Adverse Reaction of Dextran Sodium Sulfate-Induced Colitis in a Collagen-Induced Mouse Arthritis Model

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The pathogenic relationship of ulcerative colitis and rheumatoid arthritis is not known. Therefore, we examined dextran sodium sulfate (DSS)-induced colitis separately and in combination with a mouse arthritis model that mimics rheumatoid arthritis and evaluated the deterioration-related factors of each condition. Arthritis was induced in a collagen-induced arthritis mouse model using DBA/1J mice and ulcerative colitis was induced by the administration of drinking water containing 3.0% (w/v) DSS. The arthritis/DSS-treated mice developed worse colitis scores compared to that of the other groups of mice. The arthritis/DSS-treated mice did not demonstrate changes in hind foot volumes or in the concentration of matrix metalloproteinase-3 (MMP-3) in the plasma; however, plasma levels of interleukin-6 (IL-6) and tumor necrosis factor (TNF)-α were increased. Our results showed that IL-6 and TNF-α may influence the deterioration effect of colitis in arthritis mice.

Key words ulcerative colitis; arthritis; interleukin-6; tumor necrosis factor-α; matrix metalloproteinase-3

INTRODUCTION

The pathogenesis of ulcerative colitis and rheumatoid arthritis have not been fully elucidated, but it is suggested that these diseases have common aspects because of deterioration effects on the body caused by excessive autoimmune reactions. It has actually been reported that these diseases may occur clinically in association with each other. While it has only been rarely reported, a form of peripheral arthritis like rheumatoid arthritis is known to be present in some patients with ulcerative colitis. Therefore, the relationship of rheumatoid arthritis and ulcerative colitis remains unknown. This, in part, is a result of no reported basic research experiments aimed toward directly evaluating the potential relationship between these two conditions.

Previously, we separately described various biological factors associated with rheumatoid arthritis and ulcerative colitis by using a mouse rheumatoid arthritis model and a dextran sodium sulfate (DSS)-induced colitis model in mice, respectively. In the current study, we examined DSS-induced colitis in conjunction with the rheumatoid arthritis model in mice and confirmed deterioration factors of each condition with an aim of revealing the relationship between rheumatoid arthritis and ulcerative colitis.

MATERIALS AND METHODS

Experimental Animals and Collagen-Induced Arthritis
Ten-week-old specific pathogen free DBA/1J mice and collagen-induced arthritis DBA/1J mice were obtained from Japan SLC (Hamamatsu, Shizuoka, Japan). The mice were housed with a 12-h light cycle, a constant temperature of 23 ± 2°C, and a relative humidity of 55 ± 10%. The mice were euthanized by deep anesthesia with sodium pentobarbital. All experimental protocols were approved by the animal care committee in compliance with the regulations of Suzuka University of Medical Science.

DSS-Induced Colitis  DBA/1J mice and collagen-induced arthritis DBA/1J mice each were randomly assigned to two groups (n = 5 per group): DSS-treated group and mock-treated group. To induce ulcerative colitis, 3.0% (w/v) DSS (molecular weight: 36000–50000Da; MP Biomedicals, Solon, OH, U.S.A.) was administered to mice through drinking water for five consecutive days in the DSS-treated groups. Overall, this resulted in a total of four experimental groups of mice, the control group, the arthritis group, the DSS group, and the arthritis/DSS group. On the final day of the experiment, colitis development was monitored in each mouse by measuring animal body weight and observing the condition of the feces. The severity of colitis was determined by the feces condition and post mortem colon length. The fecal material was scored according to two parameters, stool consistency (0 = negative; 1 = soft; 2 = very soft but formed; 3 = liquid) and fecal bleeding based on the guaiac paper test (0 = negative; 1 = faintly blue; 2 = moderately blue; 3 = dark blue; 4 = visible blood) with the sum of the two parameters used to score the disease severity for the individual mice.

