A Study on Three-Dimensional Crypt Confugurations of the Large Bowel in Ulcerative Colitis

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Abstract

Ulcerative colitis (UC) is a disease causes severe inflammation in the mucosa of the large bowel including crypt abscess and interstitial inflammatory cell infiltration.
Three-dimensional configurations of isolated crypts were studied in the normal and UC human colon utilizing HCl crypt isolation technique with scanning electron microscopy (SEM) observations. The isolated crypts from the normal colon were visualized as a single straight tubule resembling a test tube. The crypts of UC are so fragile and most crypts had break apart, but 23 complete crypts were observed. Most crypts of UC were visualized as distorted and irregularly branched. It was suspected that inflammations directly introduce deformities of colonic crypt configuration and destroyed crypts proliferate by fission mechanism.

Introduction

A rapid increase of the incidence of ulcerative colitis (UC) accompanied by increase of consumption of Western foods is an important social problem in Japan[1], because the UC involve young people and steadily torment them for their life long accompanying risks for cancer progression[2]. A similar problem may occur in many developing countries in the near future.
We have to know more about UC. However our notion even on histological findings, we feel, is insufficient on it. The UC is essentially the disease of mucosal inflammation in the mucosa of the large bowel including crypt abscess and interstitial inflammation. Crypt configuration in such mucosal disease has not yet enough been studied.
We intended to clarify the crypt structures especially three-dimensional configurations of crypts in such inflammation of UC utilizing crypt isolation technique with scanning electron microscopy (SEM). In conventional method for reconstruction of 3- dimensional structure of crypts, over thousands of serial sections are need even for one crypt.
Araki and Ogata[3] successfully isolated crypts from human colonic adenoma with an HCl-digestion method and observed this whole configuration by SEM. The advantage of the crypt isolation method may allow direct observation of the UC crypts.

Materials and Methods

Materials used in this study were derived from segments of colon and rectum surgically removed for

UC from 2 patients and 2 grossly normal areas taken from specimens of the colon and rectum with cancers.

One of the cases was 30-year-old man suffering from the disease for 16 years, and another one was 18-year-old woman for 9 years. The first case had severe dysplasia in the mucosa and the 2nd case was difficult for medical treatment. They were operated when the disease were clinically thought to be in remission phase.

An HCl-digestion method was used for the tissues fixed with 5% formaldehyde in 0.01 phosphate buffer (pH 7.4) for several days. The tissues were sliced at 3 - 4mm in width, then further fixed with 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) for 2 hours, and washed with phosphate buffer. The specimens were then placed in 8N HCl at 60℃ for 60 to 90 minutes, and then manually agitated for 30 minutes in the same solution. They were treated for 30 seconds at 26 kHz ultrasonic waves in an ultrasonicator (UO 150 FS, Kokusai Electric Co., Tokyo, Japan), and then rinsed three times in phosphate buffer.

All specimens were immersed in 2% tannic acid solution for 1 hour and post-osmicated in 1% osmium tetroxide solution for 2 hours. They were dehydrated in a graded ethanol series, followed by replacement with t-butyl alcohol. They were then dried by the t-butyl alcohol freeze-dry method[4] in an evacuator (VFD-20, Vacuum Device, Ibaraki, Japan). The isolated crypts were mounted on brass stubs with double stick tape. They were coated with gold in an ion-coater (IB-5, Eiko, Ibaraki, Japan) and observed with SEM (S-430 Hitachi, Japan) operated at an accelerating voltage of 15 kV.

For light microscopic observation, 3 to 4 mm thickness tissues were embedded in paraffin and cut into 4 μm sections, which were stained with hematoxylin and eosin (HE).

Results

Normal colonic mucosa

On routine HE staining, the normal human colonic crypts were visualized as a single tubular gland consisted mainly of Goblet cells, and they were arranged longitudinally at almost equal interval with scant interstitial connective tissue, vessels and so on (Fig. la).

An individual isolated crypt was observed as a single straight tubular gland resembling a test tube. The length of the crypts were arranged from 220 to 300 μm (n = 43). (Fig. 1b).

Ulcerative colitis

Light microscopic finding

Light microscopy showed various grades of inflammation and regeneration of colonic glands in the mucosa. They frequently revealed denuded superficial epithelia and scant crypts, concomitant widened lamina propria with diffuse infiltration of lymphocytes, plasma cells and polymorphonuclear neutrophils in lamina propria usually involving upper part of the mucosa, together with hyperplasia of lymphoid tissue usually placed at lower half of the mucosal layer. The layer of muscularis mucosae usually was reserved with untangled or thickened (Fig. 2a). A very few crypt abscesses were also seen.

