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The aberrant expression of Lewis a antigen in intestinal metaplastic cells of gastric mucosa is caused by augmentation of Lewis enzyme expression

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Immunohistochemical staining showed an aberrant expression of Le^a antigen in the intestinal metaplastic glands of the gastric mucosa of secretors, as reported by others. In this study, we have demonstrated for the first time that the Lewis enzyme is well colocalized with Le^a antigen, indicating that the Lewis enzyme is responsible for Le^a antigen synthesis in the gastric mucosa. The staining intensity of the Lewis enzyme was much stronger in the cells with intestinal metaplasia than the cells without metaplasia, regardless of the secretor status. The amount of transcript of the *Lewis* gene was related to the degree of metaplasia; *i.e.,* the more severe the metaplastic change was, the more abundantly the transcripts of the *Lewis* gene were expressed. This augmentation of the Lewis enzyme in metaplastic tissues was also confirmed by Western blotting analysis using a specific antibody against the Lewis enzyme. We conclude that intestinal metaplastic change of gastric mucosa is usually accompanied by a marked augmentation of the Lewis enzyme expression, which results in the enhanced expression of Le^a antigens, particularly in secretors.

***Keywords*: intestinal metaplasia, gastric mucosa, stomach, Lewis antigen, Lewis enzyme, FUT3, Fuc-TIII**

***Abbreviations*: Le, Lewis; Le^a, Lewis a; Le^b, Lewis b; sLe^a, sialyl Lewis a; Se, secretor; PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism; competitive RT-PCR, competitive reverse transcription-polymerase chain reaction.**

Introduction

Based on Lauren's classification, gastric cancer is histologically divided into two types, diffuse and intestinal-type gastric cancers [1, 2]. Several immunohistochemical studies of the expression of blood group-related antigens such as ABH and Lewis antigens in gastric mucosa with intestinal metaplasia and intestinal-type cancer have revealed a high incidence of the aberrant expression of type 1 Lewis antigens, a very faint expression of type 2 Lewis antigens (which are sometimes focally expressed), and the deletion of ABH antigens [3–10].

Recent progress in the molecular cloning of glycosyltransferase genes has enabled us to examine, on the molecular level, which glycosyltransferases are synthesizing carbohydrate antigens in native tissues. We have been investigating the glycosyltransferases responsible for the expression of type 1 Lewis antigens, such as Lewis a (Le^a), Lewis b (Le^b) and sialyl Lewis a (sLe^a), on erythrocytes and in the human colon [11–13]. Based on the findings of null alleles of *Lewis* (*Le*) and *secretor* (*Se*) genes in previous studies [11, 14, 15], we have demonstrated that the Lewis (Le) enzyme (FUT3; Fuc-TIII) encoded by the *Le* gene is solely responsible for the expression of type 1 Lewis antigens not only on erythrocytes but also in the epithelial cells of the colon [11, 12]. Regarding Le^b expression, the secretor (Se) enzyme encoded by the *Se* gene was confirmed to be exclusively responsible for Le^b expression on erythrocytes and for the secretor status, which is defined by the presence of ABH antigens in saliva [13, 16]. However, Le^b expression in the colon was found to be not as simple as that on erythrocytes and in the saliva, since two α1,2 fucosyltransferases, the H and Se

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enzymes [16, 17], were found to be involved in α1,2 fucosylation resulting in Le^b synthesis in the colon (data will be published elsewhere).

Accumulated data on the null alleles of the *Le* and *Se* genes revealed that the point mutations inactivating those genes seem to be ethnic group-specific, since the null *Le* and *Se* alleles distributed in Japanese populations were hardly ever found in Caucasian populations [11, 13, 16, 18, 19]. By an analysis of more than 600 Japanese samples, we recently determined almost all of the predominant *Le* and *Se* alleles distributed in the Japanese population. The *Le* alleles could be divided into four kinds, one active allele (*Le*), *Le*, and three inactive alleles (*le*), *le1*, *le2* and *le3* [20]. The *Se* alleles were divided into four kinds, two active alleles (*Se*), *Se1* and *Se2*, and two inactive alleles (*se*), *sej* and *se5* [11, 13, 18]. The frequencies of *le3* and *se5* were found to be very low in the Japanese population [20].

We have established polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) methods to identify the null alleles of the *Le* and *Se* genes by detecting point mutations [11–13, 20]. With these PCR-RFLP methods, we can determine the *Le* and *Se* genotypes of individuals.

A competitive reverse transcription-polymerase chain reaction (competitive RT-PCR) method was also established to measure the transcripts for α1,3 fucosyltransferase genes according to the method of Sasaki *et al.* [21]. This method enabled us to distinguish the highly homologous genes, the genes for α1,3 fucosyltranferases including the Le enzyme, and to measure the transcripts in a small amount of RNA.

A monoclonal antibody named FTA1-16 that specifically recognizes the Le enzyme has been established [22]. FTA1-16 is able to stain the Le enzyme immunohistochemically and to detect it by Western blotting analysis.

