Immunohistochemical Detection of MUC2 Mucin Core Protein in Ulcerative Colitis

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MUC2 mucin is predominantly expressed in the colon and is considered to play an important role in the protection of that organ. Recent findings suggested that MUC2 protein levels are significantly decreased in active ulcerative colitis (UC). We therefore performed an immunohistochemical study to reveal if the expression of MUC2 protein is altered in UC. Seventy-nine biopsy tissue specimens from 31 UC patients, along with normal colon tissues, were immunostained with anti-MUC2 mucin core protein monoclonal antibody (MoAb) CCP58 (IgG1). UC tissue specimens were divided into two groups based on the histological severity of inflammation, i.e., 64 with active inflammation (grades 2–5) and 15 without (grade 1). In the former group, 52 out of 64 (81.3%)

tissue specimens contained sections of glands with a few cells positive for MoAb CCP58. These glands were small and consisted of MUC2 negative-short cells and a few positive cells without apparent mucus formation, considered to be immature regenerative glands. In contrast, the staining pattern was almost the same as that of the normal colon and no immature glands were seen in the group without active inflammation. The sections of immature regenerative glands with a few MUC2-positive cells were exclusively found in the UC tissues with active inflammation, but not in those without it, suggesting that the expression of MUC2 protein may be decreased in active UC. *J. Clin. Lab. Anal.* 12:150–153, 1998. © 1998 Wiley-Liss, Inc.

Key words: ulcerative colitis; MUC2; mucin core protein; goblet cell

INTRODUCTION

The MUC2 gene encodes a large mucin core protein predominantly expressed in the colon (1,2). This protein contains more than 5,100 amino acids, which mainly consist of central repetitive regions 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 thought to be important for the joining of MUC2 monomers into large polymers with high intrinsic viscosity (1). Previous immunohistochemical studies using monoclonal antibodies (MoAbs) against MUC2 mucin core protein showed that their immunoreactivity is restricted to goblet cells in the small intestine and colon (3–5). The perinuclear region was immunostained, but mucus was negative probably due to mature glycosylation. These findings suggest that MUC2 mucin core protein could be a good marker for goblet cells. MUC2 and MUC3 genes were shown to be predominantly expressed in the colon among nine mucin core protein genes so far cloned (6). Thus, it is suggested that MUC2 mucin could be a major component of the mucus gel-layer covering the epithelium of the colon, which may play an important role in providing both

lubrication and protection against the colonic microflora and injurious agents.

Although the underlying causes of ulcerative colitis (UC) remain unknown, several lines of evidences suggested that mucin glycoproteins may be altered in association with UC (7). Interestingly, Tysk et al. reported that the content of one chromatographically defined component of colonic mucin was reduced in both patients with UC and their apparently healthy twins compared with control subjects (8). More recently, analyses of mucin at the molecular level have been performed in UC (9,10), suggesting that MUC2 precursor biosynthesis and total MUC2 mucin core protein levels are significantly decreased in patients with active inflammation compared to controls, whereas MUC2 mRNA levels are similar in all patients and independent of disease activity (10). In this study, we attempted the immunohistochemical analysis using anti-

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Received 3 September 1997; Accepted 14 October 1997

MUC2 peptide MoAb CCP58 (11) in order to determine if the expression of MUC2 protein is altered in UC.

MATERIALS AND METHODS

Human Tissue Samples

Tissue specimens were endoscopically obtained from the colon of 31 patients with UC who were treated at the First Department of Internal Medicine, Sapporo Medical University. The mean age of patients was 33.2 (15–64) and the ratio of males to females was 1:1. The diagnosis of UC was based on conventional clinical, endoscopic, radiologic, and histopathologic criteria. Normal colonic tissues were obtained from autopsies performed at Sapporo Medical University. Paraffin-embedded sections (5 mm thick) were prepared from those tissue specimens which were routinely fixed in neutral buffered formalin.

Immunohistochemistry

Immunohistochemical staining was done on formalin-fixed and paraffin-embedded tissue sections by an immunoperoxidase method as described previously (12), with some modifications. 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 phosphate buffered saline (PBS). Normal rabbit serum (5%) was applied for 20 min and removed by blotting. The sections were incubated with anti-MUC2 peptide murine MoAb CCP58 (IgG1) (1:1,000 dilution of ascites) (11) for 60 min at room temperature, washed 3 times in PBS, and incubated with secondary antibody (peroxidase-conjugated rabbit anti-mouse Ig diluted 1:40 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 or methyl green, rinsed in tap water, and mounted.

Histological severity of inflammation in UC was classified into grades 1 to 5 according to Matts (13).

RESULTS

The reactivity of anti-MUC2 mucin core protein MoAb CCP58 with colonic epithelium was restricted to goblet cells as has been reported (3–5) (Fig. 1a). The cytoplasm was clearly immunostained, whereas the mucus was negative.