Measurement of Hind Foot Volume  As a reflection of the level of edema, the hind foot volume of each mouse was measured on the final experimental day using the method described in our previous report. Briefly, in the beaker filled with distilled water, the hind foot of each group mice was immersed to the joints, and the hind foot volume was measured.

Collection of Mouse Blood and Colon Tissue  Mice were anesthetized with 50mg/kg sodium pentobarbital (Nacalai Tesque, Kyoto, Japan) administered intraperitoneally, and blood and colon tissue were collected by cardiac puncture on the day of the experiment. The collected blood was centrifuged for 10 min at 3000 × g at 4°C and the plasma was stored at −30°C. The plasma was subsequently used for pro-
tein analysis as described.

**Staining of Colon Tissues**  The colon tissue was isolated and fixed in phosphate-buffered saline (PBS) containing 4% paraformaldehyde (Wako Pure Chemical Industries, Ltd., Osaka, Japan). Fixed tissue specimens were embedded in Tissue Tek OCT Compound (Sakura Finetek, Tokyo, Japan) and frozen. For histopathological analysis, the tissue blocks were cut into 5-µm-thick sections that were stained with hematoxylin and eosin (H&E) according to conventional procedures and were analyzed to evaluate the degree of colon inflammation caused by DSS. We evaluated ten regions of the colon tissue microscopically, and the acquired images where the colon appeared flat were randomly chosen. The following morphological criteria were considered: score 0 = no damage, score 1 (mild) = focal epithelial necrosis, score 2 (moderate) = diffuse necrosis of the villi, score 3 (severe) = necrosis with neutrophil infiltrate in the submucosa, and score 4 (highly severe) = widespread necrosis with massive neutrophil infiltrate and hemorrhage.11)

Moreover, the tissue sections were washed with PBS and incubated at room temperature for 2h with a primary antibody against the neutrophil marker Ly-6G (1 : 100; R&D Systems, Inc., Minneapolis, MN, U.S.A.). After washing in PBS and incubation at room temperature for 2h with fluorescein isothiocyanate-conjugated anti-rabbit immunoglobulin G (IgG) (1:30; Dako Cytomation, Glostrup, Denmark), neutrophils were quantified under a fluorescence microscope using ImageJ software (National Institutes of Health, Bethesda, MD, U.S.A.).

**Enzyme-Linked Immunosorbent Assay (ELISA) Analysis of Matrix Metalloproteinase-3 (MMP-3), Interleukin-6 (IL-6), and Tumor Necrosis Factor-α (TNF-α) in Plasma** The concentration of plasma MMP-3, IL-6, and TNF-α were measured using the appropriate ELISA kits (MMP-3, Bertin Pharma, Montigny le Bretonneux, France; IL-6, Enzo, Farmingdale, NY, U.S.A.; TNF-α, R&D Systems, Inc.) according to the manufacturers’ instructions. The optical density was measured with a microplate reader (Molecular Devices, Sunnyvale, CA, U.S.A.).

**Statistical Analysis** All data are presented as the mean ± standard deviation. For comparisons between test groups, Tukey’s post-hoc test or Steel–Dwass test was applied with p < 0.05 considered to be a statistically significant difference.

![Fig. 1. The Effect of Dextran Sodium Sulfate (DSS)-Treatment on Arthritis Mice (a–e)](image)

The body weight (a), and temporal response of the colitis score and images (b), hematoxylin and eosin staining of the colon tissue section and the histological score (c), neutrophil identification based on the expression of Ly-6G, as detected by immunohistochemistry (d), and quantified lengths of the large intestines and images (e) in the experimental groups of mice. Scale bar = 200 µm. Data are presented as the mean ± standard deviation (n = 5/group). *p < 0.05 (Tukey’s post-hoc test (a, d, and e), Steel–Dwass test (b and c)). (Color figure can be accessed in the online version.)
RESULTS

Influence of DSS-Treatment on Body Weight, Colitis Score, Colon Tissue, Neutrophils in Colon, and Colon Length

