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Expression of *MUC2* Gene in Gastric Regenerative, Metaplastic, and Neoplastic Epithelia

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It has been reported that MUC2 mucin is expressed in goblet cells of gastric intestinal metaplasia, but not in its normal epithelium. To confirm this finding, we have exam0ined the expression of the *MUC2* gene by reverse transcriptase-polymerase chain reaction and immunohistochemical methods in gastric tissues obtained by routine upper gastrointestinal tract endoscopy and compared the results with pathological findings based on hematoxylin and eosin (H&E) staining. In 16.7% of the tissue specimens tested, MUC2 mRNA was detected in spite of the absence of intestinal metaplasia in HE specimens. A possible explanation for this was the identification by immunohistochemistry of MUC2 protein in regenerative gastric mucosal cells in biopsies that did not contain intestinal metaplasia. Sialyl-Le^x epitope, which is suggested to be located on MUC2 mucin core protein (MUC2 protein), was also immunohistochemically detected in both goblet cells of intestinal metaplasia and regenerative epithelium. With regard to carcinoma, MUC2 protein was predominantly expressed in intestinal-type adenocarcinoma. These data indicate that MUC2 mucin is expressed in gastric regenerative epithelium in addition to intestinal metaplasia and intestinal type adenocarcinoma. J. Clin. Lab. Anal. 13:259–265, 1999.

Key words: *MUC2* gene; intestinal metaplasia; regenerative epithelium; gastric cancer; sialyl-Le^x

INTRODUCTION

MUC2 mucin is a large heavily glycosylated protein consisting of more than 5,100 amino acids predominantly expressed in the colon (1). A major domain is the central region with repetitive sequences which are rich in potential *O*-glycosylation sites (Thr or Ser) and Pro. The remaining N-terminal and C-terminal regions are rich in Cys, and these Cys residues are considered to be important for the joining of MUC2 monomers into large polymers with high intrinsic viscosity (1). It has previously been reported that MUC2 mucin core protein (MUC2 protein) is expressed in intestinal metaplasia and carcinoma of the stomach, but not in its normal epithelium (2,3), suggesting that it may be a new marker for intestinal metaplasia and may play an important role as a component of the mucous gel overlying the metaplastic epithelium in the stomach. On the other hand, it is considered that chronic active gastritis induced by *Helicobacter pylori* (*H. pylori*) could result in atrophic gastritis concurring with extensive intestinal metaplasia, in the latter of which *H. pylori* is not usually found (4,5). In this context, it is intriguing to hypothesize that intestinal metaplasia is a mechanism for self-cure, and it is important to analyze the expression and function of intestinal metaplasia-associated antigenic molecules. Although several antigens other than MUC2 mucin have thus far been demonstrated to be expressed in intestinal metaplasia, but not in normal gastric epithelium (6–8), their diagnostic and pathophysiological significances remain unknown. In this study, we therefore attempted the detailed examination on the expression of *MUC2* gene by using reverse transcriptase-polymerase chain reaction (RT-PCR) and immunohistochemistry in gastric tissues from patients with various gastric diseases.

MATERIALS AND METHODS

Human Gastric Tissue Samples

Gastric tissue specimens were endoscopically obtained from 102 patients who were examined at the First Depart-