Seventy nine endoscopic biopsy tissue specimens from 31 UC patients were immunostained with MoAb CCP58. All the patients tested were clinically in active stage, and biopsy specimens were always obtained from both endoscopically inflammatory and normal regions of their colon. Those specimens were divided into two groups based on the histological severity of inflammation (13) of the total area of one tissue section, i.e., 64 with active inflammation (grades 2–5) from 31

patients, and 15 without active inflammation (grade 1) from 14 patients. In the former group, 52 out of the 64 (81.3%) tissue specimens contained the sections of glands with a few cells positive for MoAb CCP58 (Table 1). All negative specimens were grade 2 in severity of inflammation. As shown in Fig. 1b, those glands were small and consisted of MUC2-negative short cells, and considered to be immature regenerative glands. The more mature regenerative glands were larger than those in Fig. 1b and consisted of higher cells with abundant positive cells whose mucus formation was still poor compared to that seen in Fig. 1a (Fig. 1c). These suggest that the expression of MUC2 protein may be decreased in those regenerative glands. On the other hand, the staining pattern was almost the same as that of a normal colon and there were no immature glands in the group without active inflammation (Table 1). The histological finding of one tissue section obtained by endoscopic biopsy may not reflect the inflammatory status of the entire gland, but the number of tissue specimens examined in this study is considered to be sufficient for outweighing it, since the section of glands observed in each biopsy tissue is variable at its depth.

To further examine the relationship between colonocyte differentiation and MUC2 expression, the reactivity of MoAb CCP58 with normal colon tissues was observed. For this analysis, the colon tissues obtained from autopsy were used because it was difficult to observe the entire structure of each gland in biopsy tissue specimens. As demonstrated in Fig. 1d, a number of positively stained cells were found at the base of crypt, suggesting the expression of MUC2 mucin core protein in immature goblet cells (14).

DISCUSSION

In this study, it was first immunohistochemically demonstrated that the sections of immature regenerative glands with poor MUC2 protein expression were found exclusively in the UC tissues with active inflammation, but not in those without it. This suggests that the decreased MUC2 mucin production in active UC tissues is probably due to the relative increase of MUC2-negative immature cells and immature goblet cells with poor mucin formation. Tytgat et al. have recently revealed the decreased levels of MUC2 precursor biosynthesis and total MUC2 mucin core protein in active UC (10), a finding that is consistent with our present data. MUC2 mucin is thought to be a major component of the mucus gel-layer in the colonic epithelium. The altered expression of its core protein may have a profound effect on disease activity, although whether it is the primary event in the pathogenesis of UC

Fig. 1. Immunohistochemical localization of MUC2 mucin core protein in the UC tissues without active inflammation (**a**) ($\times 170$) or with active inflammation (**b,c**) ($\times 170$), and in normal colon tissues (**d**) ($\times 340$). (Figure 1 is on page 152.)

TABLE 1. Immunohistochemical Detection of the Sections of Immature Regenerative Glands With Anti-MUC2 Mucin Core Protein MoAb CCP58 in UC

Severity of inflammation ^a	Active	Inactive
No. immature glands/no. total (%) ^b	52/64 (81.3)	0/15 (0)

^aActive and inactive indicate grades 2 to 5 and grade 1 in Matts's classification (13), respectively.

^bNumber of the tissue specimen containing the sections of immature regenerative glands/number of all tissue specimens tested.

remains unknown. Circulating antibodies against goblet cell mucin have been found in UC (15, 16). On the other hand, goblet cell depletion is a characteristic histological finding in UC. Since matured goblet cells are assumed to contain a large amount of MUC2 mucin, it is intriguing to hypothesize that immune responses against MUC2 mucin might cause the destruction of matured goblet cells, giving rise to the frequent observation of immature glands in active UC. The analysis of the corresponding antigens of antigoblet cell mucin antibodies will be important for making clear this point.

Although MUC2 protein has been shown to be a good marker for goblet cells (3–5), the relationship between expression of MUC2 gene and colonocyte differentiation is not known. There have been some findings suggesting a relation between the level of differentiation and MUC2 expression in colon cancer cells. Cl.16E, a stably differentiated clone of human colon cancer HT29 cells, display in vitro differentiation with many of the characteristics of normal mature goblet cells (17). Cl.16E cells express very low levels of MUC2 mRNA at 5 days (log phase) after culture and the steady increase of the mRNA begins at day 12 (postconfluent phase). The immunoreactivity of MoAb CCP58 was decreased in nonmucinous colon carcinomas, especially de novo carcinomas compared to adenomas and normal colonic epithelium (18). In this study, we revealed that the immature regenerative glands consisting of MUC2-negative short cells and a few positive cells in the UC tissues with active inflammation. Interestingly, most of MUC2-positive regenerative cells showed poor mucus formation, which suggests the expression of MUC2 protein in immature goblet cells. Furthermore, a number of MUC2-positive cells were found in the deep portion of normal colonic glands. They may also correspond to immature goblet cells, since it is known that numerous undifferentiated cells that contain few mucin granules (immature goblet cells) reside at the base of the crypt (14). Thus, the expression of MUC2 protein may begin at the early differentiation stage of goblet cells though the exact starting point is unclear.

ACKNOWLEDGMENTS

This research was supported by Grants-in-Aid for Inflammatory Bowel Disease Research from the Ministry of Health and Welfare (K.I.), Japan.

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