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Selective Ablation of Matrix Metalloproteinase-2 Exacerbates Experimental Colitis: Contrasting Role of Gelatinases in the Pathogenesis of Colitis

Pallavi Garg,* Mauricio Rojas,† Anupama Ravi,* Katrina Bockbrader,* Steven Epstein,* Matam Vijay-Kumar,‡ Andrew T. Gewirtz,‡ Didier Merlin,* and Shanthi V. Sitaraman*‡

The matrix metalloproteinases (MMPs), MMP-2 and MMP-9, share structural and substrate similarities and are up-regulated during human as well as animal models of inflammatory bowel disease. We recently demonstrated that epithelial-derived MMP-9 is an important mediator of inflammation and tissue damage in colitis. In this study, we examined the role of MMP-2 in acute colitis. Colitis was induced using two models, administration of dextran sodium sulfate (DSS) and Salmonella enterica subsp. serovar Typhimurium (S.T.). Bone marrow chimeras were performed using bone marrow cells from wild-type (WT) and MMP-2−/− mice. Colitis was evaluated by clinical symptoms, myeloperoxidase assay, and histology. MMP-2 protein expression and activity were up-regulated in WT mice treated with DSS or S.T. MMP-2−/− mice were highly susceptible to the development of colitis induced by DSS (or S.T.) compared with WT. During inflammation, MMP-2 expression was increased in epithelial cells as well as in the infiltrating immune cells. Bone marrow chimera demonstrated that mucosa-derived MMP-2 was required for its protective effects toward colitis. Furthermore, we demonstrate that severe colitis in MMP-2−/− is not due to a compensatory increase in MMP-9. Finally, we show that MMP-2 regulates epithelial barrier function. In contrast to MMP-9, mucosa-derived MMP-2 may be a critical host factor that is involved in the prevention or cessation of the host response to luminal pathogens or toxins, an important aspect of healing and tissue resolution. Together, our data suggest that a critical balance between the two gelatinases determines the outcome of inflammatory response during acute colitis. The Journal of Immunology, 2006, 177: 4103–4112.

Inflammatory bowel diseases (IBD), which include ulcerative colitis and Crohn’s disease, are chronic, incurable intestinal disorders. Ulcerative colitis and Crohn’s disease are characterized by continuous or discontinuous mucosal inflammation, respectively, with inflammatory cell infiltration, epithelial cell destruction, connective tissue defects, and ulceration of the mucosa of the major portion of intestine and/or colon. The etiopathogenesis of IBD is poorly understood, but considered to be multifactorial, involving genetic susceptibility and environmental factors that lead to the synthesis and release of inflammatory cytokines and recruitment of inflammatory cells culminating in tissue destruction. Accumulating data from several studies indicate the involvement of matrix metalloproteinases (MMPs) in the pathogenesis of IBD via their influence on the function and migration of inflammatory cells, mucosal ulceration, as well as matrix deposition and degradation (1–3). Clinical and experimental studies have demonstrated an increase in the abundance of these matrix-degrading proteases in IBD, and inhibition of MMP activation has been shown to improve experimental colitis (4–7).

MMPs are a family of Zn2+-dependent extracellular matrix-degrading endopeptidases that share common functional domains and activation mechanisms (8–10). MMPs are the only mammalian enzymes capable of catalyzing cleavage of the interstitial collagen, types I, II, III, or IV, in their native triple helical form, at the neutral pH of the extracellular space (11). Depending on substrate specificity, amino acid similarity, and identifiable sequence modules, the MMPs can be classified into four major subgroups: collagenases (MMP-1, -3, -8), gelatinases (MMP-2, -9), stromelysins (MMP-3, -7, -10, -12), and membrane-type metalloproteinases 1 through 5. Although MMPs play important roles in normal tissue remodeling, dysregulated expression has been implicated in several pathological processes, such as arthritis (1, 12), atherosclerosis, myocardial infarction (13), colorectal cancer, tumor invasion (14, 15), and IBD (4, 16). In many acute and chronic inflammatory events such as IBD, MMP levels have been shown to be up-regulated (17–23).