In the present study, we used the above-mentioned methods to examine the molecular basis of the aberrant expression of Le^a antigen in gastric mucosa with intestinal metaplasia. We found that the expression of the Le enzyme was markedly augmented in the cells with intestinal metaplasia, resulting in the aberrant expression of Le^a antigen.

Materials and methods

Gastric mucosa samples.

Gastric mucosa tissue from thirty individuals was collected as biopsy samples when a gastric endoscopy was done as part of a routine examination for gastric diseases. Non-neoplastic gastric mucosa tissue from six patients with gastric cancer was collected at surgical resection. A normal duodenum sample was obtained from one patient (patient No. 6 in Table 2 and Figures 2 and 3).

The samples were histologically analyzed by hematoxylin-eosin staining to determine the degree of intestinal metaplasia. We classified the samples into four categories depending on the percentage of metaplastic cells, $-$, $+$, $++$ and $+++$ indicating that 0%, 0–50%, 50–90% and 90–100% of the gastric epithelial cells had become metaplastic, respectively.

Immunohistochemical analysis of Le^a antigen and the Le enzyme

Two monoclonal antibodies (MAbs), anti-Le^a (7LE) [9, 23] and anti-human Le enzyme (FTA1-16) [22], were used for immunohistochemical analysis.

All specimens were fixed in 10% formaldehyde and embedded in paraffin. Deparaffinized 4 μm sections were washed in phosphate-buffered saline (PBS) three times and treated with 0.3% (v/v) H_2O_2 in methanol for 15 min to block endogenous peroxidase. The sections were again washed in PBS three times and then incubated for 20 min with 0.5% normal swine serum in PBS at room temperature to avoid nonspecific staining. Antigen detection was carried out by applying the primary monoclonal antibody at a concentration of $10\,\mu g\,ml^{-1}$ for 12 h at room temperature, followed by the streptavidin-biotin complex method (Elite ABC Kit; Vectastain, Vector Labs., Burlingame, CA, USA). The glass slides were washed with PBS between each step, and $0.1\,mg\,ml^{-1}$ of DAB 4HCl (Dojin, Kumamoto, Japan) in 0.1 M Tris-HCl buffer (pH 7.6) was used for the peroxidase reaction. Nuclei were counterstained with Mayer's hematoxylin.

Genotyping of *Le* and *Se* genes

The *Le* and *Se* genotypes of the above 36 individuals were determined by PCR-RFLP methods using genomic DNAs extracted from peripheral blood leukocytes. The detailed methods for *Le* and *Se* genotyping, by detection of three missense mutations (T59G, G508A and T1067A) in the *Le* gene and of two missense mutations (C357T and A385T) in the *Se* gene, were described previously [11–13, 20].

Western blotting analysis

The tissues were solublized in HEPES buffer (pH 7.2) containing 2% Triton X-100 by brief sonication. After determination of the protein concentration using the Micro BCA Protein Assay Reagent Kit (Pierce, Rockford, IL, USA), samples were finally suspended in Laemmli's sample buffer. Thirty micrograms of the solubilized protein from each sample was heated at 100 °C for 5 min and subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Then, separated proteins were transferred to an Immobilon PVDF membrane (Millipore, Bedford, MA, USA) by Transblot SD cells (Bio-Rad, Richmond, CA, USA). The membrane was blocked with PBS containing 5% skim milk at 4 °C overnight and then incubated with 7LE (anti-Le^a) or FTA1-16 (anti-Le enzyme). After being washed three times with PBS containing 0.01% Tween 20, the membranes were incubated with horseradish peroxidase (HRP)-conjugated anti-mouse IgG or anti-mouse IgM in

PBS containing 0.01% Tween 20. The detection of HRP was carried out with enhanced chemiluminescence (ECL) Western blotting detection reagents (Amersham, Buckinghamshire, UK). The exposed film (Hyper film TM-ECL, Amersham) was scanned with a Flying Spot Scanner CS9000 (Shimadzu, Tokyo, Japan) to quantify the intensities of the positive bands.

Measurement of transcripts of the *Le* gene by competitive RT-PCR

The *Le* gene was cloned in a pBluescript SK (-) vector in our previous study [11]. The cloned DNA encoding the full-length open reading frame (ORF) of the *Le* gene was used as a standard DNA and also served for the subsequent construction of a competitor DNA to be used for the competitive RT-PCR method. The plasmid containing the human *β-actin* gene insert and the competitor DNA of the human *β-actin* gene were kind gifts from Kyowa Hakko Inc. (Tokyo, Japan) [21]. A competitor DNA plasmid of the *Le* gene was prepared by deleting the 172 bp *Tth111* I-*Mbo* II fragment from the full-length *Le* gene plasmid. The competitor DNA plasmids of the *Le* and *β-actin* genes were used for quantification of the respective mRNAs by the competitive RT-PCR method.

