DYSPLASIA AND CARCINOMA DEVELOPMENT IN EXPERIMENTAL COLITIS

Dysplasia and carcinoma development in a repeated dextran sulfate sodium-induced colitis model

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Abstract

Background: As an important mechanism underlying the increased risk of colorectal carcinoma development in patients with long-standing ulcerative colitis, promotion as a result of the regenerative process has been proposed. In the present study, a dysplasia-carcinoma sequence in a novel repeated colitis model in mice is documented.

Methods: Repeated colitis was induced by nine administration cycles of 3% dextran sulfate sodium (DSS; molecular weight, 54,000): each administration cycle comprised 3% DSS for 7 days followed by distilled water for the subsequent 14 days, to give conditions similar to the clinically observed active and remission phases in humans.

Results: Multiple colorectal tumors (nine low- and four high-grade dysplasias and two carcinomas) developed in 25 mice. These neoplastic lesions consisted of tubular structures, presenting as various types of elevated, flat and depressed tumor, similar to those in ulcerative colitis patients. A time-course study with assessment of the severity of colitis and in vivo bromodeoxyuridine uptake during a single 3% DSS administration cycle revealed a high level of regenerative activity in the colitis-affected mucosal epithelia.

Conclusion: Thus, with the present repeated colitis model, regeneration and neoplastic lesions were apparent, the biological features of which provide evidence of a colorectal dysplasia–invasive carcinoma sequence in ulcerative colitis.

INTRODUCTION

Although patients suffering from ulcerative colitis for a long period are at increased risk of colorectal carcinoma development, the factors responsible for the tumor induction are still not detailed.1–4 Appropriate experimental models to evaluate this phenomenon have yet to be firmly established. Clinically, patients with chronic ulcerative colitis typically display a recurrence–remission cycle, manifesting mucosal ulceration accompanied by necrosis and regeneration of the colonic mucosa. This necrosis–regeneration process would in theory be expected to enhance transformation of the mucosal epithelia to give rise to colorectal tumors.4,5

In order to check this hypothesis, we previously developed a novel reliable model for induction of acute and chronic ulcerative colitis in mice.6 Further, we induced colorectal dysplasia and carcinomas in mice with experimental chronic colitis,7 achieved by a single pretreatment with the carcinogen, azoxymethane, followed by three cycles of alternating administration of 3% synthetic dextran sulfate sodium (DSS) for 7 days, succeeded by distilled water for the subsequent 14 days. The DSS exposure induces inflammatory infiltration of the mucosa propria, ulceration and bloody diarrhea, which are characteristic of ulcerative colitis. With this model, ulcerative colitis-like lesions are mainly found in the distal colon, whereas with...
carrageenan, they are more pronounced in the proximal colon. In the present study, in order to determine whether repeated colitis alone as a result of intermittent DSS administration can induce dysplasia or carcinomas, mirroring the case with ulcerative colitis patients, we investigated development of colorectal neoplasia with nine administration cycles of 3% DSS: each featured alternation of 3% DSS for 7 days with distilled water for the next 14 days, to give conditions similar to the clinically observed active and remission phases. The time course of in vivo bromodeoxyuridine (BrdU) uptake was also investigated to assess the relationship between mucosal damage and regeneration.

**MATERIALS AND METHODS**

**Experimental animals**
A total of 105 specific pathogen-free CBA/J female mice (Charles River, Kanagawa, Japan), 6 weeks old at commencement, were housed in our Animal Laboratory Center under standard conditions and allowed free access to animal chow (CE-2; Nippon Clear, Tokyo, Japan) and distilled drinking water.

**Administration of dextran sulfate sodium**
The mice were given distilled water containing 0 or 3% (wt/vol) synthetic DSS (molecular weight, 54,000; Meitoh Sangyo, Tokyo, Japan) ad libitum.

**Protocol for induction of colorectal tumors and the time-course study**
Mice, 8 weeks old, received nine administration cycles of DSS: 3% DSS for 7 days followed by distilled water for the next 14 days. After exposure to 3% DSS for 7 days, the mice manifested diarrhea and occult blood in the feces. However, these signs disappeared about 8–9 days after return to distilled water (Fig. 1). For comparison purposes, a control group of five animals received distilled water instead of DSS. Surviving mice were killed under ether anesthesia at the end of the experiment (on day 189).

Polypoid or flat elevated or depressed lesions that developed were examined grossly and their natures confirmed histopathologically by observation of hematoxylin and eosin (HE) stained sections. Histopathological diagnosis of dysplasia and carcinomas was made on the basis of Riddell’s classification by two researchers. In particular, inflammatory changes were mixed in the DSS-administration group. Dysplasia lesions were carefully differentiated from regenerating crypts and diagnosed according to the cytological abnormalities and architectural alterations, as follows: cellular and nuclear pleomorphism, nuclear enlargement and hyperchromatism, marked stratification of nuclei, loss of nuclear polarity, irregular sizes of glands, and irregular glandular budding.

