

Dextran Sodium Sulfate-Induced Colitis in Germ-Free IQI/Jic Mice

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Abstract: This study presents a histological examination of dextran sodium sulfate (DSS)-induced colitis in germ-free (GF) mice. A comparison of the pathology between GF and conventionalized mice (CVz) was made to determine the role that intestinal microflora play in DSS-induced colitis. To induce colitis, GF and CVz IQI/Jic mice were given either 5% or 1% DSS orally. Administration of 5% DSS, a common concentration used to induce colitis in mice, caused gross rectal bleeding and a marked decrease in hematocrit as early as day one in GF mice. These mice died on day three due to massive bleeding into the intestinal lumen. In contrast, CVz mice did not die during the seven-day experimental period. Histopathological examination three days after administration of 5% DSS did not reveal any colitis lesions in GF mice, but CVz mice had developed moderate colitis in the large intestine. Administration of a low concentration of DSS (1%), which only induces mild basal crypt loss in CVz mice, caused severe colitis in the distal colon in GF mice, and they died on day 14. These data suggest that intestinal microflora are not necessary for the induction of colitis. Furthermore, DSS may be highly toxic to GF mice, and when given at a concentration of 5% it causes massive bleeding into the intestinal lumen resulting in death prior to development of colitis.

Key words: colitis, DSS, germfree, mice, microflora

Introduction

Oral administration of dextran sodium sulfate (DSS) in drinking water to experimental animals such as mice [1–3, 5–7, 13, 14, 18, 19, 21], rats [4, 28], hamsters [20, 29], and guinea pigs [9, 10] induces colitis that is pathologically similar to the condition of ulcerative colitis (UC) in humans. Both acute and chronic colitis can

be induced by changing the concentration of DSS or by the administration of multiple doses of DSS [21]. DSS-induced colitis is considered to be a good model for human UC and it has been used to evaluate the effects of anti-inflammatory drugs [1, 2, 18, 19]. Despite its widespread use as a model, the pathogenesis of DSS-induced colitis is still unclear.

It has been suggested that intestinal microflora play

a role in the induction of colitis. These suggestions have resulted from studies using animal models of human inflammatory bowel disease (IBD), including those for UC and Crohn's disease. For example, interleukin (IL)-2 and IL-10 deficient mice develop colitis when they are transferred from specific pathogen-free (SPF) or sterile conditions to conventional conditions [15, 25, 26]. Similarly, germ-free (GF) guinea pigs do not develop colitis when administered carrageenan [22]. Carrageenan is a sulfated polysaccharide with high molecular weight similar to DSS that can be used to induce experimental colitis.

Despite these findings, Bylund-Fellenius *et al.* [5] have reported the development of DSS-induced colitis with concurrent high mortality in GF mice. However, this study was limited in its investigations into the clinical and histological changes found in colitis. In an effort to further elucidate the involvement of intestinal microflora in the development of colitis in mice administered DSS, we performed an extensive histological examination of colitis lesions in both GF and conventionalized mice (CVz).

Materials and Methods

Mice

Female GF IQI/Jic mice (Japan CLEA Co., Tokyo, Japan) were maintained in vinyl isolators in a room kept at a constant temperature ($22 \pm 2^\circ\text{C}$) and humidity ($55 \pm 5\%$). All mice were six to seven weeks old at the start of DSS administration. Mice were fed a commercial diet (Labo MR Stock; Nihon Nosan Industry, Yokohama, Japan) sterilized by γ -irradiation (50 kGy), and given sterile water *ad libitum*. The light cycle was 12 hr light/dark. To confirm GF status, microbiological assays were performed on a monthly basis by culturing feces, bedding, and drinking water in thioglycollate medium (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan), cooked meat medium (Difco Laboratories, Detroit, MI, USA), and potato dextrose broth (Difco).