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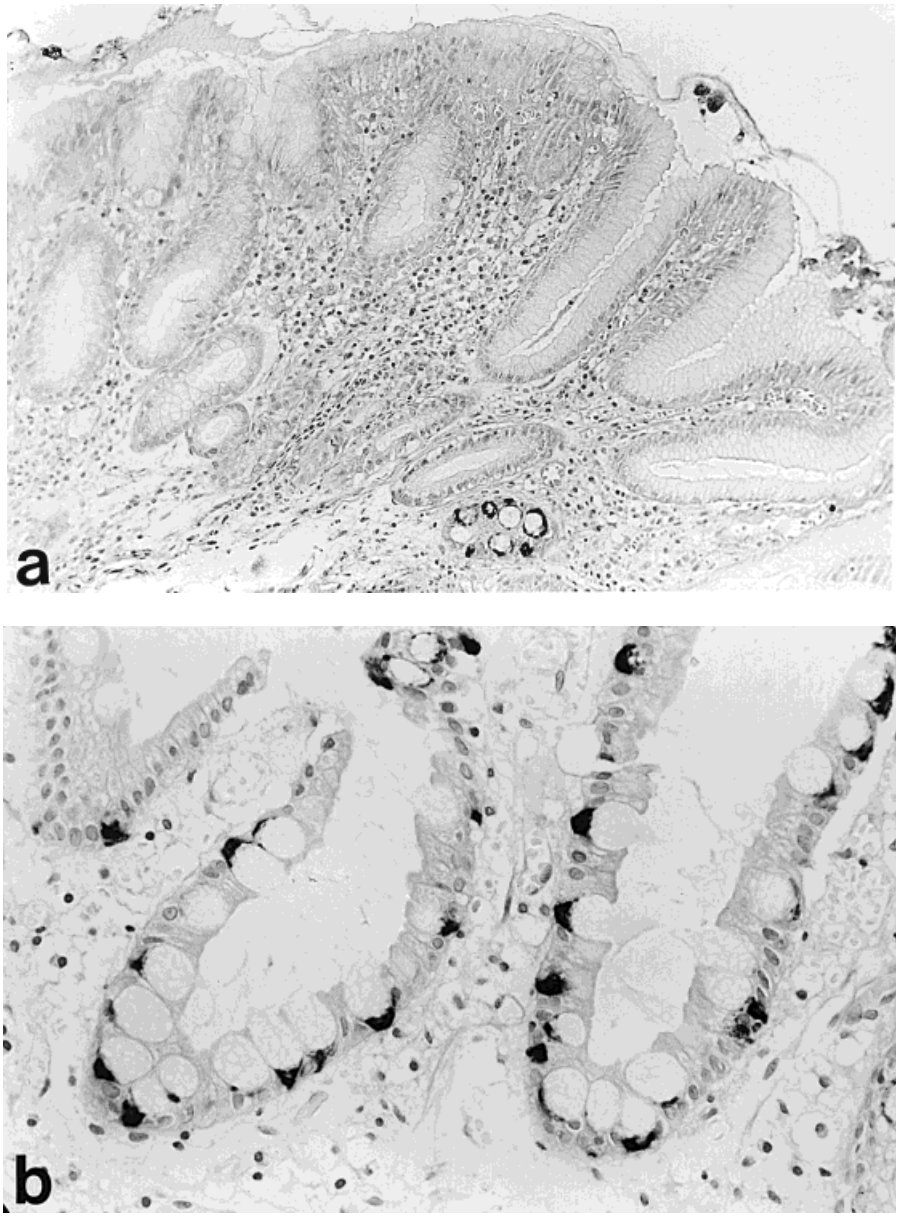

Fig. 1. Immunohistochemical staining with anti-MUC2 peptide mAb of endoscopically biopsied gastric tissue specimens. Normal epithelium (**a**) except for intestinal metaplasia was shown negative for the mAb (× 85) and the cytoplasm except for mucus of goblet cells were positive in intestinal metaplasia (**b**) (× 340).

ment of Internal Medicine, Sapporo Medical University. Gastric ulcer and cancer tissues were obtained from 5 and 52 patients, respectively, who were surgically operated in Sapporo Medical University. Informed consent was obtained from all patients. The mean age of patients was 63.4 (ages 36–81) and the ratio of males to females was 1.1:0.9. Paraffin-embedded tissue sections (5-μm thick) were prepared from those tissue specimens that were routinely fixed in neutral buffered formalin and subjected to immunohistochemical and hematoxylin and eosin (H&E) stainings.

Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR)

According to Blank et al. (9), specific PCR primers were synthesized to recognize a 189-bp fragment of nonrepetitive region of the MUC2 gene (nucleotides 3440–3628): sense primer, 5′-CCATTCTCAACGACAACCCCTACTACCCC-3′, primer 1; reverse primer, 5′-TCCAATGGGAACATC-ACGATACATGGTGGC-3′, primer 2. One μ g of total RNA was reverse transcribed with random nanomers and avian myeloblastosis virus reverse transcriptase using RT-PCR

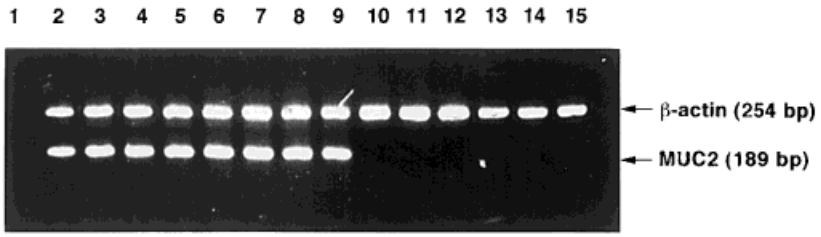

Fig. 2. Detection of MUC2 mRNA in endoscopically biopsied gastric tissue specimens by RT-PCR. A 189-bp fragment of nonrepetitive region of the MUC2 gene (nucleotides 3440–3628) was amplified with primer 1 and 2. The PCR products were electrophoresed through a 2.0% agarose gel and stained with ethidium bromide. β-actin was amplified as an internal control. Lane 1, negative control (no template cDNA); lanes 2–9, positive cases; lanes 10–15, negative cases.

kit (Takara, Otsu, Japan) following the conditions of the manufacturer. The template cDNAs were amplified with Taq polymerase in the presence of primer 1 and 2 on the basis of PCR protocol (10). The thermocycling parameters used in the PCR were as follows: denaturation, 30 sec at 94°C; annealing, 30 sec at 60°C; extension, 1.5 min at 72°C. These reactions were repeated for 30 cycles. The PCR products were electrophoresed through a 2.0% agarose gel and stained with ethidium bromide. Similarly, β-actin was amplified as an internal control.

Immunohistochemistry

Immunohistochemical staining was done on paraffin-embedded tissue sections by an immunoperoxidase method as described previously (8). Briefly, each section was deparaffinized, rehydrated, and incubated with fresh 0.3% hydrogen peroxide in methanol for 20 min at room temperature and then washed with PBS. Normal goat serum (5%) was applied for 20 min and removed by blotting. The sections were incubated with anti-MUC2 monoclonal antibody (mAb) CCP58 against synthetic peptide for repetitive sequence (11) or anti-sialyl-Le^x mAb CSLEX1 (Dakopatts, Copenhagen, Denmark) for 60 min at room temperature, washed 3 times in PBS, and incubated with secondary antibody (peroxidase-conjugated rabbit antimouse Ig diluted 1/50 in PBS) for 30 min at room temperature. After washing 3 times, the sections were incubated with diaminobenzidine tetrahydrochloride in 0.03% hydrogen peroxide for 5–10 min, washed, counterstained with hematoxylin, rinsed in tap water, and mounted. Diluted rabbit serum was used as a negative control for the primary antibody. Histological classification of gastric cancer was given according to the Japanese classification of gastric carcinoma (12).

TABLE 1. Comparison of detection of MUC2 mRNA by RT-PCR with identification of intestinal metaplasia by H&E staining

		H&E staining (identification of IM)	
		+ (n = 32)	– (n = 40)
RT-PCR for MUC2 mRNA	+	32 (44.4%)	12 (16.7%)
	–	0	28 (38.9%)

RESULTS

Expression of MUC2 Protein in Intestinal Metaplasia

Expression of MUC2 protein in intestinal metaplasia of the stomach was confirmed by immunohistochemistry using biopsy tissue specimens from patients with chronic gastritis pathologically diagnosed. As shown in Figure 1, the mucosa with chronic gastritis was negative for anti-MUC2 mAb CCP58 whereas its reactivity localized to the cytoplasm of goblet cells was seen in intestinal metaplasia. These observations were consistent with the findings of previous reports (2,3).

