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ORIGINAL ARTICLE

AN IMMUNOHISTOCHEMICAL EVALUATION OF ENZYMES IN METAPLASTIC PANETH CELLS IN ULCERATIVE COLITIS

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Abstract Innate Paneth cells (IPCs) contain various enzymes such as matrix metalloproteinase-7 (MMP7), lysozyme, secretory phospholipase A_2 (sPLA$_2$), and α_1-antitrypsin (α_1AT), which are considered to participate in important roles against bacteria and mucosal injury, while it is unknown whether metaplastic Paneth cells (MPCs) in ulcerative colitis (UC) have similar functions to IPCs. An immunohistochemical comparison between IPCs and MPCs was made using antibodies against these enzymes. The study included 20 patients with total colitis type of active UC. For six segments from the cecum to the rectum, ratios of the number of MPCs with enzyme positive granules (enzyme$^+$-MPCs) and ratios of the number of MPCs with positive granules occupying more than a half of the cytoplasm (enzyme$^{1/2}$-MPCs) to that of all MPCs were calculated. The populations of MMP7$^+$-MPCs, MMP7$^{1/2}$-MPCs and lysozyme$^{1/2}$-MPCs were significantly smaller than those of IPCs in the ileum taken from 20 controls (p<0.01). Both MPCs and IPCs abundantly contained sPLA$_2$ positive granules, but had few α_1AT granules. MPCs were characterized by scant MMP7 and abundant sPLA$_2$; MMP7 is capable of causing tissue injury and sPLA$_2$ is associated with mucosal repair. Although a population of MPCs is usually small in the colorectal mucosa of UC, they are suggested to come into existence to play a role of mucosal repair.

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Key words: Paneth cell metaplasia; ulcerative colitis; immunohistochemistry.

原 著

潰瘍性大腸炎の化生性パネート細胞に含まれる酵素に関する免疫組織化学的検討

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抄録 潰瘍性大腸炎(UC)の大腸には化生性パネート細胞(MPCs)が出現するが，その意義や健常者小腸に元々存在するパネート細胞（IPCs）との機能的な違いについては不明である．我々は，マトリックスメタロプロテナーゼ-7（MMP7），リゾチーム，分泌型ホスフォリパーゼ A_2 (sPLA$_2$)，α_1-アンチトリプシン（α_1AT）の４つの酵素に注目して，健常者（n=20）回腸のIPCsと全大腸炎型UC患者（n=20）のMPCsにおける差異を免疫組織化学的に検討した．MMP7およびリゾチームを含む細胞内顆粒はMPCsで有意に少なく，特に組織傷害を惹起するとされるMMP7についてはMPCsを特徴づける所見であった．一方，sPLA$_2$とα_1ATはMPCsとIPCsの両者で差がなく，粘膜修復に関与するsPLA$_2$は豊富に認められた．UC粘膜でのMPC数は多くはないが，MPCsはUCの傷害粘膜を修復する役割を担って出現している可能性が示唆された．

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Introduction

Paneth cells normally exist only in the crypt base of the small bowel. Intracytoplasmic granules of these innate Paneth cells (IPCs) contain many kinds of enzymes such as matrix metalloproteinase-7 (MMP7), lysozyme, α_1-antitypsin (α_1AT), secretory phospholipase A_2 (sPLA$_2$), α-defensin, and cysteine-rich intestinal polypeptide[1-2]. Considering the presence of these substances, IPCs have been thought to play a role against bacteria and mucosal injury. In the large bowel affected with ulcerative colitis (UC), metaplastic Paneth cells (MPCs) are commonly found and it has been suggested that they appear during the course of regeneration and repair of mucosa[3-4]. To date, however, the function of MPCs has not fully been understood, and it remains unclear whether the cytoplasmic granules of MPCs contain the same enzymes as those of IPCs.

In the immunohistochemical study, we made semiquantitative comparisons between MPCs and IPCs regarding MMP7, lysozyme, α_1AT, and sPLA$_2$. These enzymes have been considered to participate in important roles of IPCs, but are different in function from each other; all the enzymes have a direct or indirect antibacterial action and/or stimulate mucosal repair[1), 5-12], although MMP7 may also cause tissue injury[13-15].