Conventionalization

Conventionalization was performed by feeding four-week-old GF IQI/Jic mice with feces (10 mg in 0.1 ml saline) obtained from SPF BALB/c Cr Slc mice (Japan SLC Co., Shizuoka, Japan). These animals were used

in experiments as CVz mice three weeks after this procedure. Microbiological analysis of feces from CVz and SPF animals was then performed. Fresh feces were immediately transferred to an anaerobic box filled with nitrogen and carbon dioxide, which was then moved to another laboratory (Seac Yoshitomi, Ltd., Fukuoka, Japan) for cultivation. No more than 3 hr elapsed between collection and cultivation. Feces were weighed and then homogenized in a 50-fold volume (v/w) of diluent buffer. This homogenized solution was then diluted serially and plated onto selective agar media according to the method of Mitsuoka [17]. Number of bacteria were expressed as log₁₀ counts of viable bacteria per gram wet weight of feces.

Experimental design

To induce colitis, GF and CVz mice were given 5% or 1% (w/v) DSS (MW=40,000, ICN Biomedicals Inc., CA, USA) in their drinking water *ad libitum*. The experimental period in the mice given 5% and 1% DSS was 3 and 14 days respectively. The length of this period was governed by the survival of the mice. The clinical condition of the animals was checked daily. Particular attention was paid to observing whether there was any gross bleeding from the anus, which is indicative of rectal bleeding. Body weight in the mice given 5% DSS was measured daily. In the mice given 1% DSS, body weight was measured on days 0–3, 5, 7, 9, 11, 13, and 14. At the end of the experimental period, surviving mice were sacrificed by cervical spine dislocation. At this time, tissues for histological analysis, and blood samples for measuring hematocrit (Ht) were collected. The experimental protocol and design was approved by the Saga Medical School Animal Experimentation Committee and performed according to the Saga Medical School Guidelines for Animal Experimentation.

Histologic examination

In the CVz mice given 5% DSS, histological examinations were performed on days 0 (not treated), 1, 3, 5, and 7. In the GF mice given 5% DSS, histological examinations were performed on days 0, 1, and 3 only, since they died three days after DSS administration. In the mice given 1% DSS, histological examinations were performed on day 14 in both the GF and CVz mice. The cecum and colon from each mouse was dissected

and fixed in 10% neutral-buffered formalin solution. After fixation, the colon was divided into three segments of equal length from the proximal, middle, and distal colon. At least three transverse sections at equal intervals were taken from each of these segments. Three sections at equal intervals were also taken from the cecum of each mouse. The specimens were then paraffin embedded and cut into 3–4 μm sections for staining according to routine procedures. Sections were stained with hematoxylin and eosin (H-E), Masson's trichrome, periodic acid Schiff (PAS) and phosphotungstic acid hematoxylin (PTAH).

Statistical analysis

Data are expressed as the mean \pm SD. Statistical analysis was performed using either the Student's *t*-test when the *F* values were equal, or Welch's *t*-test when the *F* values were not equal. A value of $p < 0.05$ was considered statistically significant.

Results

Clinical condition and macroscopic examination:

Administration of 5% DSS

The fecal bacteria composition of some of the SPF BALB/c and CVZ IQI/Jic mice three weeks after conventionalization is shown in Table 1. No major differences in the fecal bacteria composition between the two groups were observed.

A summary of the day on which gross rectal bleed-

Table 1. Composition of fecal bacteria in SPF and CVZ mice

	BALB/c (SPF)			IQI/Jic (CVZ)		
	1 ^{a)}	2	3	1	2	3
Bacteroides	7.4 ^{b)}	8.6	9.5	9.0	7.9	7.4
Clostridium	10.0	9.3	9.2	9.6	8.6	10.1
Eubacterium	5.3	4.7	3.9	4.0	3.5	2.9
Fusobacterium	— ^{c)}	—	—	—	—	—
Lactobacillus	9.8	8.6	9.4	9.7	8.6	9.8
Enterobacteriaceae	4.4	4.3	6.0	5.9	5.4	5.9
Enterococcus	5.2	6.5	7.3	6.6	4.8	5.3
Pseudomonas	—	—	—	—	—	—
Staphylococci	3.5	4.7	4.7	4.6	5.4	4.3
Yeasts	—	—	—	—	—	—

^{a)} Samples of feces from SPF or CVZ mice. ^{b)} Log number of bacteria per 1 g feces. ^{c)} Not detected.

ing began and the number of days until death following the administration of 5% or 1% DSS in GF and CVZ mice is listed in Table 2. GF mice showed rectal bleeding as early as day one, and they died three days after administration of 5% DSS. In contrast, CVZ mice showed rectal bleeding at day three and none died during the seven-day observation period. A significant loss of body weight ($p < 0.05$ vs. initial value) was observed on days two and three in GF mice (Fig. 1), whereas in CVZ mice a significant body weight loss ($p < 0.01$ vs. initial value) was observed between days five and seven.