Comparison of Detection of MUC2 mRNA by RT-PCR With Identification of Intestinal Metaplasia by H&E Staining

For the purpose of the sensitive detection of MUC2 mRNA, RT-PCR was performed using primer 1 and 2 on biopsy tissue specimens from 72 patients who had been examined by routine endoscopy of the upper gastrointestinal tract. The mean age of patients was 54.9 (ages 22–79) and the ratio of male to female was 0.83. Two tissue specimens per patient were obtained from almost the same point in the lesser curvature of the antrum by endoscopic biopsy. One was used for RT-PCR and the other for H&E staining for pathological examination. Intestinal metaplasia was observed in H&E stained sections in 32 of 72 (44.4%) biopsies and in each of these samples, MUC2 mRNA was demonstrated by RT-PCR in the paired biopsy specimen (Fig. 2 and Table 1). No cases were positive for intestinal metaplasia but negative for MUC2 by RT-PCR. However, 12 cases (16.7%) were positive for MUC2 mRNA by RT-PCR and negative for intestinal metaplasia. A possible explanation for this latter finding could have been that intestinal metaplasia was in fact present in the biopsy used for RT-PCR even though it was not observed in the adjacent H&E stained samples. To evaluate this possibility, pairs of tissue specimens were obtained from an 30 additional patients, stained with H&E, and compared to each other. Twenty-nine

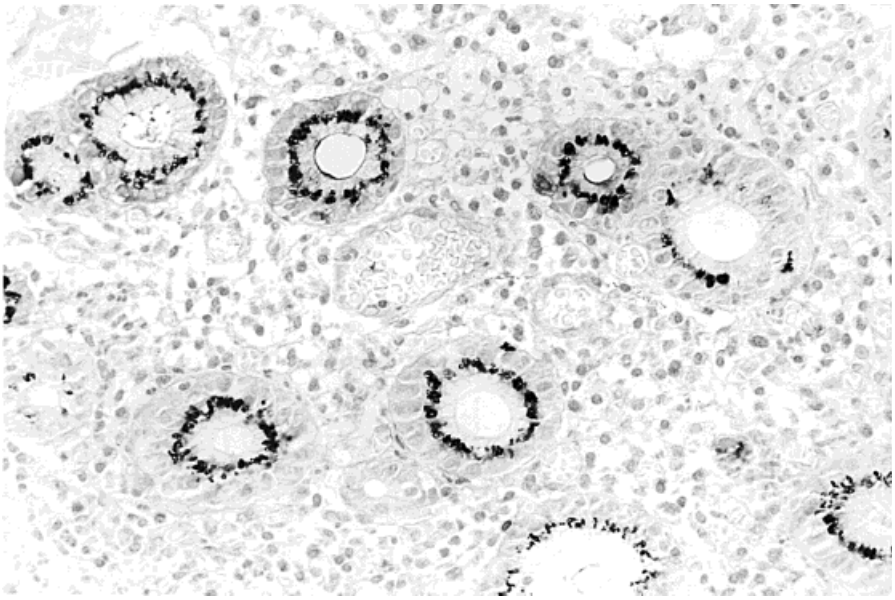

Fig. 3. Immunohistochemical detection of MUC2 protein in regenerative epithelium that were shown positive for immunohistochemistry and negative for pathology (× 340).

out of 30 (96.7%) pairs showed concordance in the presence or absence of intestinal metaplasia. Thus, it is unlikely that the apparent false-positive RT-PCR results occurred due to undiagnosed metaplastic epithelium.

Detection of MUC2 Protein and Sialyl-Le^x in Regenerative Gastric Epithelium by Immunohistochemistry

In a further effort to identify the cell types that might be responsible for expression of MUC2, 42 gastric biopsies that were part of 72 specimens used for the RT-PCR studies were examined by both H&E staining and immunohistochemical staining for MUC2 protein. Interestingly, in seven biopsies that were negative for intestinal metaplasia, immunohistochemical staining for MUC2 protein was detected (Table 2). Staining occurred in the supranuclear region of small glandular cells with a relatively high nucleo-cytoplasmic ratio (Fig. 3). These glandular cells were smaller than those of intestinal metaplasia (Fig. 1b) and were consistent with regenerative epithelium.