The isolated crypts

The crypts of UC were so fragile and many of them were damaged during the procedure. However, 23

almost complete crypts were successfully gotten out of hundreds of crypt.

They were observed by SEM. They usually showed irregularities in shape, length and width compared to those of normal. Many crypts (18/23) have several short or long branchings (Fig. 3b, c arrows) and small protuberances (Fig. 3b*). Some crypts appeared stunt and distorted in shape with notches (Fig.3a, b small arrows). The length of the crypts were ranged from 180 to 460 μm (n = 23). The width of the crypts, which include non-branching part and branchings of the crypts, ranged from 32 to 270 μm (measured point = 93).

Discussion

An increase of the incidence of ulcerative colitis is a social problem in Japan. The incidence has increased rapidly from 1966 according to popularizing of Western foods, tending to remain at the same level since 1975[1]. The consumption of Western foods is associated with the risk of developing UC (P = 0.04), especially margarine (P = 0.005) and bread for breakfast (P = 0.07)[5].

The disease usually requires long time treatments and care through patient's life. Besides, she/he carries a troublesome problem of an increasing risk for developing colorectal cancer[6]. Significant risks of carcinoma after 10 years, which may in some cases, require frequent surveillance biopsies or event prophylactic bowel removal.

On the histological findings, detailed explanation made by Fenoglio-Preiser CM et al.[7] is valuable to understand the UC. They mentioned that the UC frequently repeat acute and remission phase. In active colitis, lymphocytes and plasma cells accumulate in the lamina propria, together with foci of polymorphonuclear leukocytes, which collect within the epithelial crypts producing cryptitis, crypt abscesses, and crypt rupture. In colitis in remission, microscopic examination shows variable degrees of mucosal atrophy. This may take the form of loss of parallelism and branching of the crypts to a more severe reduction in the number of crypts per unit area and shortening with a characteristic gap between the base of the crypts and the luminal surface of the muscularis mucosae. A few crypts had branches which came from the lateral part or the upper part of the crypts. The muscularis is often thickened with fraying of its fibers.

Our two cases of UC, clinically thought to be in remission phase, showed intermingled findings of active and remission phase. They showed various intermingled findings such as abundant inflammatory cell infiltration in lamina propria, atrophic/hypertrophic tendency of the crypts, even a few crypt abscesses. Because the disease does not make abrupt change, the findings may have shown intermingled or differences at the places of the large bowel. The size and shape of the regenerated crypts varied from each other. Several crypts apparently had branches. Compared to the normal, the volume of the crypts had increased.

Three- dimensional structures of the isolated crypts on SEM observation, normal crypts were same as other report[3]. The configurations of the crypts in the UC colon, the notable features of them were irregularity in shape, length and width. Especially, the branchings and stunt of the crypt are worthy of mention.

The irregularity of the crypts can be comprehensively understood as the result of severe inflammation of crypt and interstitial tissue. The stunt of the crypt may be results of crypt destroy and immature regeneration of the crypt.

The most special mention is the branching and notch of the crypts. These findings are supposed to be particular phenomena of regeneration of crypts. The generation mechanism of crypt in the colon mucosa is still poorly documented. Some colon crypts consist of pleural number of crypts, and it has been suggested that the number of crypts increase with time due to crypt multiplication and/or branching[7] but the actual process had unknown. Some investigators think that the mechanism by which new glands derive from the mucosal epithelium implies a longitudinal fission of preexisting glands[8]. Furthermore, morphological analysis of isolated colonic crypts in rats, postnatally, indicated that the crypts reproduce themselves by a fission mechanism, the division beginning at the crypt base and proceeding upwards until there are two separate crypts[9]. How is that in injured crypts? Our experimental study of the regeneration mechanism on acetic acid injured rectal mucosa using Wister adult rats had certified importance of the fission mechanism on crypt reproduction[10].

The notch at a bottom of a crypt indicates the beginning of the fission. And branching shows the process of crypt fission. Three- dimensional configuration of crypt in UC colon revealed direct influences of crypt injury and active regeneration of crypts by fission mechanism.

Conclusions

In light microscope specimens, the normal colonic mucosal crypts were straight tubular glands, and the isolated crypts were test-tube shaped. Most isolated crypts of the UC were visualized as distorted and irregularly branched.

It was suspected that inflammations introduce deformities of colonic crypt configuration directly and the destroyed crypts by inflammation are reproduced by fission mechanism.