Total cellular RNA was isolated from the gastric mucosa tissues by the acid guanidium thiocyanate-phenol-chloroform method [24]. The RNA solubilized was treated with DNase I (GIBCO-BRL Life Technologies, Rockville, MD) to avoid the contamination by genomic DNA. Complementary DNAs were synthesized with oligo(dT) primer from 5 μg of the total RNA in a total volume of 20 μl reaction mixture using the SUPERSCRIPT™ Preamplification System for First Strand cDNA Synthesis (GIBCO-BRL). After cDNA synthesis, the reaction mixture was diluted 50-fold with H_2O.

The competitive RT-PCR for the *Le* gene was performed with ampli Taq Gold (Roche Molecular Systems, NJ, USA) in a total volume of 50 μl containing 10 μl of standard plasmid DNA or sample cDNA, 10 μl of competitor DNA which contained 2 fg of the DNA and 0.2 μM of the primer set, a forward primer, 5′-CCTCCCGACAGGACACCACTCC-3′ and a reverse primer, 5′-GCGTCCGTACACGTCCACCTTG-3′. The PCR buffer contained 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2.5 mM $MgCl_2$, 0.2 μM of each dNTP and 0.1% Triton X-100. After preheating for 10 min at 94 °C, 35 cycles of PCR were run for 1 min at 94 °C, 1 min at 60 °C as an annealing temperature, and 2 min at 72 °C. The competitive RT-PCR for the *β-actin* gene was carried out by almost the same method as that used for the *Le* gene, except for the amount of competitor DNA (2 pg), the number of PCR cycles (25 cycles) and the primer set, a forward primer, 5′-GATATCGCCGCGCTCGTCGTCGAC-3′ and a reverse primer, 5′-CAGGAAGGAAGGCTGGAAGAG TGC-3′.

To establish standard curves for transcripts of the *Le* gene and the *β-actin* gene, competitive RT-PCR was performed at the fixed optimal concentration of 2 fg for the *Le* gene and 2 pg for the *β-actin* gene in 50 μl reaction volume of PCR, of the competitor DNA and various concentrations of the respective standard DNA. A 10 μl aliquot, after the competitive RT-PCR, was electrophoresed in a 1% agarose gel and the bands were observed after ethidium bromide staining. Competitive RT-PCR with the optimal competitor DNA concentration gave rise to two bands. The intensity of the amplified fragments was quantified by scanning the positive pictures with the use of the NIH Image system. Thus, standard curves for transcripts of the *Le* gene and *β-actin* gene were obtained and used for the subsequent quantification of the transcripts in cDNA samples of gastric mucosa tissues. To normalize the efficiency of cDNA preparation from total RNAs, each value of the transcripts for the *Le* gene and the *β-actin* gene was plotted on the respective standard curve to obtain the actual amount of the transcript. The value of *Le* gene transcript thus obtained was divided by that of the *β-actin* transcript for normalization.

Results

Le and *Se* genotyping of thirty individuals whose gastric mucosa samples were obtained by biopsy, and histochemical and immunohistochemical studies of the samples.

We first determined the *Le* and *Se* genotypes of the 30 individuals whose gastric mucosa samples were collected by biopsy. Among the 30 individuals, there were three Lewis-negative individuals whose genotype was determined to be *le*/*le*. The gastric mucosa samples of the *le*/*le* individuals were devoid of any positive staining with anti-Le^a, Le^b and sLe^a antibodies (data not shown). Therefore, the three Lewis-negative individuals were excluded from the subsequent analyses in which we examined the correlation between the Le^a expression and the Le enzyme. The results of the immunohistochemical analysis of the remaining 27 Lewis-positive individuals are summarized in Table 1. Ten of these individuals were found to be homozygotes for the inactive *Se* allele, *sej*/*sej*, and to be nonsecretors, whereas the other 17 individuals were found to be secretors possessing at least one of the active *Se* alleles. Information about the biopsy samples, the age and sex of the subjects and their genotypes, are listed in Table 1. All biopsy samples were examined histochemically for the presence of intestinal metaplasia by hematoxylin and eosin staining. The samples of six secretors, Nos. 1–6 in Table 1, and four nonsecretors, Nos. 18–21, were found to show intestinal metaplasia mainly in the antrum and fornix portions. We did not find metaplastic change in the other 17 individuals (Nos. 7–17 and 22–27). The immunohistochemical staining with FTA1-16 (anti- Le enzyme) showed a good correlation with the metaplastic cells; *i.e.*, the Le enzyme was strongly stained in

Table 1. The age, sex, *Se* genotype, intestinal metaplastic change and Le enzyme staining of Lewis-positive subjects whose samples were collected by endoscopical biopsy