The hematocrits (Hts) (%) covering the experimental period for the GF and CVZ mice administered 5% DSS are shown in Fig. 2. The Hts of both the GF and CVZ mice decreased as early as day one ($p < 0.01$ vs. initial value) and then decreased further from days 1 to 3. GF mice showed a significant decrease in Ht when compared to CVZ mice ($p < 0.05$) one day following DSS administration. By day three, the Ht in GF mice did not differ significantly from that in CVZ mice. The Hts of CVZ mice recovered from days three to seven.

Post-mortem macroscopic examination on day one after administration of 5% DSS revealed that the cecum of GF mice was enlarged, dark red, and filled with a dark red fluid that appeared to be blood. On day three in GF mice, the liver, spleen, and kidney were very pale, probably as a result of severe anemia. In contrast, CVZ mice did not show any remarkable changes on day one, however both the cecum and proximal colon were dark red in color on day three. By days five and seven shortening of the colon and swelling of the spleen were observed in CVZ mice in addition to the above noted changes.

Administration of 1% DSS

In GF mice, rectal bleeding was observed on day

Table 2. Day of onset of rectal bleeding and death after administration of 5% or 1% DSS in GF and CVZ mice (IQI/Jic strain)

Groups	Rectal bleeding	Death
5% DSS GF	day 1 (10/10)	day 3 (3/10)
CVZ	day 3 (1/5)	— (0/5)
1% DSS GF	day 9 (6/9)	day 14 (3/9)
CVZ	— (0/5)	— (0/5)

Number of affected mice/total is in parentheses.

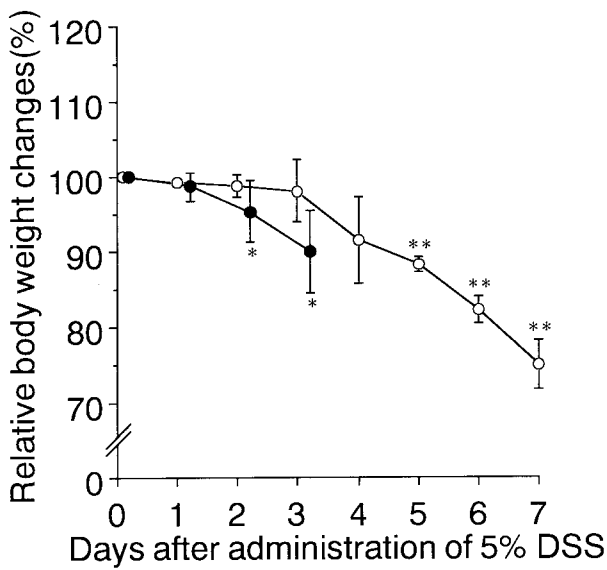


Fig. 1. Relative body weight change (%) in GF and CVz mice following administration of 5% DSS. A significant decrease in relative body weight vs. initial value was observed. ●: GF mice, N=10 except on day three when N=7. ○: CVz mice, N=5. *: $p < 0.05$. **: $p < 0.01$. Data are expressed as the mean \pm SD.