Among the MMPs, MMP-2 and MMP-9 are the two known gelatinases that degrade denatured collagen and are consistently up-regulated during active flares of IBD in human as well as in animal models of colitis (17, 18, 23–26). Gelatinases differ from other MMPs in terms of their structure as well as substrate specificity. Both MMP-2 and MMP-9 have the prototypic structure that is present in other MMPs: signal peptide, followed by the prodomain, catalytic domain, hinge region, and the hemopexin domain.
respectively (1). However, they differ from other MMPs by the presence of fibronectin-like repeats in their catalytic domain. Both MMP-2 and MMP-9 share common substrates and are expressed by similar cell types (1, 11). A major structural difference between MMP-2 and MMP-9 is the lack of an additional type V collagen-like domain in MMP-2 (27). Little is known regarding the role of the MMP-2 or MMP-9 in inflammation or injury of colon during IBD.

We recently demonstrated that MMP-9 activity and protein expression were absent from normal colonic mucosa, but were up-regulated during experimental colitis in response to luminal toxin (dextran sodium sulfate; DSS) as well as bacteria Salmonella enterica subsp. serovar Typhimurium (S.T.) (24). MMP-9−/− mice exposed to DSS or S.T. had significantly reduced inflammation and mucosal injury and were protected against acute colitis. Immune response to systemic administration of S.T. was also not affected in MMP-9−/− mice. Finally, epithelial- but not immune cell-derived MMP-9 was required for tissue damage. The role and function of MMP-2 in the pathogenesis of intestinal inflammation are not known. In this study, we sought to characterize the role of MMP-2 in the pathogenesis of experimental acute colitis.

Materials and Methods

Experimental animals

The Animal Care Committee of Emory University approved all procedures performed on animals and the procedures were in accordance with the Guide for the Care and Use of Laboratory Animals, published by the U.S. Public Health Service. MMP-2−/− mice (C57BL/6 background) were a gift from L. Matrisian (Vanderbilt University, Nashville, TN). The homozygous MMP-2−/− mice were used were from breeding pairs of C57BL/6 background with disruption of the MMP-2 gene that were backcrossed for more than six generations (28). These mice developed normally and were fertile. To confirm the absence of MMP-2 gene expression, genomic DNA was isolated and the disruption of the MMP-2 gene was confirmed via PCR using primers designed to specifically detect hetero- and homozygote mice. For MMP-2 wild type (WT), we used primers 5′-CCGATGCTCCAGA-3′ and primer 5′-TTG-3′. MMP-2−/− mice age- and sex-matched WT and MMP-2−/− littermates used in the study were between 6 and 8 wk old at the beginning of the experimental protocol and were maintained under conditions as described previously (24).

Induction of DSS colitis

Colitis was induced in two groups of age- and sex-matched male and female WT and MMP-2−/− littermates, by oral administration of DSS (ICN Biomedicals) at 3% (w/v) in tap water ad libitum for 6 days. Age-matched male and female WT and MMP-2−/− littermates receiving tap water served as control. Mice were observed daily and evaluated for changes in body weight and development of clinical symptoms.

S.T. infection

Gut-restricted S.T. infection was induced, as described previously (24, 30). To prepare S.T. inocula, bacteria (S.T. SL3201) were grown overnight at 37°C in 10 ml of Luria-Burtani broth in a 20-ml container with shaking (150 ppm) and were then used to inoculate fresh medium (1:100) and were grown under the same conditions for 2–3 h until an OD at 550 nm of 0.35–0.6 was reached. Bacterial cultures were then diluted in normal saline, and the CFU were enumerated by plating a dilution series of the inoculum. Water and food were withdrawn 4 h before treating with 7.5 mg of streptomycin (75 μl of sterile water containing streptomycin or 75 μl of sterile water by gavage). Afterward, animals were supplied with food and water ad libitum. At 20 h after streptomycin treatment, food and water were withdrawn again for 4 h before mice were infected with 10^9 CFU of S.T. (50-μl suspension in PBS) or treated with vehicle. Thereafter, food and water were offered immediately. Mice were sacrificed by CO2 inhalation, and tissue samples were processed, as described for the DSS colitis model (6).