Materials and Methods

Patients and control subjects

Twenty patients who underwent total colectomy for total colitis type of active UC (male/female: 11/9; mean age ± SD: 32.4 ± 16.3 years) were chosen from the consecutive pathology files of Hirosaki University School of Medicine from 1998 to 2001. The resected specimens studied were taken from the cecum, the ascending, transverse, descending and sigmoid colon, and the rectum. As a control, we used normal terminal ileum specimens obtained from 20 subjects who underwent surgery for colon cancer (male/female: 11/9; mean age ± SD: 72 ± 10 years). The specimens studied were 3 to 4 cm in length. The informed consent was obtained from the patients and control subjects after the explanation of the study protocol.

Immunohistochemistry

The specimens were fixed with 10% buffered formalin, embedded in paraffin, and serially cut into 4 μm thick sections. A strep tavidin-biotin-peroxidase complex technique was performed using a Histofine kit (Nichirei, Co., Tokyo, Japan) according to the manufacturer's instructions. The antibodies used and their dilutions are listed in Table 1.

Diaminobenzidine-cobalt was used as a chromogen to yield dark purple reaction

Table 1 Antibodies used for immunohistochemistry

Antibody to	Type	Source	Dilution
matrix metalloproteinase-7 (MMP7)	mouse monoclonal	Daiichi Fine Chemical (Takaoka, Japan)	1:50
lysozyme	rabbit polyclonal	Dako (Glostrup, Denmark)	1:600
secretory phospholipase A_2 (sPLA$_2$)	mouse monoclonal	Upstate (New York, NY, USA)	1:50
α_1-antitrypsin (α_1AT)	rabbit polyclonal	Dako	1:50

products, for the purpose of making a distinction from the red color tone of eosin, which was essentially required to recognize Paneth cell granules; the optimal staining time for eosin was 3 minutes. Nuclei were lightly counterstained with hematoxylin. To keep positive and negative control, the immunohistochemical procedures were simultaneously carried out for both the sections from the normal terminal ileum and those from the large bowel affected with UC.

Evaluation of Paneth cell granules with or without enzyme

The population of Paneth cells varied on the individual slides; e.g. 50 to 60 IPCs/mm in the normal terminal ileum and 0.3 to 0.5 MPCs/mm in the large bowel affected with UC. We blindly observed at least 5 and up to 20 Paneth cells on each slide. MPCs with at least one positive granule were judged as "enzyme^{+}-MPC." For each slide, the ratio of the number of enzyme^{+}-MPCs to that of all MPCs was calculated; IPCs were also examined in the same way. MPCs with positive granules occupying more than a half of the cytoplasm were designated as "enzyme$^{1/2}$-MPC." For each slide, the ratio of the number of enzyme$^{1/2}$-MPC to that of all MPCs was calculated.

Statistics

The ratios of the number of enzyme^{+}-MPCs to that of all MPCs were compared among all sites of the large bowel and also compared with the ratios for enzyme^{+}-IPCs in the normal terminal ileum. The ratios for enzyme$^{1/2}$-MPCs were also compared with those for enzyme$^{1/2}$-IPCs in the same way. Statistic significances were tested for each enzyme using the Mann-Whitney U-test in SPSS software (SPSS Inc., Chicago, IL, USA).

Results

MPCs with granules containing enzyme

In all sites of the large bowel, the ratios of the number of MMP7^{+}-MPCs to that of all MPCs were significantly lower than the ratios for IPCs in the control ileum ($p<0.01$). As regards lysozyme, $sPLA_2$, and α_1AT, there were no significant differences among all sites of the large bowel and the control ileum (Table 2).

MPCs with positive granules occupying more than a half of the cytoplasm

The ratios of the number of MMP7$^{1/2}$-MPCs and lysozyme$^{1/2}$-MPCs to that of all MPCs were significantly smaller in all sites of the

Table 2 Ratios (%, mean ± SD) of the number of metaplastic Paneth cells (MPCs) with enzyme positive granules to that of all MPCs.

	matrix metalloproteinase-7	lysozyme	α_1-antitrypsin	secretory phospholipase A_2
Cecum	68.3 ± 23.2*	97.5 ± 3.8	88.1 ± 16.7	98.2 ± 5.1
Ascending colon	67.1 ± 18.3*	97.2 ± 4.5	86.4 ± 17.9	96.1 ± 7.2
Transverse colon	67.9 ± 24.5*	98.2 ± 4.2	89.1 ± 18.2	96.5 ± 1.4
Descending colon	67.5 ± 22.8*	96.3 ± 6.9	88.3 ± 8.6	97.9 ± 3.3
Sigmoid colon	59.3 ± 23.6*	96.5 ± 5.3	90.1 ± 6.2	98.7 ± 2.4
Rectum	67.9 ± 20.1*	95.4 ± 7.5	90.3 ± 8.8	98.0 ± 8.1
Normal ileum	93.1 ± 10.2	99.1 ± 2.3	94.2 ± 6.2	97.3 ± 7.0

* $p<0.01$ compared with normal ileum.