No.	*Age*	*Sex*	*Se genotype*	*Intestinal metaplasia*			*Positive staining of the Le enzyme with FTA1-16*		
				Fornix	*Corpus*	*Antrum*	*Fornix*	*Corpus*	*Antrum*
1	85	M	*Se/Se*	+	−	+	+	−	+
2	64	M	*Se/Se*	−	−	+	−	−	+
3	62	M	*Se/Se*	−	−	+	−	−	+
4	79	M	*Se/sej*	+	NT	NT	+	NT	NT
5	62	M	*Se/Se*	+	−	+	+	−	+
6	67	M	*Se/sej*	+	+	+	+	+	+
7	68	M	*Se/Se*	−	−	−	−	−	−
8	54	F	*Se/Se*	−	−	−	−	−	−
9	34	M	*Se/Se*	−	−	−	−	−	−
10	29	M	*Se/Se*	−	−	−	−	−	−
11	67	M	*Se/Se*	−	NT	NT	−	NT	NT
12	83	M	*Se/sej*	−	−	−	−	−	−
13	65	M	*Se/sej*	−	−	−	−	−	−
14	57	M	*Se/sej*	−	−	−	−	−	−
15	54	M	*Se/sej*	−	−	−	−	−	−
16	20	M	*Se/sej*	−	−	−	−	−	−
17	65	F	*Se/sej*	−	NT	NT	−	NT	NT
18	72	M	*sej/sej*	−	−	+	−	−	+
19	69	M	*sej/sej*	−	−	+	−	−	+
20	68	F	*sej/sej*	−	−	+	−	−	+
21	29	F	*sej/sej*	−	−	+	−	−	+
22	61	F	*sej/sej*	−	−	−	−	−	−
23	54	F	*sej/sej*	−	−	−	−	−	−
24	52	F	*sej/sej*	−	−	−	−	−	−
25	46	M	*sej/sej*	−	−	−	−	−	−
26	33	M	*sej/sej*	−	−	−	−	−	−
27	77	M	*sej/sej*	−	NT	NT	−	NT	NT

NT, not tested because the samples were not obtained.

all the cells with intestinal metaplastic change (Nos. 1–6 and 18–21), while the cells without metaplastic change, for example, in the corpus portions of Nos. 1–3 and No. 5 (Table 1), were not stained.

This result strongly indicated that the cells with intestinal metaplastic change express the Le enzyme, resulting in the abberrant expression of Le^a antigens.

Colocalization of Le^a antigens with the Le enzyme and the augmented expression of the Le enzyme in intestinal metaplastic cells.

All of the biopsy samples listed in Table 1 were stained with anti-Le^a (7LE) in addition to FTA1-16. Representative results are shown in Figure 1. Serial sections of the pyloric mucosa with intestinal metaplasia of a secretor (No. 5) were stained positively both with 7LE (Figure 2) and FTA1-16 (Figure 1b), and the serial sections of a nonsecretor (No. 19) also stained positively with 7LE (Figure 1c) and FTA1-16 (Figure 1d). Regarding the secretor, No. 5, goblet cells in the metaplastic glands were mainly stained with strong intensity, and the absorptive cells in the metaplastic glands also showed positive staining at the apical surface, while foveolar cells, without metaplasia, were not stained with 7LE (Figure 1a). In contrast, both metaplastic epithelial cells and foveolar cells of the nonsecretor, No. 19, showed positive staining with 7LE, though the former cells showed stronger staining than the latter cells (Figure 1c). These staining results with anti-Le^a antibody were consistent with those of previous studies (3–10).

In this study, we first demonstrated the localization of the Le enzyme in gastric mucosa. It is noteworthy that the Le enzyme was well colocalized with Le^a antigens, especially in the secretors. As can be seen in Figure 1a and 1b, the cells stained with 7LE were also strongly stained by FTA1-16, and the Le enzyme was apparently localized at the apical