To further evaluate this finding, regenerative epithelium associated with peptic ulcers was examined by immunostaining with mAb CCP58. Eight gastric ulcer tissues obtained by endoscopic biopsy (n = 3) (part of 72 specimens used for RT-PCR) or surgical operation (n = 5) were examined. Immature regenerative glands in the marginal mucosa of the ulcer were found in all the tissue specimens tested and were positively immunostained in a manner similar to that previously observed (data not shown). Next, expression of sialyl-Le^x in metaplastic and regenerative epithelia of the stomach was studied in ten surgically resected gastric cancers (n = 5) or ulcer tissues (n = 5) that contained metaplastic and regenerative epithelia. The entire cytoplasm of goblet cells in intestinal metaplasia (Fig. 4b) and of regenerative glandular cells in ulcer tissues (Fig. 4d) were shown positive for anti-sialyl-Le^x mAb.

Expression of MUC2 Protein in Gastric Cancer Tissues

Because the relation of MUC2 protein expression to histological type of gastric cancer was not sufficiently analyzed in previous studies (2,3), immunostaining of 52 surgically excised gastric cancer tissue specimens (containing 5 specimens used for the examination of sialyl-Le^x expression as described above) with anti-MUC2 mAb was carried out. The summary of the results is shown in Table 3. Primary tumors tested were histologically classified into six groups according to the Japanese classification of gastric carcinomas (12), i.e., well-differentiated type of tubular adenocarcinoma (tub 1) (n = 12), moderately differentiated type of tubular adenocarcinoma (tub 2) (n = 12), solid type of poorly differentiated adenocarcinoma (por 1) (n = 8), nonsolid type of poorly differentiated adenocarcinoma (por 2) (n = 6), signet-ring cell carcinoma (sig) (n = 12) and mucinous adenocarcinoma (muc) (n = 2). Tubular adenocarcinoma, the solid type of poorly differentiated adenocarcinoma, and the other types correspond to intestinal, solid, and signet/scirrhous histological classifications

Fig. 4. Immunohistochemical detection of MUC2 protein (**a, c**) and sialyl-Le^x (**b, d**) in metaplastic (**a, b**) and regenerative (**c, d**) epithelia of surgically excised gastric tissue from a patient with peptic ulcer (× 170).