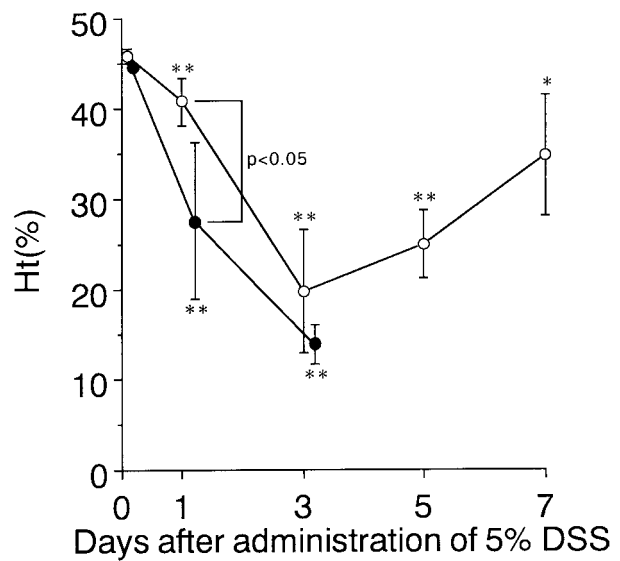


Fig. 2. Ht (%) in GF and CVz mice following administration of 5% DSS. A significant decrease in Ht vs. initial values was observed. ●: GF mice, N=5 except on day three when N=7. ○: CVz mice, N=5. *: $p < 0.05$. **: $p < 0.01$. Data are expressed as the mean \pm SD.

nine, and they died 14 days after administration of 1% DSS. The relative body weight of GF mice increased until day nine for both GF and CVz mice. A significant loss of body weight ($p < 0.01$ vs. initial value) was observed on days 13 and 14 in GF mice (Fig. 3), whereas the relative body weight in CVz mice increased until day 14.

Post-mortem macroscopic examination on day 14 after administration of 1% DSS revealed colonic shortening, pallor of the abdominal organs, yellowish-brown livers, and swelling of the spleens of GF mice. In contrast, CVz mice showed no clinical or macroscopic changes during the 14-day observation period.

Histologic examination:

Administration of 5% DSS

Histological findings in GF and CVz mice after administration of 5% or 1% DSS are summarized in Table 3. One day after administration of 5% DSS to GF mice, edema of the submucosa was observed, particularly in the cecum. However, destruction of the mucosa was not found in the large intestine, although gross rectal bleeding was clinically noted in all mice. After three days, when the GF mice died, we still could not find any

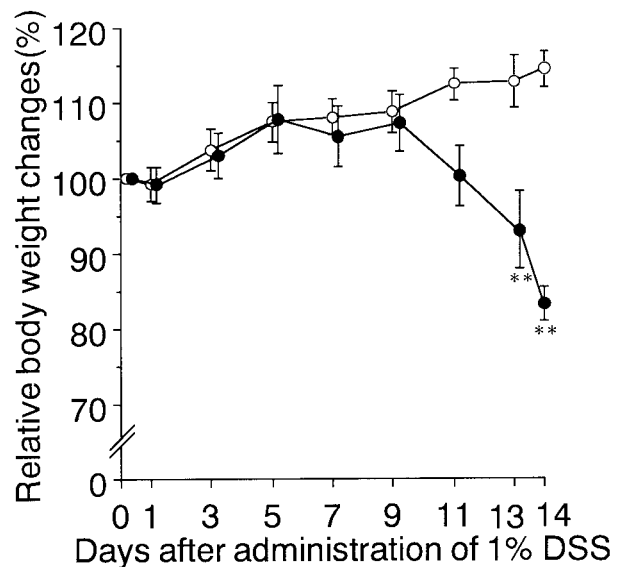


Fig. 3. Relative body weight change (%) in CVz and GF mice following administration of 1% DSS. A significant decrease in relative body weight vs. initial value was observed. ●: GF mice, N=9 except on day 14 when N=6. ○: CVz mice, N=5. **: $p < 0.01$. Data are expressed as the mean \pm SD.

Table 3. Major histological findings in GF and CVz mice (IQI/Jic strain) after administration of 5% or 1% DSS