Gelatin zymography

The activity of MMPs is measured by zymography under nonreducing conditions, as described previously (24, 31). Briefly, snap-frozen samples of colon were homogenized with homogenizer and extracted in ice-cold nonreducing extraction buffer (20 μg/ml tissue). After centrifugation, the supernatant was collected and the total protein concentration was measured by Lowry method using protein assay reagent (Bio-Rad). Protein from tissue samples was electrophoretically separated on 7.5% polyacrylamide agarose gel copolymerized with gelatin (1.5 mg/ml) as a substrate. The gels were washed three times for 15 min each with 2.5% Triton X-100 (Sigma-Aldrich) to remove the SDS and to allow the electrophoresed enzymes to renature, before being incubated in zymography buffer (5 mMol/L CaCl2 and 50 mMol/L Tris-HCl (pH 7.5)) for 18 h at 37°C. The gels were then stained with 0.5% Coomassie brilliant blue R-250 (Bio-Rad) and destained with methanol:acetic acid/water (v/v: 4:1.5). Prestained standard high-range protein markers (Bio-Rad) were used to determine the molecular weights of the gelatines. Clear digested regions representing MMP activity were quantified using an imaging densitometer (Camera Imaging Densitometer; Model 8300; Alpha Innotech).

Protein extraction and Western blot analysis

As described previously (24), for Western blot analysis, colon tissue obtained as above were homogenized and extracted with lysis buffer. Samples were then centrifuged at 12,000 rpm for 10 min at 4°C and the resulting supernatant was used for assays. The total protein concentration of all samples was measured by Lowry method using protein assay reagent (Bio-Rad). A total of 40 μg was boiled for 5 min in Laemmli’s sample buffer (Bio-Rad) and electrophoresed in 10% SDS-PAGE gels. Proteins were transferred to nitrocellulose (Bio-Rad), and the membrane was then blocked in 5% nonfat dry milk for 1 h. Incubation was performed overnight at 4°C with Abs for inhibitor of metalloproteinase-1 (TIMP-1) (1:1000), TIMP-2 (1:1000), MMP-2 (5 μg/ml), and MMP-9 (1:2000) (Chemicon International). Subsequently, the membranes were washed with Tris-NaCl-Tween 20 and incubated with a goat anti-rabbit (1:2500) or goat anti-mouse (1:4000) IgG HRP conjugate (Bio-Rad) for 1 h at room temperature. Membranes were developed with Western Lightning Chemiluminescence Reagent Plus (PerkinElmer) and quantified by image analysis (32).

Clinical activity score

Assessment of body weights, stool consistency, and the presence of occult/ gross blood by a guaiac test (Hemocult Sensa; Beckman Coulter) were determined daily for each mouse. Colitis was quantified with a clinical score, as described by Cooper et al. (33), using the parameters of weight loss, stool consistency, and fecal blood. Briefly, no weight loss was considered as 0 points, weight loss of 1–5% was scored 1 point, loss of 5–10% as 2 points, 10–20% weight loss as 3 points, and a loss of >20% of the weight was scored as 4. The stool character was characterized as normal (0), soft with well-formed pellets (1), soft without pellets (2), or diarrhea (4). For occult blood, no blood was scored 0, positive hemoccult scored as 2 points, and gross bleeding was scored 4. The total score was added to get a clinical activity score ranging from 0 to 12. Six days after the induction of colitis, mice were euthanized by CO2/air. The abdominal cavity was opened by a midline laparotomy, and the entire colon was removed from the caecum to the anus. The colon was flushed with cold PBS and opened longitudinally for morphologic studies. The length and weight of the colon were measured, and tissue obtained from each colon was processed for further assays.