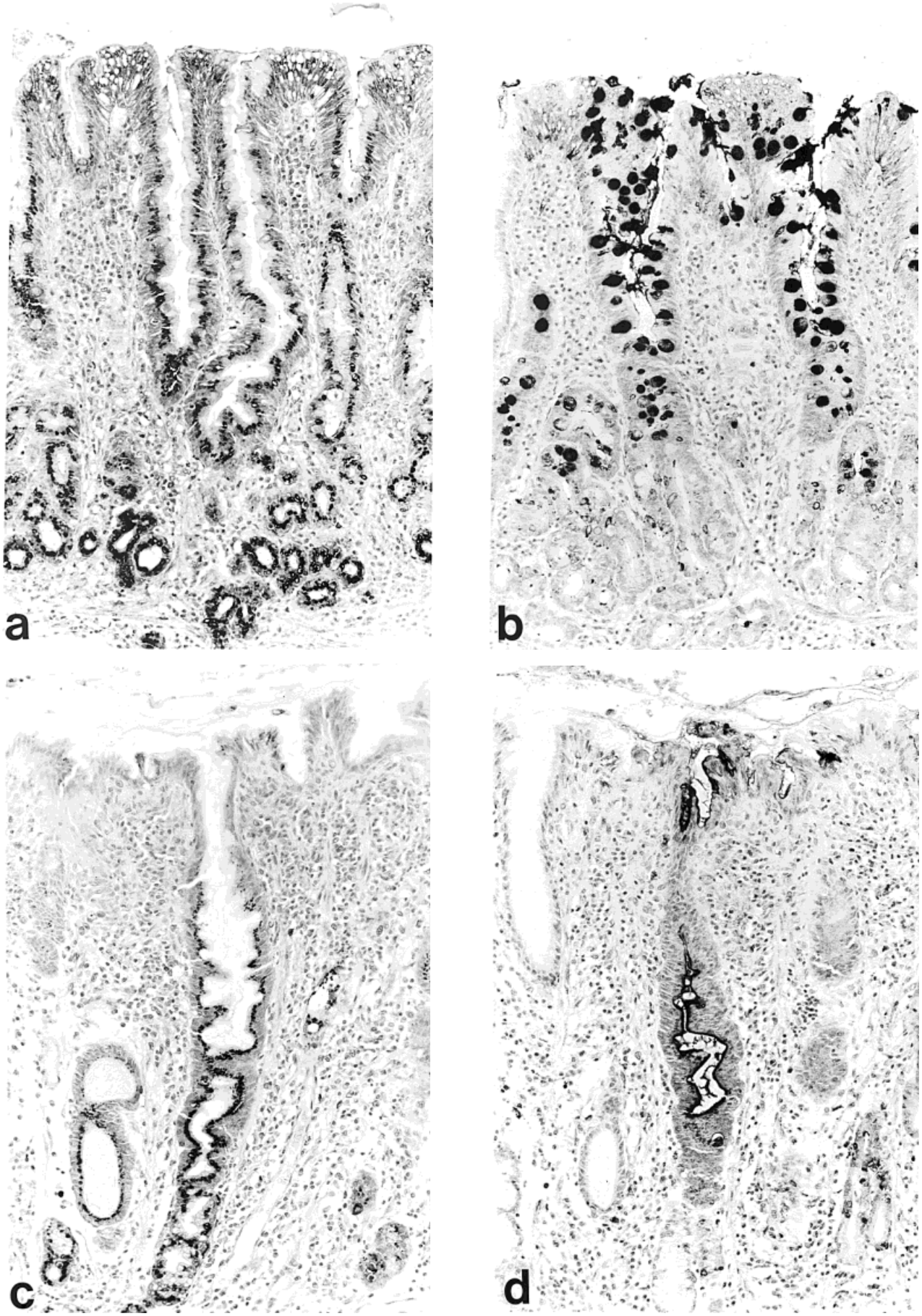

Figure 4.

TABLE 2. Comparison of immunohistochemical detection of MUC2 protein with identification of intestinal metaplasia by H&E staining

		H&E staining (identification of IM)	
		+ (n = 16)	– (n = 26)
Immunostaining with anti-MUC2 mAb	+	15 (35.7%)	7 (16.7%)
	–	1 (2.4%)	19 (45.2%)

by Chong et al. (13), respectively. If one considers immunostaining over 5% of the cancer region to be positive, then the incidence of positivity in each histological type was 58.3% (7/12) of tub 1, 41.7% (5/12) of tub 2, 37.5% (3/8) of por 1, 16.7% (1/6) of por 2, 33.4% (4/12) of sig and 0% of muc (Table 3). Expression involving > 30% of the cancer area was seen in 33.3% of tub 1, tub 2, and sig, suggesting that MUC2 protein is predominantly expressed in those types of carcinomas.

DISCUSSION

It was first revealed by RT-PCR and immunohistochemistry that the *MUC2* gene is expressed not only in gastric intestinal metaplasia and carcinoma, but also in regenerative epithelium associated with chronic gastritis or peptic ulcers. Expression of the MUC2 protein as observed in regenerative epithelial cells without goblet cells shown in Fig. 4d could indicate the cells' intestinal phenotype, perhaps for the following reasons. Firstly, all glandular cells, irrespective of the presence of mucus, expressed the MUC2 protein in intestinal metaplasia of ulcer margin. Secondly, MUC2-positive regenerative epithelium was often observed adjacent to intestinal metaplasia in surgically resected tissues (data not shown). Thirdly, previous reports and our present observation showed that normal gastric epithelium including proliferative cells (neck cells) was totally negative for anti-MUC2 peptide mAb. Finally, a wide expression of the MUC2 protein in gastric cancer was restricted to well- or moderately differentiated adenocarcinoma (intestinal type) and signet ring cell carcinoma (Table 3). Some signet ring cell carcinomas have been revealed to consist of intestinal epithelial type cells (14). Thus, *MUC2* gene expression may be of use as an intestinal phenotypic marker for analyzing various gastric epithelial lesions. A recent electron microscopic study of gastric ulcer tissues demonstrated two types of regenerating epithelium: gastric and intestinal cell types. Both were composed of immature cells that later matured to form primitive gastric and intestinal glands, respectively (15). The *MUC2* gene might be expressed in intestinal cell-type regenerating cells.