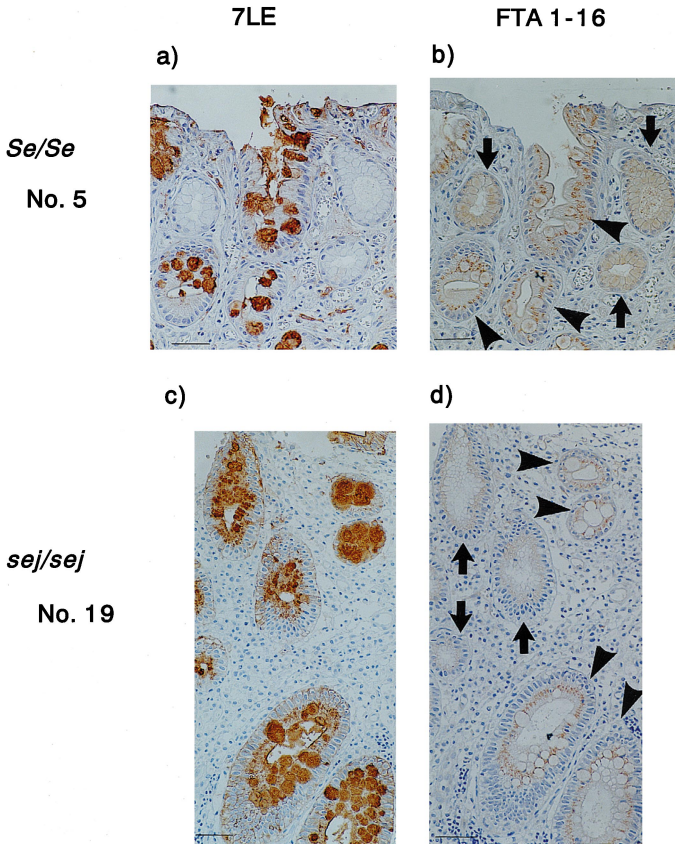

Figure 1. Immunohistochemical staining on antrum portion samples with intestinal metaplasia of a secretor (No. 5) and a nonsecretor (No. 19) with 7LE (anti-Le^a) and FTA1-16 (anti-human Le enzyme). Arrowheads indicate the intestinal metaplastic glands, and arrows indicate the non-metaplastic glands, *i.e.* the glands consisting of foveolar cells. (a) Staining with 7LE on the secretor, (b) staining with FTA1-16 on the secretor, (c) staining with 7LE on the nonsecretor, (d) staining with FTA1-16 on the nonsecretor. Bars in each figure indicate 50 μm.

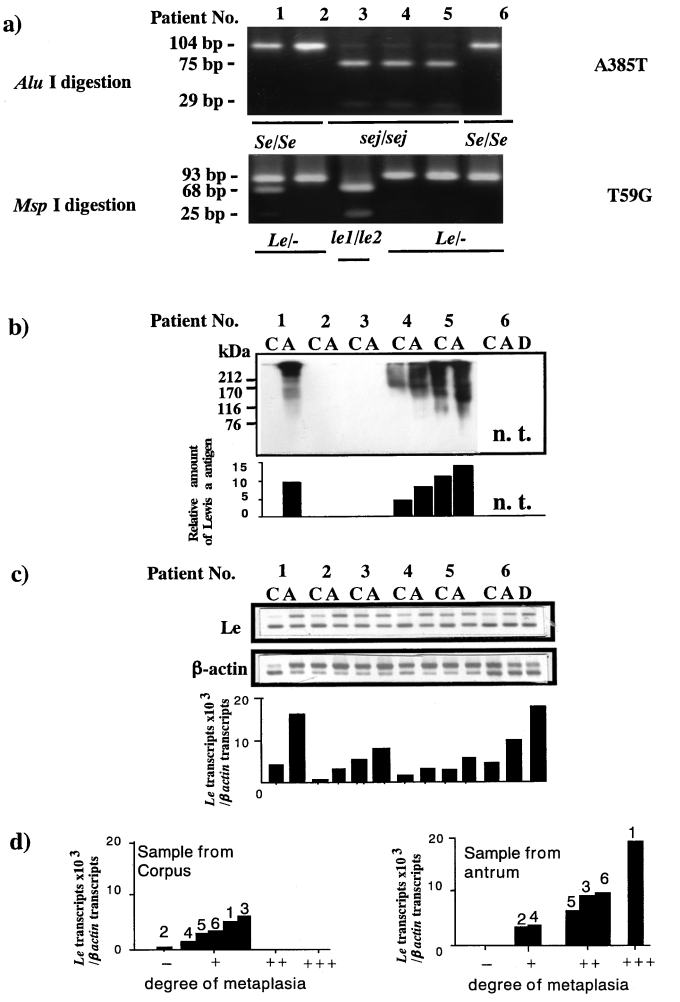

Figure 2. The numbers 1-6 in Fig. 2(a), (b), (c) and (d) indicate the patient Nos. as listed in Table 2. (a) PCR-RFLPs for the *Se* and *Le* genotyping of six patients. The upper and lower panels are the results of the *Se* and *Le* genotypings, respectively. (b) Western blotting analysis of Le^a antigens with 7LE on each portion sample of the six patients. The relative amount of Le^a antigens is presented at the bottom. C, A and D are the abbreviations of corpus, antrum and duodenum, respectively; n.t., not tested. (c) Competitive RT-PCR for the measurement of *Le* transcripts expressed in each portion (corresponding to the samples measured for the amount of Le^a antigens) of the six patients. (d) The amounts of *Le* transcript measured in (c) are arranged depending on the degree of intestinal metaplasia from – to + + + as described in Materials and Methods. The numbers at the tops of bars indicate the patient numbers.

supranuclear region, probably the Golgi region, of the cells. Interestingly, the cells in the metaplastic glands including goblet cells and absorptive cells, irrespective of the secretor status, showed a clear positive staining for the Le enzyme, while the foveolar cells showed very faint staining. Regarding the nonsecretors, the Le^a antigens and the Le enzyme were not completely colocalized; *i.e.* the foveolar cells indicated by arrows in Figure 1d were stained positively with 7LE (Figure 1c), but not with FTA1-16 (Figure 2d), although the cells in metaplastic glands indicated by arrowheads in Figure 2d were stained strongly with both 7LE and FTA1-16. However, we observed that the Le^a intensity in the metaplastic cells was stronger than that in foveolar cells, even in the nonsecretors.