For a time-course study of induction of ulcerative colitis-like lesions and regeneration of colorectal mucosa, mice were killed daily during a single period of 3% DSS administration and the subsequent 7 days. Each subgroup comprised five mice.

**Morphological analysis**
All analyses were performed in a blind fashion following the methods described previously. Colonic preparations adhering to thick filter paper (Advantec; Toyo Roshi, Tokyo, Japan) were fixed in 10% formalin (pH 7.2). After HE staining of paraffin sections, the severity of colitis in one medial longitudinal section of each colon was histologically graded on a scale from 0 to 3 and expressed using a pathological index corresponding to the following standard scoring system: 0, normal; 1, focal inflammatory cell infiltration including polymorphonuclear leukocytes; 2, gland loss with inflammatory cell infiltration or crypt abscess formation; 3, mucosal ulceration.

**In vivo bromodeoxyuridine labeling and histological observation during a single period of 3% DSS administration**
One hour before killing, during a single period of 3% DSS administration and the subsequent 7 days, intraperitoneal administration of BrdU (Sigma Chemicals, St Louis, MO, USA) at a dose of 40 mg/kg body weight was performed. After fixation in 10% formalin solution (pH 7.4) for 48 h, longitudinal sections of all colons were dehydrated and embedded in paraffin. To identify BrdU-labeled nuclei, immunohistochemistry was performed with the streptavidin biotin-peroxidase method using a Histofine SAB-PO kit (Nichirei Co., Tokyo, Japan). In brief, deparaffinized sections (4 µm in thickness) were pretreated with 2 mol/L HCl for 60 min, treated twice with 0.1 mol/L borate buffer (pH 8.5) for 5 min, washed, and then incubated with 20-fold diluted mouse monoclonal antibody against BrdU (DAKO, Copenhagen, Denmark) for 1 h at room temperature. After exposure to biotinylated rabbit antinmouse immunoglobulin G for 40 min, the sections were incubated with peroxidase-labeled streptavidine for 40 min. The diaminobenzidine reaction was performed for color development. Faint nuclear counterstaining was achieved with Meyer’s hematoxylin solution.

Bromodeoxyuridine labeling indices were calculated for the following regions of the colon: the left side of the large intestine (2 cm proximal to the anus); transverse colon (the middle portion of transverse colon); right side of the colon (the middle portion of the ascending colon); cecum (the middle portion of the cecum); and the ileum (1 cm proximal to the ileocecal junction). Mucosal crypts identified from the base (adjacent to the muscularis mucosa) to the luminal sur-
face were selected at random in each region. Branched or extremely distorted crypts were deleted for analysis. After counting BrdU-labeled cells per 10 crypts, labeling indices were generated as the numbers of BrdU labeled cells for one crypt. The value for the BrdU labeling index on each experimental day was divided by that on day 0.

**Statistical analysis**

Data are presented as means with standard deviations. The significance of differences between two groups was determined by the non-parametric Mann–Whitney U-test and Fisher’s exact test.

**RESULTS**

**Induction and histopathological characteristics of colorectal tumors in chronic colitis mice with nine cycles of DSS/distilled water administration**

The repeated colitis mice group (n=25) that received nine administration cycles of DSS developed 15 neo-

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**Figure 1** Neoplastic lesions in the dextran sulfate sodium-induced chronic colitis model (HE). (a) Elevated low-grade dysplasia; (b) elevated high-grade dysplasia; (c) depressed high-grade dysplasia; (d) elevated carcinoma (a–d, ×50); (e) higher magnification of (d), submucosal invasion by the carcinoma is evident (×120).
Table 1 Colorectal tumors in the chronic ulcerative colitis model (scores are mean ± standard deviation)

<table>
<thead>
<tr>
<th>Group</th>
<th>Lesion</th>
<th>Right colon</th>
<th>Transverse colon</th>
<th>Left colon</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>3% DSS × 9 (n=25)</td>
<td>LGD</td>
<td>2 (F, D)</td>
<td>2 (D × 2 lesions)</td>
<td>5 (E, D × 4 lesions)</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>HGD</td>
<td>0</td>
<td>2 (E, D)</td>
<td>2 (E, F)</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Carcinoma</td>
<td>0</td>
<td>1 (E)</td>
<td>1 (E)</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Total†</td>
<td>2</td>
<td>5</td>
<td>8</td>
<td>15 (0.6 ± 0.7/mouse)</td>
</tr>
<tr>
<td></td>
<td>Colitis score</td>
<td>2.5 ± 0.5‡</td>
<td>2.8 ± 0.5†</td>
<td>3.0 ± 0.2‡†</td>
<td>2.8 ± 0.3‡</td>
</tr>
<tr>
<td>DW × 9 (n=5)</td>
<td>Tumors</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Colitis score</td>
<td>0.2 ± 0.4</td>
<td>0.4 ± 0.5</td>
<td>0.2 ± 0.4</td>
<td>0.2 ± 0.1</td>
</tr>
</tbody>
</table>

†P = 0.0211, significantly different from the control value. ‡P = 0.0005, significantly different from the control value. §P = 0.0006, significantly different from the control value. ¶P = 0.0397, significantly different from the right colon value.