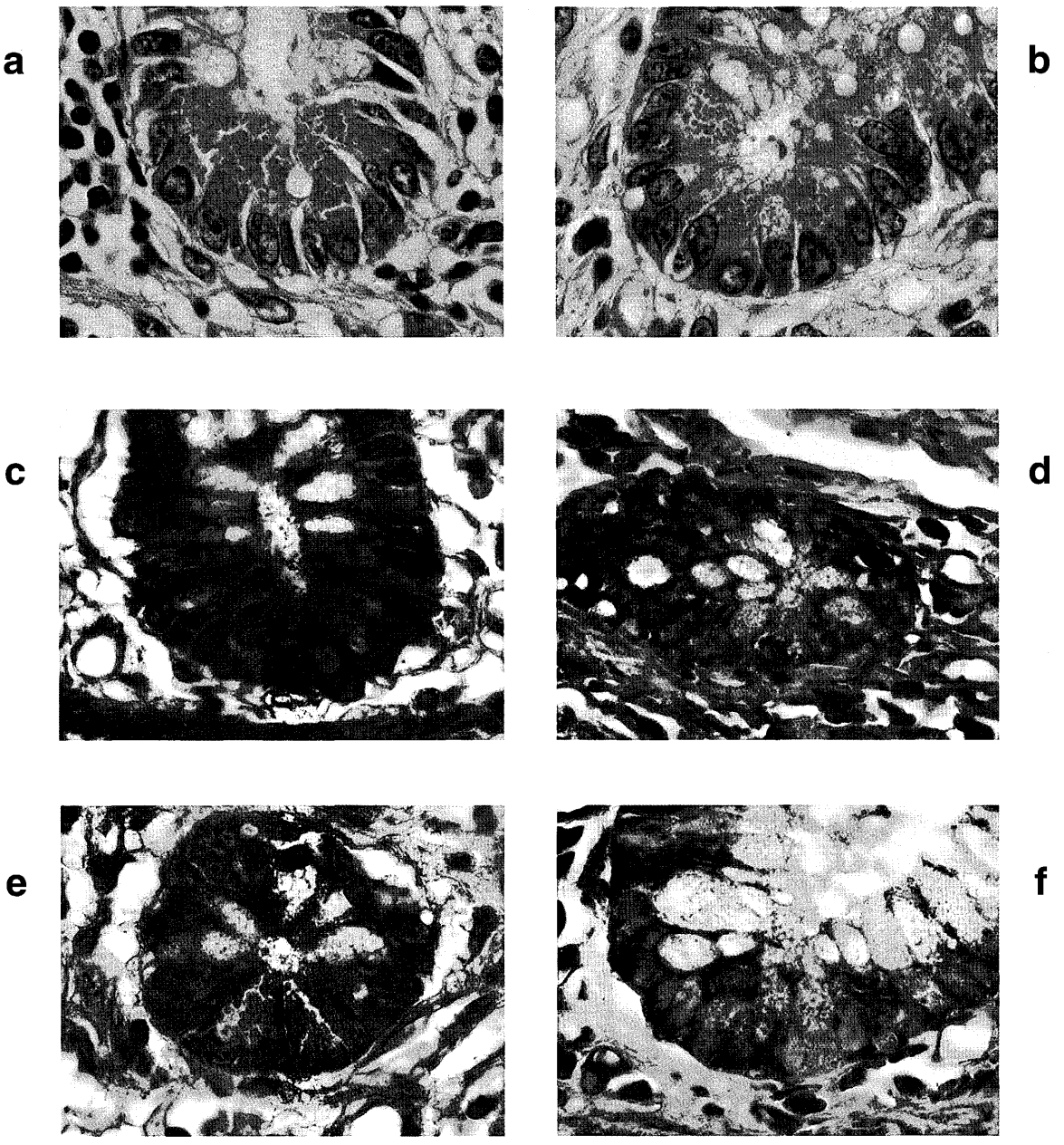

Figure T Innate Paneth cells in the normal terminal ileum (a, c and e) and metaplastic Paneth cells in the large bowel affected with ulcerative colitis (b, d and f) (x 1000). The sets of a and b, c and d, and e and f represent HE, matrix metalloproteinase-7, and lysozyme, respectively.