Histological assessment of colitis

Colonic specimens obtained as above were fixed in formalin and coded for blind microscopic assessment of mucosal lesions (descending colon for DSS colitis and caecum for S.T. colitis). Sections were stained with H&E. Microscopic sections were analyzed and histologic scoring was performed, as described by Cooper et al. (33), based on three variables, according to the severity of the induced damage. Briefly, for inflammation, rare inflammatory cells in the lamina propria were counted as 0; increased numbers of granulocytes in the lamina propria were scored as 1; confluence of inflammatory cells extending into the submucosa as 2; and a score of 3 was given for transmural extension of the infiltrate. For crypt damage, intact crypt was scored 0, loss of 1/3 basal counted as 1, loss of 2/3 basal was counted as 2, entire
crypt loss was scored as 3, change of epithelial surface with erosion as 4, and a score of 5 was given for confluent erosion. For evaluation of ulcers, an absence of ulcer was scored 0, 1–2 foci of ulcers were scored as 1, 3–4 foci of ulcers were scored as 2, and confluent/extentive ulceration was scored 3. These values were added to give a total histological score of 11.

Myeloperoxidase (MPO) activity in the colon

Neutrophil infiltration into colon was quantified by measuring MPO activity, as described previously (24, 34). Briefly, a portion of colon was homogenized in 1:20 (w/v) of 50 mM phosphate buffer (pH 6.0) containing 0.5% hexadecyltrimethyl ammonium bromide (Sigma-Aldrich), on ice using a Polytron homogenizer. The homogenate was sonicated for 10 s, freeze-thawed three times, and centrifuged at 14,000 rpm for 15 min. Supernatant (14 μl) was added to 1 mg/ml o-dianisidine hydrochloride (Sigma-Aldrich) and 0.0005% hydrogen peroxide, and the change in absorbance at 460 nm was measured. One unit of MPO activity was defined as the amount that degraded 1 μmol peroxidase per minute at 25°C. The results were expressed as absorbance per gram of tissue.

Immunofluorescence

Colon tissue from WT and MMP-2−/− mice embedded in paraffin was obtained, as described by Castaneda et al. (24). The sections were rehydrated using graded alcohol solutions. Sections were treated with 0.5% Triton X-100 + 0.08% saponin in PBS at room temperature for 35 min. The sections were rinsed in PBS and incubated with rhodamine-phalloidin (1/100 in 2% BSA in PBS solution), or rhodamine-conjugated anti-mouse secondary Ab (Bio-Rad; 1/100 in 2% BSA in PBS solution) or rabbit IgG (Sigma-Aldrich; control), or anti-actin (Sigma-Aldrich; 1:50,000 in 2% BSA) for 1 h at room temperature. After washing three times with PBS, they were incubated with FITC-conjugated anti-rabbit secondary Ab (Bio-Rad; 1/100 in 2% BSA in PBS solution) or rhodamine-conjugated anti-mouse secondary Ab (Bio-Rad; 1/100 in 2% BSA in PBS solution), for 45 min at room temperature and then mounted with Slow Fade (Molecular Probes) mounting medium and examined using Zeiss LSM microscope.

Bone marrow transplantation

Bone marrow transplantation was performed, as described previously (24, 35). Briefly, the femur and tibia were removed and stripped off all muscle and sinew, and bone marrow cells were harvested from both femurs and tibias by flushing the bone cavity with basal marrow medium (Isco’e medium; Cambrex). After washing with PBS, bone marrow cells were resuspended in basal marrow medium. Approximately 5 × 10⁶ cells in 50 μl were transplanted retro-orbitally. Four treatment groups with 10 animals per group were used (WT→WT, WT→MMP-2−/−, MMP-2−/−→WT, and MMP-2−/−→MMP-2−/−). Mice were given neomycin at 2 mg/ml for the first week of posttransplantation, after which they were switched to tap water. Engraftment was verified by genotyping bone marrow cells using specific primers (29), as described previously in this work. Five weeks after transplantation, we induced colitis by 5% DSS and mice were assessed daily for rectal bleeding, weight loss, and diarrhea, and the clinical score was obtained. At the end of the experimental period (6 days for DSS) (24), mice were sacrificed and colons were processed for histology, MPO assay, and immunohistochemistry. Engraftment was verified by performing genotyping on the bone marrow cells at the end of 4 wk after transplantation.