Findings	5% DSS									1% DSS	
	GF			CVz					GF	CVz	
	0 ^{a)}	1	3	0	1	3	5	7	14	14	
Mucosa											
Crypt loss	0/5 ^{b)}	0/5	0/7	0/5	2/5	4/5	5/5	5/5	6/6	3/5	
Inflammatory cells infiltration	0/5	0/5	0/7	0/5	1/5	3/5	5/5	5/5	6/6	3/5	
Hemorrhage/Thrombus	0/5	0/5	0/7	0/5	0/5	0/5	0/5	1/5	4/6	0/5	
Erosion/Ulcer	0/5	0/5	0/7	0/5	0/5	2/5	5/5	5/5	6/6	0/5	
Submucosa											
Edema	0/5	2/5	1/7	0/5	0/5	3/5	5/5	5/5	6/6	1/5	
Inflammatory cells infiltration	0/5	0/5	0/7	0/5	0/5	2/5	5/5	5/5	6/6	0/5	
Hemorrhage/Thrombus	0/5	0/5	0/7	0/5	0/5	0/5	0/5	1/5	4/6	0/5	

^{a)} Days after administration of DSS. ^{b)} Number of affected mice/total.

lesions indicative of colitis in the large intestine (Fig. 4, A, C and E). In contrast, the CVz mice developed moderate colitis predominantly in the cecum (Fig. 4B) and proximal colon (Fig. 4D) by day three. In these mice moderate basal crypt loss was observed in the middle and distal colon (Fig. 4F). On days five and seven, inflammatory cell infiltration and ulceration became more extensive and diffuse in the large intestine of CVz mice.

Administration of 1% DSS

On day 14 after administration of 1% DSS to GF mice, severe colitis was observed in the distal colon (Fig. 5E). The observed lesions were essentially the same as those observed in the CVz mice given 5% DSS for 7 days, but hemorrhaging with thrombi was less frequently observed, and inflammatory cell infiltration was less marked in the GF mice compared to CVz mice. In contrast, in CVz mice given 1% DSS, only mild basal crypt loss was observed in the distal colon (Fig. 5F). Small patchy lesions with slight inflammatory cell infiltration and lamina propria edema were observed in the cecum, and proximal and middle colon of both the GF and CVz mice (Fig. 5, A-D).

Discussion

Results from the present study have confirmed that intestinal microflora are not necessary for the induction of colitis in a DSS-induced murine colitis model. However, we found that GF mice are more susceptible to DSS-induced colitis than CVz mice. GF mice given a low 1% dose of DSS developed severe colitis in the

distal colon with a concurrent high mortality, whereas CVz mice did not die or develop severe colitis when given the same dose of DSS. However, administration of 5% DSS, which is the dose more commonly used to induce colitis in mice, led to death on day three in GF mice. This result is similar to that reported by Bylund-Fellenius *et al.* [5]. However, we did not observe any lesions indicative of colitis in the large intestine of GF mice, although all mice showed gross rectal bleeding, which is considered to be a clinical sign of colitis, as early as one day after administration of DSS. These findings suggest that the cause of death in the GF IQI/Jic mice may not be due to colitis. In addition it suggests that when using GF mice as a colitis model, rectal bleeding may not be a suitable clinical marker, and may not reflect the pathological development of colitis.

The cause of death in GF mice given 5% DSS may have been due to massive bleeding into the intestinal lumen. Our macroscopic observations, such as decreased Ht, gross rectal bleeding, and dark red fluid in the cecum, support this suggestion. These findings were observed in GF mice on day one after administration of 5% DSS. Furthermore, the dark red stained contents were observed in the large intestine but not in the small intestine. We did not observe any mucosal destruction in the intestines of these mice and therefore concluded that this was not the cause of the bleeding into the large intestine of GF mice. The mechanism by which massive blood loss into the intestinal lumen is occurred was not clear, however, DSS is an analog of heparin and it has anticoagulant activity [11, 24], its action on the clotting cascade may