large bowel as compared with the controls ($p<0.01$) (Fig. 1 and 2). However, there were no significant differences among all sites of the large bowel and the control ileum in the data of $sPLA_2$ and α_1AT. Note that $sPLA_2$ were abundantly contained in most MPCs distributed throughout the large bowel, as well as IPCs.

Discussion

Colorectal MPCs are commonly found in idiopathic inflammatory bowel disease and are known to be a sign of a long history of colitis4). Although the presence of MPCs is a non-specific phenomenon in the proximal colon, MPCs in the distal colon appear

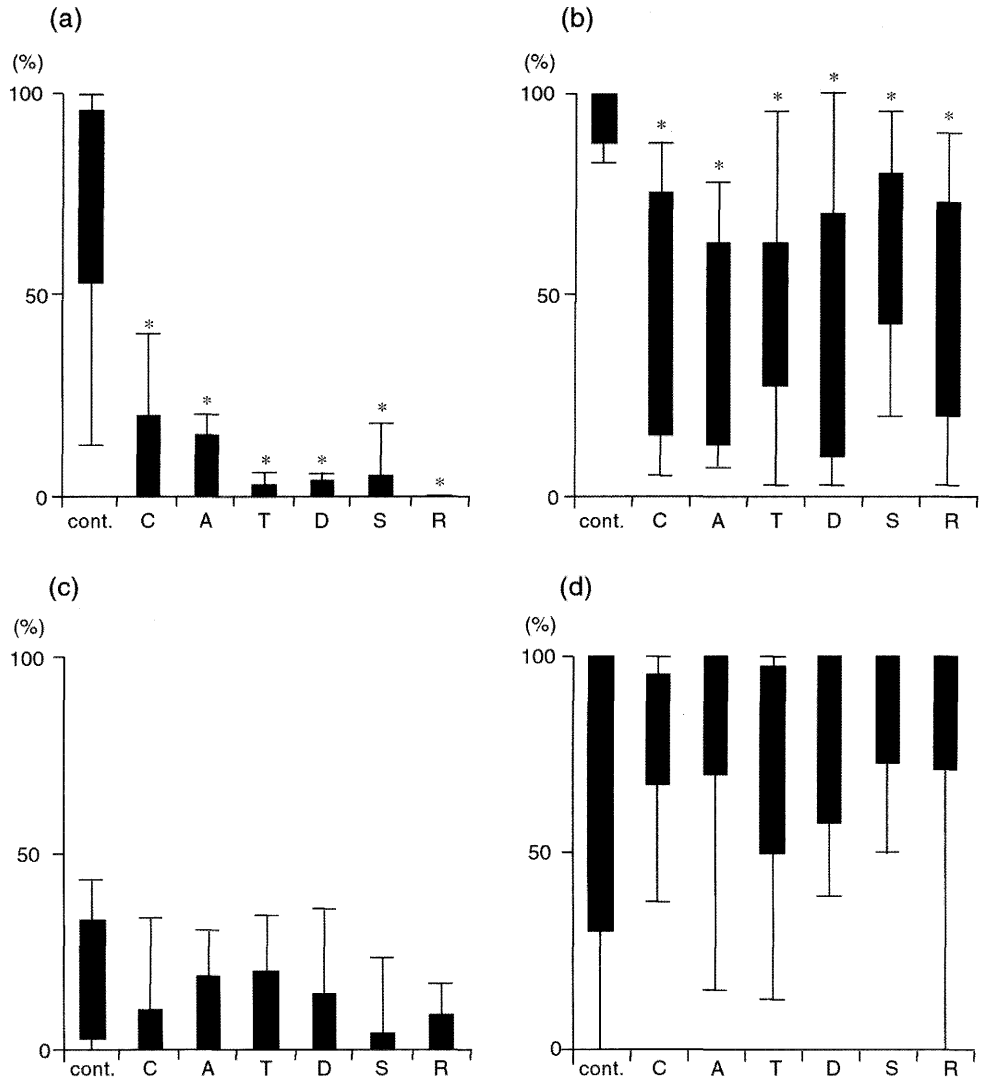

Figure 2 Frequencies of the number of metaplastic Paneth cells (MPCs) with positive granules occupying more than a half of the cytoplasm to that of all MPCs. (a) matrix metalloproteinase-7, (b) lysozyme, (c) α_1-antitrypsin, and (d) secretory phospholipase A_2. Data represent the results for innate Paneth cells in the normal ileum (cont.) and those of MPCs in the cecum (C), the ascending (A), transverse (T), descending (D), sigmoid (S) colon, and rectum (R).
* $p<0.01$ compared with data obtained from innate Paneth cells in the ileum.

exclusively in the affected mucosa[3]. A recent study using multiple logistic regression analysis suggested that mucosal injury and regeneration may be the most potent stimuli causing Paneth cell metaplasia[3].

MMP7 that often exists in carcinoma cells and macrophages is an enzyme involved in degradation of extracellular matrix and is associated with the invasion of these cells[13-15]. On the other hand, this enzyme is considered an activator of α-defensin, which is one of the antibacterial substances contained in Paneth cell granules[16-17]. MMP7 therefore can indirectly participate in host defense of Paneth cell, whereas excessive MMP7 may cause tissue injury. The study demonstrated that the populations of $MMP7^{+}$-MPCs and $MMP7^{1/2}$-MPCs were significantly smaller than those for IPCs ($p<0.01$; Table 2 and Fig. 2). Although it is unclear which of two contrary actions is predominant at a low level of MMP7, this enzyme in MPCs might be

controlled not to exacerbate tissue injury of the inflamed mucosa.