These immunohistochemical results indicated that the expression of the Le enzyme is markedly augmented in cells with intestinal metaplasia, regardless of the secretor status.

Quantitative analysis of Le^a antigens and the Le enzyme expressed in corpus and antrum portions of gastric mucosa by Western blotting analysis and competitive RT-PCR

Gastric mucosa samples which did not contain cancerous tissue were collected from tissue surgically resected from six patients with gastric adenocarcinoma and subjected to

Table 2. The age, sex, *Se* and *Le* genotypes and degree of intestinal metaplasia of six patients whose samples were collected by surgical resection

Patient. No.	*Age*	*Sex*	*Se genotype*	*Le genotype*	*Intestinal metaplasia*	
					Corpus	*Antrum*
1	70	M	*Se/Se*	*Le/le2*	+	+ + +
2	44	F	*Se/Se*	*Le/Le*	−	+
3	76	M	*sej/sej*	*le1/le2*	+ +	+ +
4	31	F	*sej/sej*	*Le/Le*	+	+
5	72	M	*sej/sej*	*Le/Le*	+	+ +
6	46	M	*Se/Se*	*Le/Le*	+	+ +

The degree of metaplasia determined by microscopic observation is presented as − to + + + as described in Materials and Methods.

a quantitative analysis of the amount of transcript of the *Le* gene and the amount of the Le enzyme, for which a larger amount of mucosa tissue was required. The results of PCR-RFLP for determining the *Le* and *Se* genotypes of the six patients (Nos. 1–6) are shown in Figure 2a and summarized in Table 2. There were three secretors (Nos. 1, 2 and 6 in Table 2) and three nonsecretors (Nos. 3–5), and one of the nonsecretors was a Lewis-negative individual (*le*/*le*) (No. 3 in Table 2). The samples were subjected to immunohistochemical study, Western blotting analysis and competitive RT-PCR to measure the amount of the Le enzyme expressed. The amount of Le^a antigen on mucins was also measured by Western blotting analysis.

The degree of progression of intestinal metaplasia in each portion of the corpus or antrum of the six patients was determined by microscopic observation and is presented as $-, +, + +$ or $+ + +$.

As can be seen in Figure 2b, Le^a antigens on the mucins were detected as smear bands of high molecular weight by Western blotting analysis, when probed with 7LE. The intensity of the positive bands was measured by a densitometer, and the amount of Le^a antigen is presented at the bottom of Figure 2b. The amount of Le^a antigen in the secretors was well correlated with the degree of progression of intestinal metaplasia. Patient No. 1 (a secretor), a 70-year-old man, with intestinal metaplasia that was severe in the antrum portion but not so severe in the corpus region, showed an abundant expression of Le^a antigen on mucins in the antrum portion, but not in the corpus portion. In contrast, Patient No. 2 (a secretor) with very faint intestinal metaplasia even in the antrum portion showed no Le^a antigen in either the antrum or the corpus. Patient No. 3, a Lewis-negative patient with null *Le* alleles (*le*/*le*) , did not express Le^a antigen at all because he genetically lacks the Le enzyme. Patients Nos. 4 and 5 (nonsecretors) showed positive smear bands of Le^a antigen at similar levels between the antrum and corpus portions, though the amount of Le^a antigen in the antrum portion was slightly larger than that in the corpus portion. Figure 2c showed the results of competitive RT-PCR for the measurement of transcripts of the *Le* gene and the *β-actin* gene. The intensity of the bands was measured as described in Materials and Methods, and the amount of transcript of the *Le* gene normalized by the amount of *β-actin* transcript is presented at the bottom of Figure 2c. The expression of the *Le* gene was upregulated in the antrum portion in all six cases, regardless of the *Le* or *Se* genotype, compared to the corpus portion. In particular, a marked upregulation of the *Le* gene was observed in the antrum portion of patients with severe intestinal metaplasia (Nos. 1 and 6). The null *Le* alleles of Patient No. 3 were fully transcribed. Duodenal mucosa of patient No. 6 (a secretor) was obtained by surgical resection for gastric cancer and examined together with the samples of the corpus and antrum. The level of *Le* transcripts in the duodenal mucosa was highest in all samples examined in this experiment (Figure 2c). To determine the correlation of the *Le* transcript levels of the six patients with the degree of progression of intestinal metaplasia, the *Le* transcript levels were arranged according to the degree of metaplasia, and the results are presented in Figure 2d. The *Le* transcript levels increased with the progression of intestinal metaplasia, in both the corpus and antrum portions. The expression levels of *Le* transcripts in the corpus were lower than those in the antrum, since the corpus portions usually had less intestinal metaplasia than the antrum portions.