TABLE 3. Expression of MUC2 mucin core protein in gastric cancer tissues

	Immunoreactivity of cancer region[a]			
	(+)			(–)
Histological type	> 50 (%)	< 50 & > 30 (%)	< 30 & > 5 (%)	< 5 (%)
Tub 1 (n = 12)	16.6	16.6	25	41.7
Tub 2 (n = 12)	8.3	25	8.3	58.3
Por 1 (n = 8)	0	0	37.5	62.5
Por 2 (n = 6)	0	0	16.7	87.5
Sig (n = 12)	8.3	25	0	66.6
Muc (n = 2)	0	0	0	100

[a]When the positively immunostained area of cancer region was less than 5%, it was determined to be negative.

Expression of the *MUC2* gene in regenerative epithelium (probably intestinal type) in addition to mature goblet cells in intestinal metaplasia may suggest that intestinal-type carcinoma might be developed from immature cells of metaplastic glands. A close relationship between incomplete-type intestinal metaplasia and intestinal-type gastric cancer has previously been suggested using morphologic, enzymatic, or histochemical methods (16–19), but any conclusions are made difficult by the fact that the incomplete type of intestinal metaplasia was also found in patients with chronic gastritis. It has recently been shown that the relationship between intestinal metaplasia with the expression of brain-type glycogen phosphorylase in the proliferative zone and intestinal-type carcinoma was apparently closer than the conventional subtype of intestinal metaplasia and gastric cancer, suggesting that intestinal-type carcinoma might arise from some of those proliferating cells (20). Moreover, Hamamoto et al. (21) have demonstrated alterations at one or more loci in 26.7% (4/15) of intestinal metaplasia adjacent to gastric cancer by microsatellite assay at nine loci. Importantly, an identical pattern of microsatellite alteration was detected in the cancer tissue and the adjacent metaplastic mucosa of one case, suggesting the sequential development of gastric cancer from intestinal metaplasia. These findings may further support a relation of intestinal metaplasia with intestinal type gastric cancer.

Previous studies revealed that sialyl-Le^x is expressed on the MUC2 mucin core protein in normal colon and colon cancer tissues (22), and that it is immunohistochemically detected in goblet cells of intestinal metaplasia in a cytoplasmic pattern (23). Such findings suggest that sialyl-Le^x may be expressed in the MUC2 mucin produced by metaplastic epithelium, but not by normal epithelium in the stomach. We therefore immunohistochemically examined the expression of sialyl-Le^x in metaplastic and/or regenerative epithelia. As shown in Figure 4, we demonstrated that the sialyl-Le^x epitope is expressed in regenerative as well as metaplastic epithelium. It was considered reasonable that MUC2 protein was detected at the supranuclear region in all glandular cells of intestinal metaplasia (Fig. 4a) whereas only relatively mature cells expressed sialyl-Le^x, a peripheral sugar chain (Fig. 4b). It has been shown that the sialyl-Le^x oligosaccharides inhibit neutrophil interaction with endothelial cells and suppress the inflammatory vascular injury (24). Recently, sialyl-Le^x oligosaccharides were shown to markedly suppress

postreperfusion cardiac dysfunction and neutrophil infiltration (25). Also, a recombinant sialyl-Le^x-bearing α1-acid glycoprotein reduced pulmonary myeloperoxidase levels and remote lung injury in rats after intestinal ischemia (26). Thus, production of sialyl-Le^x oligosaccharides together with MUC2 protein from regenerative and metaplastic epithelial cells seems to be useful for self-cure in gastric inflammatory lesions, although further studies are required to reveal if MUC2 protein is one of the core proteins for sialyl-Le^x epitope in those cells.

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