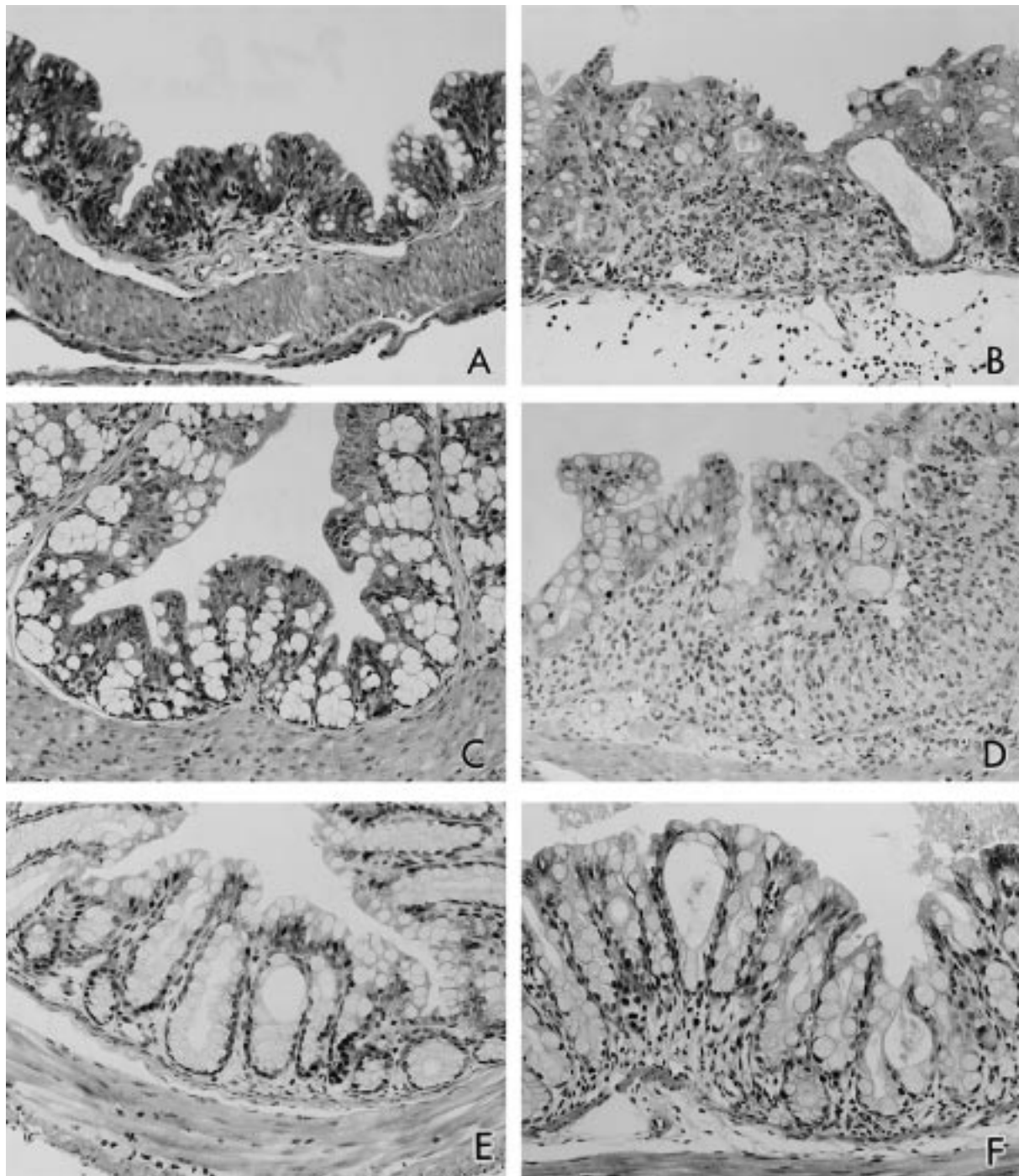


Fig. 4. Histopathological findings of the large intestine of GF and CVz mice on day three after administration of 5% DSS. In GF mice, no remarkable changes were observed in the cecum (A), proximal colon (C), or distal colon (E). In CVz mice, focal erosion or ulcers are observed in the cecum (B) and proximal colon (D), and moderate crypt loss is observed in the distal colon (F). H-E stain, $\times 50$.