Though we examine only one sample of the duodenal mucosa of patient No. 6, the level of the *Le* transcripts in the antrum portion of No. 6, in whom we observed severe intestinal metaplasia, was close to that in the duodenal mucosa. Figure 3 shows the results of Western blotting analysis for the Le enzyme using FTA1-16 on the three portion samples (corpus, antrum and duodenum) from patient No. 6. The positive 45kDa band, corresponding to the size of a membrane-bound form of the Le enzyme, was detected in all samples of the three portions. The duodenal samples showed an additional lower band, which presumably corresponds to a truncated form lacking a cytoplasmic tail and a transmembrane domain. The intensity of the positive bands was measured and is presented at the bottom of Figure 3, and the amount of *Le* transcript, which was measured in the previous experiment (Figure 2c), is also shown in Figure 3. The amount of the Le enzyme, which corresponded well to the amount of the *Le* transcript, was greatest in the duodenum, and least in the corpus. The amounts of both the *Le* transcript and the Le enzyme expressed in the antrum were almost half the amounts of those expressed in the duodenum. This result strongly indicated that the level of Le enzyme expression in the antrum portion with intestinal metaplasia became similar to that in intestinal tissue, such as duodenal mucosa, resulting in the aberrant expression of Le^a antigens.

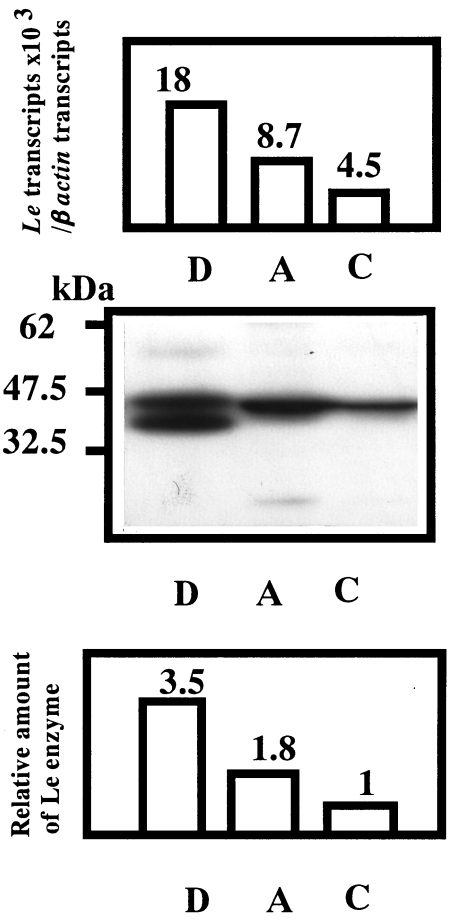

Figure 3. Western blotting analysis with FTA1-16 (anti-Lewis enzyme) of the corpus, antrum and duodenal samples of patient No. 6. The relative amounts of *Le* transcript measured in Figure 2(c), and the relative amounts of the Le enzyme determined in this Western blotting experiment, are indicated at the top and the bottom, respectively. D, A and C indicate the duodenum, antrum and corpus, respectively.

Discussion

There have been several immunohistochemical studies on the aberrant expression of Le^a antigen in the intestinal metaplastic glands in gastric mucosa, particularly in that of secretors [4, 7–9, 25, 26]. Previous immunohistochemical studies indicated that Le^b antigens are expressed in the foveolar cells of secretors but not in those of nonsecretors, while Le^a antigens are expressed in the foveolar cells of nonsecretors but not in those of secretors [4, 7–9, 25, 26]. These immunohistochemical observations suggested that the two enzymes, Le and Se, may compete for the type 1 chain substrates resulting in the synthesis of Le^a or Le^b antigens, respectively, in the foveolar cells in gastric mucosa, or that the Se enzyme is localized in a subcompartment of the Golgi aparatus prior to the Le enzyme.

In this study, we also stained all samples with an anti-Le^b antibody (data not shown). Both foveolar epithelial cells and intestinal metaplastic cells of all secretors (100%) were positively stained by anti-Le^b. About 70% of the nonsecretor samples showed positive staining with anti-Le^b regardless of the intestinal metaplasia, although it was difficult to determine the amounts of Le^b antigen by immunohistochemical analysis. The above staining results with anti-Le^b were consistent with those reported previously [26]. The molecular basis of Le^b expression in nonsecretors remains to be elucidated. We assume that Le^b expression is due to an α1,2 fucosyltransferase other than the Se enzyme, *i.e.* the H enzyme.

The aim of this study was to elucidate the molecular basis of the aberrant expression of Le^a antigens in intestinal metaplasia and to investigate the regulated expression of the Le enzyme, which is known to be responsible for the expression of type 1 Lewis antigens, Le^a, Le^b and sLe^a, in colorectal tissues [12, 20].

In our immunohistochemical study, we first demonstrated the localization of the Le enzyme at the supranuclear region of the epithelial cells of gastric mucosa; and we observed a marked enhancement of the Le enzyme expression in the cells with intestinal metaplasia: the Le enzyme was strongly stained in all cases with intestinal metaplasia, including both the biopsy samples and the surgically resected samples. This strong staining was restricted to cells in the intestinal metaplastic glands, and was not limited to the secretors; it was also observed in the nonsecretors, except for the Lewis-negative individuals (*le*/*le*) who genetically lack the Le enzyme.