Both lysozyme and α_1AT have antibacterial action, and the former enzyme has been identified in Paneth cells of various animals and considered to be a marker of Paneth cell[18-24]. Although ratios of the number of lysozyme$^{1/2}$-MPCs to that of all MPCs were a little lower than those for lysozyme$^{1/2}$-IPCs, there was no significant difference between the population of lysozyme^{+}-MPCs and that of lysozyme^{+}-IPCs (Table 2 and Fig. 2). The enzyme α_1AT can control superfluous tissue destruction by inhibiting neutrophil elastase[10-12]. α_1AT positive granules were found in most MPCs and IPCs (Table 2), but were too few to fully play a role. At any rate, there may not be an essential difference in lysozyme or α_1AT between MPCs and IPCs, because both cells contained a relatively large amount of lysozyme but scant α_1AT.

sPLA$_2$ identified in Paneth cell granules is one of three major subtypes of phospholipase A$_2$, which releases arachidonic acid (AA) from biomembrane phosphatide and is known as an enzyme starting AA metabolism pathway[25-30]. The present study revealed that sPLA$_2$ was abundantly contained in MPCs as well as IPCs (Fig. 2), and the result is consistent with the published data that the mRNA level of sPLA$_2$ was increasing in idiopathic inflammatory bowel disease[31]. It is therefore suggested that the major function of MPCs might be associated predominantly with sPLA$_2$.

sPLA$_2$ has an antibacterial action, but it stimulates production of prostaglandin and leukotriene and leads to expression of cyclooxygenase-2 (COX-2)[25-26, 29-30, 32]. COX-2 subsequently accelerates production of prostaglandinE$_2$ (PGE$_2$), which is associated with mucosal repair[9, 25-26, 32]. Furthermore, PGE$_2$ urges fibroblast to promote the production of tissue growth factors, such as hepatocyte growth factor (HGF)[7] and vascular endothelial growth factor (VEGF)[33]. In UC, it is considered that sPLA$_2$ of MPCs may participate in regeneration and repair via production of PGE$_2$, HGF and VEGF.

The present study showed that all the four enzymes are present in both MPCs and IPCs, and suggests that there may not be an essential difference between these cells. Compared with IPCs, however, MPCs were characterized by scant MMP7 and abundant sPLA$_2$; MMP7 is capable of causing tissue injury and sPLA$_2$ is associated with mucosal repair. Although a population of MPCs is usually small in the colorectal mucosa of UC, they are suggested to come into existence to play a role of mucosal repair.

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