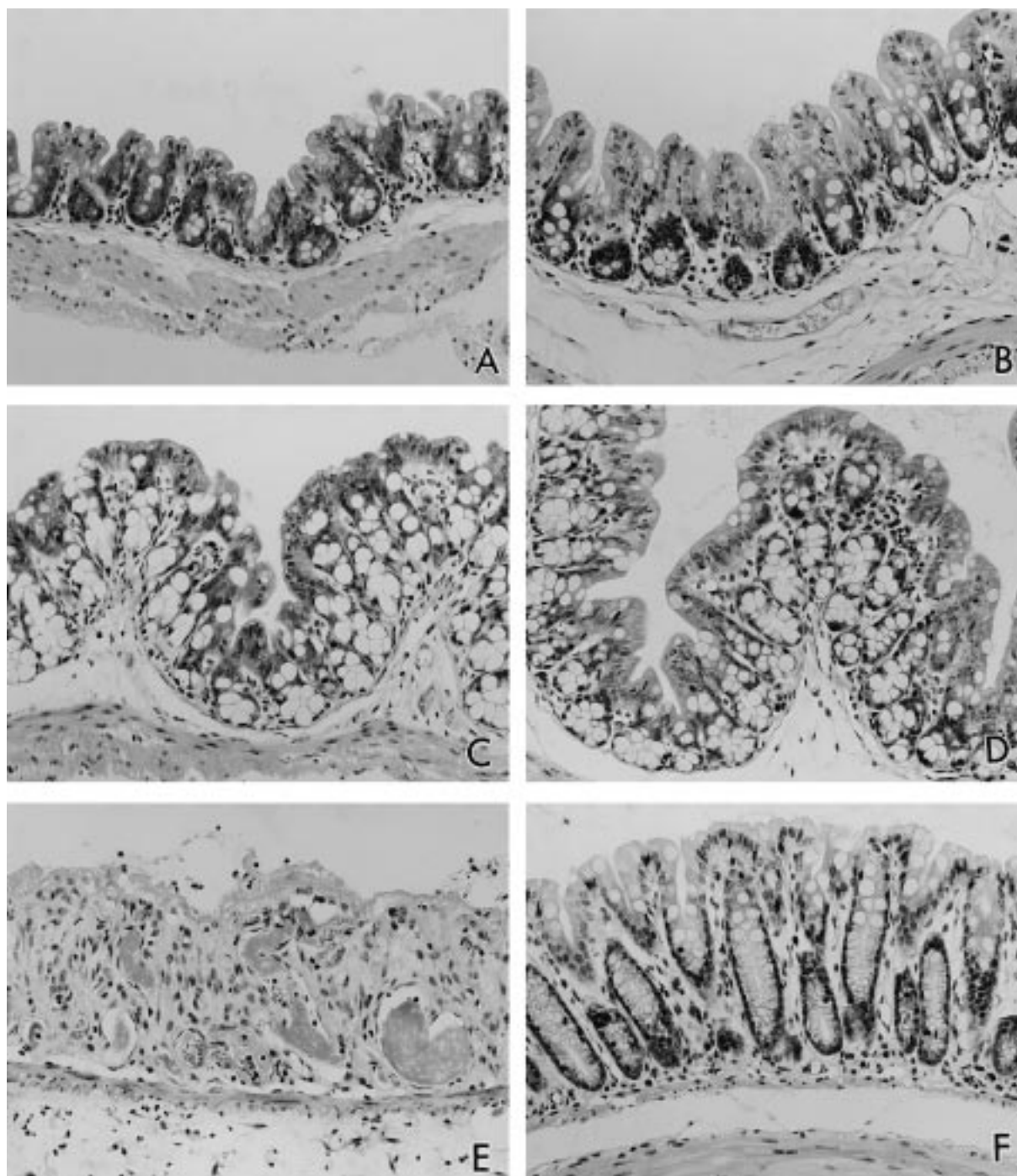


Fig. 5. Histopathological findings of the large intestine of GF and CVz mice on day 14 after administration of 1% DSS. In GF mice, focal regions of slight inflammatory cell infiltration and edema of the lamina propria of the cecum (A) and proximal colon (C) are apparent. However, in the distal colon (E) of GF mice, severe ulceration, hemorrhages with frequent thrombi, and slight inflammatory cell infiltration was observed. In CVz mice, histological changes in the cecum (B) and proximal colon (D) were similar to those found in GF mice, however erosion or ulcer formation were not observed in the distal colon (F). H-E stain, $\times 50$.

have contributed to the uncontrolled bleeding into the intestinal lumen in these mice.