No remarkable inflammation or tumors were evident in the ileum. 3% DSS, dextran sulfate sodium; carcinoma, invasive carcinoma; D, depressed lesion; DW, distilled water; E, elevated lesion; F, flat lesion; HGD, high-grade dysplasia; LGD, low-grade dysplasia; right colon, cecum and ascending colon.

plastic lesions (0.6 ± 0.7/mouse: three mice, two lesions; nine mice, one lesion) consisting of nine low-grade dysplasias, four high-grade dysplasias and two invasive carcinomas (Table 1). Histologically, they featured tubular structures with dysplastic cells, five being elevated, two flat and eight depressed (Fig. 1). No neoplastic lesions were observed in mice administered distilled water alone (n=5).

The mice that underwent repeated administration of 3% DSS showed severe colitis, particularly in the left side or transverse colon. High pathological scores on day 185 were evident in the colitis mice group, relative to the very low control values (Table 1).

**Time-course study of the induced colitis**

Pathology scores of colitis and data for in vivo BrdU uptake by cells of the mucosal epithelium are summarized in Figure 2a,b. Severe injury was evident on the left side of the large intestine and the transverse colon. Return to non-supplemented distilled water for 7 days resulted in relative improvement, but pathological signs on the left side of the large intestine remained severe. Similarly, BrdU labeling indices showed remarkable differences between the different sites. The colonic mucosa on the left side of the large intestine demonstrated particularly high regenerative activity on days 7, 8, and 9.

**DISCUSSION**

Following our introduction of a novel method for inducing acute and chronic colitis in mice by administration of DSS solution, we developed an experimental model of dysplasia and carcinoma development in chronic colitis mice with a single pretreatment of azoxymethane. The present experiment revealed that repeated mucosal necrosis and regeneration in ulcerative colitis itself may bring about the development of colorectal tumors. Thus, repeated colitis alone with nine cycles of 3% DSS and distilled water administration induced dysplasia and also carcinomas, similar to ulcerative colitis patients, although the neoplastic lesions were relatively few in number (mean, 0.6 tumors per mouse), compared to the findings with the previously reported model (10.5 tumors per mouse), featuring a single pretreatment of azoxymethane followed by three cycles of intermittent 3% DSS administration. In line with the supposition that foods are contaminated with only extremely small quantities of carcinogenic substances in life, a single pretreatment with the proximate carcinogen azoxymethane would be expected to induce more colorectal tumors.

With regard to the characteristics of the observed mucosal tumors, structural and cellular atypia, with alterations in tubular arrangement were seen, allowing a diagnosis of low-grade or high-grade dysplasia, according to established criteria. Further, submacroscopic elevated, flat or depressed lesions were evident, similar to ulcerative colitis-associated neoplasias. Most of the tumors found here corresponded to stage A of Astler and Coller’s classification. Submucosal invasion by tumor cells was also evident in two out of 15 lesions that developed during the relatively long period of the experiment, although no distant metastases were found.

With the present experimental regimen, repeated administrations of 3% DSS induced chronic ulcerative colitis predominantly on the left side of the large intestine, followed by the transverse colon, this correlating well with the locations of tumors. A similar link has been described for neoplastic lesions observed in patients suffering from chronic ulcerative colitis. Because DSS is negative in the Ames test for
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The DSS-induced colitis model has advantages for understanding mechanisms of induction of dysplasia and invasive carcinomas associated with colonic inflammation.\textsuperscript{19,20} It seems likely that the necrosis and regeneration of colorectal mucosa characteristic of ulcerative colitis facilitates transformation to dysplasia on the left side of the large intestine and transverse colon, where both pathology scores of DSS-induced colitis and mucosal regeneration activity as evidenced by BrdU uptake were the highest (Fig. 2a, b) in the present study, similar to the results in earlier reports.\textsuperscript{4,7,21}

Increases in both epithelial cell proliferation and cell death, partially associated with p53 accumulation and high p21WAF1 expression, are characteristic of active-phase ulcerative colitis. Accelerated epithelial cell turnover caused by chronic inflammation and epithelial damage might predispose the mucosa to DNA damage.\textsuperscript{5} Prostaglandins, the products of cyclooxygenase-2 (COX-2) acting on arachidonic acid, which are increased in active ulcerative colitis,\textsuperscript{22} stimulate proliferation of epithelial cells,\textsuperscript{23} suggesting an important role in tumorigenesis in ulcerative colitis. Furthermore, the fact that COX-2 and inducible nitric oxide synthase overexpression, evident in active ulcerative colitis,\textsuperscript{24–26} are associated with cellular resistance to apoptosis, indicates an elevated risk of neoplasia development in patients with ulcerative colitis.\textsuperscript{27–29}

In conclusion, with this repeated colitis model, colitis-regeneration and neoplastic lesions develop, the biological features of which are in line with a colorectal dysplasia–invasive carcinoma sequence in ulcerative colitis.

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Dysplasia–carcinoma in an UC model


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