References

1) Kitaoka T, Utsunomiya T, Yokota A. Epidemiological study of ulcerative colitis in Japan: incidence and familial occurrence. The Epidemiology Group of the Research Committee of Inflammatory Bowel Disease in Japan. J Gastroenter. 30 (Suppl 8) (1995) 5-8.

2) Viscido A, Bagnardi V, Sturniolo GC, Annese V, Frieri G, D'Arienzo A, Papi C, Riegler G, Corrao G, Cprilli R; GISC: Italian Group for the Study of the Colon and Rectum. Survival and causes of death in Italian patients with ulcerative colitis. A GISC nationwide study. Dig Liver Dis. 33 (2001) 686-92.

3) Araki K, Ogata T: Three-dimensional configuration of crypts of different types of colorectal adenomas. Scanning Microscopy. 9 (1995) 149-158.

4) Inoue T, Osatake H. A new drying method of biological specimens for scanning electron microscopy. The t-butyl alcohol freeze-drying method. Arch Histol Cytol. 51 (1988) 53-59.

5) Epidemiology Group of the Research Committee of Inflammatory Bowel Disease in Japan. Dietary and other risk factors of ulcerative colitis. A case-control study in Japan. J Clin Gastroenterol. 19 (1994) 166-71.

6) Ullman TA. Preventing neoplastic progression in ulcerative colitis. J Clin Gastroenterol. 39 (4 Suppl 2) (2005) S66-9.

7) Fenoglio-Preiser CM, Lantz PE, Listrom MB, Davis M, Rilke PO. The neoplastic large intestine.

In: Gastrointestinal Pathology: An Atlas and Text. Raven Press, New York. (1989) 727-790.

8) Maskens AP. Histogenesis of colon glands during postnatal growth. Acta Anat (Basel). 100 (1978) 17-26.

9) Fujimitsu Y; Nakanishi H; Inada K; Yamachika T; Ichinose M; Fukami H; Tatematsu M. Development of crypt involves a fission mechanism as revealed by isolation of aberrant crypts. Jpn J Cancer Res. 87 (1996) 1199-203.

10) Lei Cheng, Keijiro Araki, Yasuo Furuya, Takanori Matsuoka, Kunihiro Mashima, Michiya Kobayashi, Kimio Mtsuura. Morphological Study of the Regeneration Mechanism of Acetic Acid-injured Colon Crypts in the Rat. Med Electron Microsc. 33 (2000) 165-171.

Figure Legends

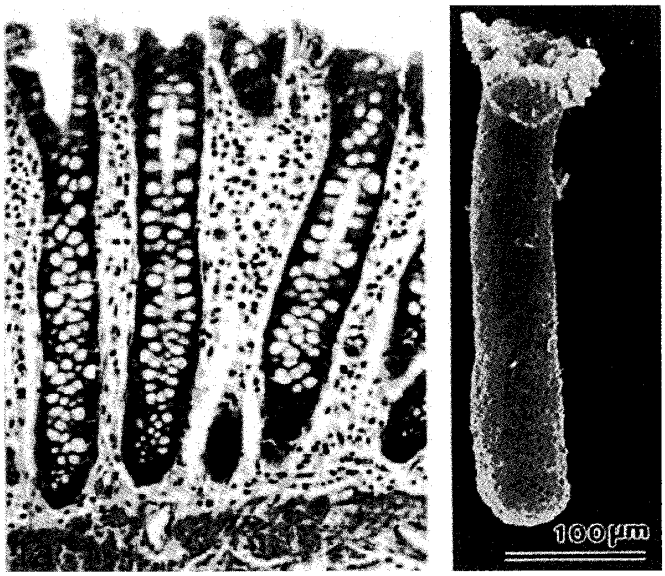

Figure 1. Micrographs of the normal colon.

(1a) A H-E stained section. Crypts of Lieberkühn are seen as single straight tubule.

(1b) An isolated crypt from the normal colon. The crypt is seen as a single straight tube like a test tube. Bar = 100 μm.