We did not observe colitis lesions in GF mice given 5% DSS. This may be because these mice died from the toxic effects of DSS before they could develop colitis. Also, the total volume of water containing 5% DSS consumed by the GF mice was lower than that consumed by the CVz mice. This may also have contributed to the toxic effects of the DSS. On day one after administration of 5% DSS, water consumption by GF mice was similar to that of the CVz mice. However, water consumption by GF mice was negligible by day two, whereas CVz mice began drinking less water on day four (data not shown).

In this study, we used the IQI/Jic strain of mice which may be more sensitive to DSS-induced colitis. The features of colitis that we observed in CVz mice with colitis induced by 5% DSS were similar to those described in previous reports [6, 21]. However, when compared to our previous data using BALB/c mice [21], the CVz IQI/Jic mice rapidly developed intestinal erosion, whereas BALB/c mice had only mild crypt loss on day three after administration of 5% DSS [13, 14]. In BALB/c mice, intestinal erosion was first noticed on day five following dosage with DSS [13, 14].

Several factors may be contributing to the observed difference between GF and CVz mice in their susceptibility to DSS-induced colitis. Bylund-Fellenius *et al.* [5] have suggested that degradation of DSS by intestinal microflora is a likely reason for this difference. However, we believe that this is unlikely because in one of our previous studies we did not detect any small fragments of DSS in the feces of mice on day three after administration of 5% DSS on polyacrylamide gels [14]. Certain bacterial strains produce dextranase, which hydrolyzes the α -1, 6 glycosidic linkage [12, 27]. Dextran, which has the same structure as DSS except without the sulfate ester, is hydrolyzed into small fragments by incubation with rat cecal contents [16]. DSS is composed of α -1, 6-linked D-glucose and contains up to three sulfate esters per sugar unit [24] which reportedly stabilize glycosidic linkages [23].

Another possible reason for the difference in susceptibility to DSS-induced colitis may be due to a difference in the function of the mucosal barrier in these mice. For instance, xylose, which is absorbed from the intestinal lumen by both passive and carrier-facilitated

means, is absorbed at approximately twice the rate in GF mice compared to CVz mice [8]. Therefore, it is possible that the absorption or permeability rate of DSS into the mucosa may differ between GF and CVz mice. However, the specific reason for the difference in susceptibility to DSS-induced colitis between GF and CVz mice remains unclear.

Despite many studies, the mechanism of induction and the pathogenesis of DSS-induced colitis are still unclear. The DSS-induced murine model for colitis was originally reported by Okayasu *et al.* [21]. They noted that the population of intestinal microflora was changed by the administration of 5% DSS and suggested that the microflora may be involved in induction of colitis. Cooper *et al.* [6] reported that the initial lesion in DSS-induced colitis is a loss of basal crypts, and suggested that the infiltration of inflammatory cells into the mucosa is secondary to this lesion. Axelsson *et al.* [3] and Dieleman *et al.* [7] reported that the immune system does not play an important role in DSS-induced colitis because they observed histological changes similar to those of Cooper *et al.* [6] in severe combined immunodeficient (SCID) mice lacking functional T and B lymphocytes. It has also been reported that DSS inhibits proliferation of a murine colon carcinoma cell line *in vitro* [7]. These observations suggest that DSS may cause mild injury to colonic epithelial cells resulting in increased mucosal permeability and infiltration of toxic luminal bacteria or bacterial products into the mucosa. These substances may then destroy the epithelial cells of basal crypts and induce an inflammatory reaction in the colonic mucosa [4, 13]. However, our study has shown that mucosal destruction occurs without the involvement of intestinal microflora. The mechanism of mucosal destruction in DSS-induced murine colitis may be different from that of colitis observed in several knockout mouse strains [15, 25, 26] and in a carrageenan-induced model of colitis [22].

In conclusion, we have demonstrated that intestinal microflora is not necessary for the induction of colitis in a DSS-induced murine model. We also demonstrated that GF mice are highly susceptible to low-dose DSS-induced colitis. Administration of 5% DSS is toxic to GF mice and causes massive bleeding into the intestinal lumen resulting in death prior to the development of colitis.

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