The abberrant expression of Le^a antigen in intestinal metaplastic glands of the secretors was usually accompained by the enhancement of Le enzyme expression, and well colocalized with the cells in which the Le enzyme was strongly stained. As for the nonsecretors, the Le^a antigen was stained not only in the intestinal metaplastic cells but also in the foveolar cells; however, the staining intensity was slightly stronger in the former cells than in the latter cells. The expression of Le enzyme was also markedly enhanced in the intestinal metaplastic cells of the nonsecretors. The above immunohistochemical results regarding the Le^a antigen and the Le enzyme can be interpreted as follows. In the secretors, who have the active *Se* alleles, the Se enzyme competes with the Le enzyme for the acceptor substrate, the type 1 chain substrate. In the foveolar cells of the secretors, which possess a small amount of Le enzyme, the Se enzyme is more dominant than the Le enzyme, leading to H-type 1 synthesis by α1,2fucosylation, and finally resulting in Le^b synthesis. In fact, the foveolar cells of the secretors showed positive staining with anti-Le^b antibody (data not shown), as reported by others [10]. However, in the intestinal metaplastic cells, the Le enzyme expression is markedly upregulated to overcome the Se enzyme, resulting in the aberrant expression of Le^a antigens. In the cells of nonsecretors who genetically lack the Se enzyme, the small amount of Le enzyme in the fovcolar cells is enough to synthesize the Le^a antigen because of the absence of the competing Se

enzyme. Therefore, the marked enhancement of Le enzyme expression in the intestinal metaplastic cells of the nonsecretors did not have a significant influence on the increase of Le^a antigen.

Our observation of the enhanced expression of Le enzyme in the intestinal metaplastic cells in the immunohistochemical experiments was confirmed by the quantitative analyses of the *Le* transcripts and the Le enzyme by competitive RT-PCR and Western blotting, respectively. The amount of *Le* transcript increased with the progression of intestinal metaplasia. The null *Le* alleles of the Lewis-negative individual were found to be fully transcribed (Figure 2c), but Le^a antigens were not expressed at all in this patient. The immunohistochemical study of the biopsy samples of the three Lewis-negative individuals also showed no Le^a antigens with 7LE and no Le enzyme with FTA1-16 (data not shown). These results again confirmed that the Le enzyme is solely responsible for the Le^a antigen expression in the gastric mucosa, as demonstrated in the colon epithelial cells in our previous study [12]. It was also indicated that the null *Le* alleles are fully transcribed, but do not produce a functional Le enzyme residing in the Golgi apparatus. The lack of staining of Le enzyme in the tissues of Lewis-negative individuals was extensively examined in a separate study, and the results will be published elsewhere. In brief, the transcripts for null *Le* alleles seem to be fully translated, but the translated proteins cannot form a folded structure because of the amino acid substitution in the catalytic domain, and these proteins become sensitive to some protease(s) in the cells, resulting in the disappearance of the protein.

As shown in Figure 3, the antrum portion with severe intestinal metaplasia of patient No. 6 expressed almost half the amount of Le enzyme as that expressed in the duodenal mucosa. The microscopy observations revealed that the antrum portion was a mixture of metaplastic and non-metaplastic glands. If we could separately measure the Le enzyme only in the metaplastic glands, we might find that the amount is very similar to that in the duodenal mucosa.

Several studies have shown that the intestinal metaplasia of gastric mucosa is associated with some intestinal marker enzymes and marker cells [2, 27–29]. As clarified in the present study, the Le enzyme could be included in such intestinal enzyme markers for intestinal metaplasia. We do not think that only Le enzyme, among the many glycosyltransferases, is upregulated in association with intestinal metaplasia. We speculate that the intestinal metaplastic change in gastric mucosa is accompanied by an expression pattern of glycosyltransferases similar to that of intestinal mucosa. We recently determined the expression patterns of many glycosyltransferases (including 12 species), in the human colon by measuring the amounts of transcript; we also compared the patterns of normal and cancerous colon tissue (manuscript submitted). We are now examining the patterns of glycosyltransferase expression in other tissues, *i.e.* gastric mucosa, small intestine, lung and liver. We expect to determine which glycosyltransferases are upregulated and which are downregulated in association with intestinal metaplasia.

We are also interested in the relation between intestinal metaplasia and intestinal-type gastric cancer. Most gastric cancers show some intestinal histology. Controversy exists as to whether intestinal metaplastic cells are destined to become cancer cells. In future studies, we will determine the expression patterns of as many glycosyltransferases as possible, in both intestinal metaplasia and intestinal-type gastric cancers. It is of interest to know whether the expressions of some glycosyltransferases differ between intestinal metaplasia and intestinal-type cancers.

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