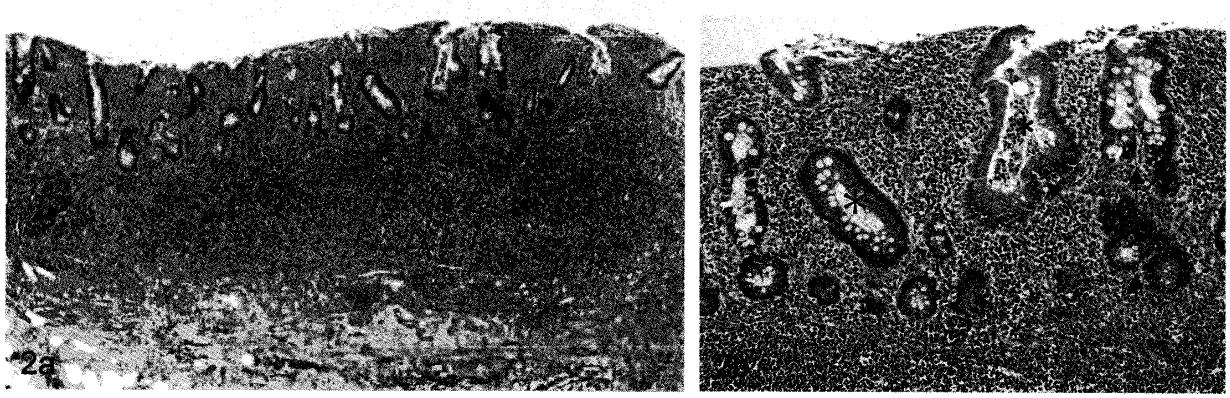

Figure 2. Micrographs of an ulcerative colitis. H-E stained section.
(2a) The lamina propria is wide and consisted of round inflammatory cells namely plasma cells. Note abundant lymphocyte accumulation at lower half of the mucosal layer. A layer of retaining thick muscularis mucosa is seen at the bottom of the picture. Original magnification, x20.
(2b) A high power magnification of the Fig 2a. Almost denuded superficial epithelium is seen. The irregularly arranged crypts are sparsely seen. Crypts were varied from each other in size, shape and distribution. There seems to be branching of crypts (arrows) and bulky crypts are seen (stars). Density of Goblet's cells in a crypt was less than that in the normal crypts.

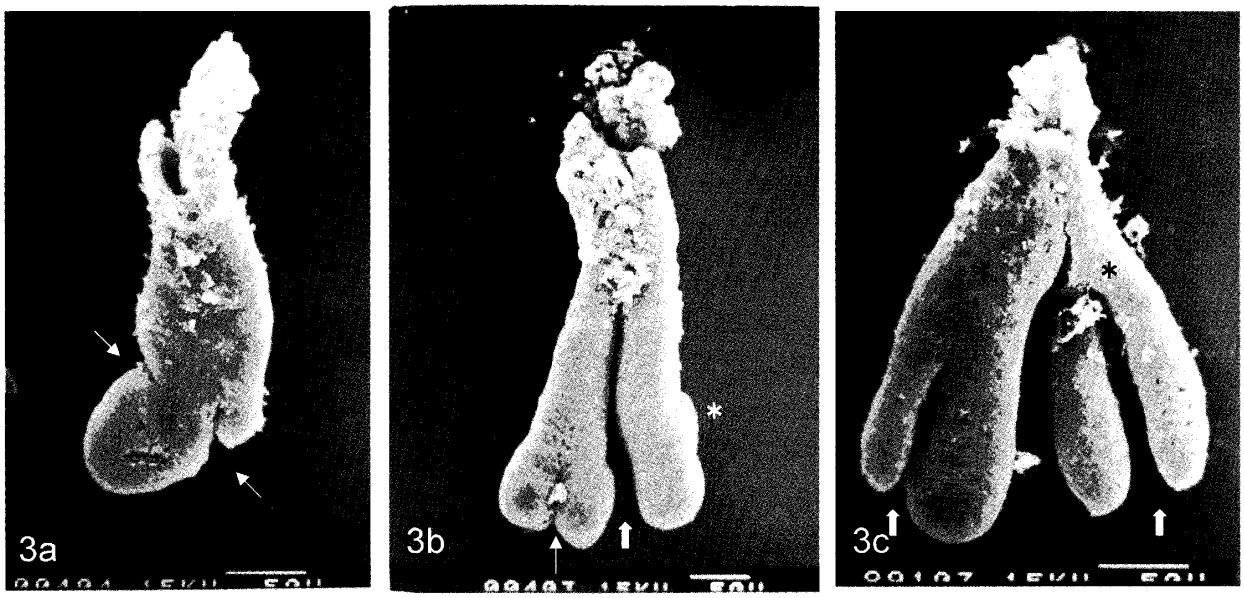

Figure 3. The isolated crypts.
(3a) A short but bulky isolated crypt. The dumpy crypt looks like distorted and has notches (arrows). The width is not even. Bar = 50 μm.
(3b) A crypt showing a notch (a small arrow), branching (a large arrow) and a small protuberance (a star). Bar = 50 μm.
(3c) A crypt with multiple branchings. The crypt is consisted of grossly two branches (stars*) and each branch make furthermore branches (large arrows). Bar = 50 μm.

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