The mean body weights were significantly lower for the arthritis/DSS-treated mice compared to that of the control mice (Fig. 1a). The colitis scores of the control and arthritis mice were zero. However, the mean colitis score of the DSS-treated mice was high at 4.0 ± 1.41 with the mean colitis score of the arthritis/DSS-treated mice tending to be worse at 5.25 ± 0.5 (Fig. 1b). The H&E-stained colon tissue and the number of neutrophils therein were observed microscopically, revealing intestinal edema, destruction of epithelial cells, and increased neutrophils in the DSS-treated mice. The colitis was much more severe in arthritis/DSS-treated mice, as indicated by the histological score and the number of neutrophils (Figs. 1c, d). The mean colon length of the DSS-treated mice was shorter than that of the DSS-untreated mice. The colon lengths between the control and arthritis mice was not different. There was also no difference in colon length between the DSS-treated mice and arthritis/DSS-treated mice (Fig. 1e).

Influence of DSS-Treatment on the Foot Volume and MMP-3

The foot volume of mice is known to increase during cases of arthritis and thus can be used as an indicator of arthritis. In addition, MMP-3 can be used as an indicator of articular cartilage destruction. In the current study, the foot volumes and plasma levels of MMP-3 were increased in the arthritis and arthritis/DSS-treated mice compared to those in the control mice. However, there was no difference in these indicators between the arthritis and arthritis/DSS-treated mice (Fig. 2).

Influence of DSS-Treatment on the Plasma Levels of IL-6 and TNF-α

Increased levels of IL-6 and TNF-α have been confirmed in inflammatory diseases such as rheumatoid arthritis and ulcerative colitis.12,13) In the current study, the plasma levels of IL-6 in the arthritis mice, DSS-treated mice, and arthritis/DSS-treated mice were compared to levels in the control mice. Increased levels of IL-6 were most remarkable in the arthritis/DSS-treated mice. In contrast, the plasma levels of TNF-α were not significantly different among the groups. However, the plasma level of TNF-α tended to be highest in the arthritis/DSS-treated mice compared to that in the other groups (Fig. 3).
DISCUSSION

In the current study, we confirmed a relationship between rheumatoid arthritis and ulcerative colitis by concomitantly administering DSS to mice of a collagen-induced arthritis model mice. While MMP-3 and MMP-9, which are involved in organ remodeling, have been shown to be useful markers of ulcerative colitis, it has also been reported that the severity of ulcerative colitis is not correlated with MMP-3 levels. MMP-3 is able to destroy type II collagen in the synovial membrane and MMP-3 is thus considered to be a protein that is expressed specifically during arthritis. In the current study, colitis was worse in arthritis/DSS-treated mice compared to other mice; however, the foot volume and plasma levels of MMP-3 did not differ. Therefore, we suggest that MMP-3 levels did not correlate to the amount of deterioration of ulcerative colitis in the arthritic mice. Contrastingly, IL-6 and TNF-α levels correlated to the pathogenesis of ulcerative colitis and arthritis and these cytokines increased or decreased in this study relative to the severity of colitis. IL-6 and TNF-α are secreted from many types of immune cells, including mast cells, T helper 2 (Th2) cells, and others. We believe that the secretion of IL-6 and TNF-α are from different immune cells under conditions of arthritis and DSS-induced colitis. The arthritis/DSS-treated mice had the most severe case of colitis due to the additive effects of increased cytokine secretion from the various immune cells. However, the foot volume did not change, possibly because the arthritis symptoms had reached a peak.

Overall, our results showed that IL-6 and TNF-α influenced the deterioration effects of colitis in arthritic mice. It has been previously reported that IL-6 and TNF-α are involved in the deterioration of colitis. However, it is difficult to deny a relation for MMPs with colitis since it has been reported that IL-6 correlates with increased levels of MMP-3 and subtypes of MMP are detected at different stages of disease. Therefore, further examination regarding the details of MMPs is needed. In addition, time course studies are also needed to confirm kinetic changes in levels of biogenic factors pertinent to ulcerative colitis and arthritis.

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Conflict of Interest The authors declare no conflict of interest.

REFERENCES