brinkman B (1996) Ethnic differences in the expression of blood group antigens in the salivary gland secretory cells from German and Japanese non-secretor individuals. Glycoconj J 13:537–545
27. Torrado J, Blasco E, Cosme A, Gutierrez-Hoyos A, Arenas JI (1989) Expression of type 1 and type 2 blood group-related antigens in normal and neoplastic gastric mucosa. Am J Clin Pathol 91:249–254
28. Torrado J, Blasco E, Gutierrez-Hoyos A, Cosme A, Lojendio M, Arenas JI (1990) Lewis system alterations in gastric carcinogenesis. Cancer 66:1769–1774
29. Varki A (1993) Biological roles of oligosaccharides: all of the theories are correct. Glycobiology 3:97–130
30. Watkins WM (1995) Molecular basis of antigenic specificity in the ABO, H and Lewis blood-group systems. In: Montreuil J, Vliegenthart JFG, Schachter H (eds) Glycoproteins. Elsevier, New York, pp 313–390
31. Yamaoka Y, Kita M, Kodama T, Sawai N, Imanishi J (1996) *Helicobacter pylori cagA* gene and expression of cytokine messenger RNA in gastric mucosa. Gastroenterology 110: 1744–1752