In vivo permeability

In vivo permeability assay to assess barrier function was performed using an FITC-labeled dextran method, as described (36). Briefly, 8- to 10-wk-old WT and MMP-2−/− mice were used. Food and water were withdrawn for 4 h and mice were gavaged with permeability tracer (60 mg/100 g body weight of FITC-labeled dextran, m.w. 4000; Sigma-Aldrich). Serum was collected retro-orbitally; fluorescence intensity of each sample was measured (excitation, 492 nm; emission, 525 nm; Cytofluor 2300; Millipore, Waters Chromatography); and FITC-dextran concentrations were determined from standard curves generated by serial dilution of FITC-dextran. Permeability was calculated by linear regression of sample fluorescence (Excel 5.0; Microsoft).

Statistical analysis

The data are presented as mean ± SEM. Statistical analysis was performed using GraphPad Instat 3 software (www.graphpad.com). Groups were compared using nonparametric tests, and significance of the differences between groups was assessed using the Mann-Whitney U test or the Wilcoxon signed-ranks test for paired data. Values of p < 0.05 were considered statistically significant.

Results

MMP-2 expression and activity are induced during DSS colitis in mice

DSS colitis model, a rapid and reproducible model of colitis that mimics human IBD in several respects, was used to investigate the expression of MMP-2 during colitis. WT C57BL/6 mice were administered 3% DSS in their drinking water (w/v) for 6 days. Mice were weighed daily, and stool was examined for consistency and the presence of blood. Mice were euthanized after 6 days, and protein lysates were prepared from the colon for zymography and Western blot analysis, as described in Materials and Methods. Zymography was performed using a gelatin-impregnated PAGE to evaluate gelatinolytic activity. A representative zymogram and its densitometric analysis are shown in Fig. 1A. Pro-MMP-2 activity was observed in colonic extracts prepared from WT mice that had been fed with water (Fig. 1A, lanes 1–4). There was no active MMP-2 detected in these mice. In contrast to mice fed water, colonic extracts from WT animals given DSS showed strong induction of pro- and active MMP-2 activity (pro, 2 ± 0.3-fold and active, 7.5 ± 3.9-fold over water-treated mice, respectively; mean ± SE, 6 mice/group; Fig. 1A, lanes 5–9). MMP-2 activity steadily increased over the course of DSS administration starting on day 1 (data not shown).

Western blot analysis of colonic protein extracts from WT mice treated with water or DSS was performed to confirm the identity of gelatinolytic activity observed on zymography. Animals that had not been exposed to DSS showed constitutive MMP-2 protein expression (Fig. 1B, lanes 1–4). Marked induction of MMP-2 protein expression was observed in colitic samples (Fig. 1B, lanes 5–8). WT mice exhibited immunoreactivity at 72 kDa consistent with pro-MMP-2, while the colitic samples showed immunoreactivity at a slightly lower molecular mass at 62 kDa consistent with active MMP-2. Densitometric analysis revealed 8.2 ± 4.1-fold increase in MMP-2 protein expression in extracts from mice exposed to DSS compared with mice fed water (mean ± SE, 6 mice/group; p < 0.01).

The proteolytic activity of MMPs is regulated by the balance between zymogen activation and enzyme inhibition through their endogenous inhibitors, α-macroglobulins and TIMPs (16). Given the importance of TIMPs on the biological activities of MMPs, we next examined whether the expression of TIMP-1 and TIMP-2 was influenced differentially by DSS administration. Fig. 2 shows the expression levels of TIMP-1 and TIMP-2 in WT mice fed with DSS or water. TIMP-1 levels were unchanged (Fig. 2), while TIMP-2 levels were decreased in mice treated with DSS (Fig. 2, lanes 5–8). Together, these data suggest that MMP-2 activity and protein expression are induced during DSS colitis along with a significant decrease in TIMP-2.

MMP-2−/− mice developed severe inflammation in response to DSS and were unable to recover from DSS-induced colitis

To further investigate the role of MMP-2 in the pathogenesis of colitis, we used C57BL/6 mice with targeted deletion of MMP-2 (28). These mice exhibit normal phenotype. We administered DSS in drinking water to age (8–10 wk)- and sex-matched C57BL/6 WT and homozygous MMP-2−/− mice. The mice were compared for the clinical signs of disease, including weight changes, stool consistency, and occult blood, according to grading system previously described (24, 33). Both WT and MMP-2−/− mice exposed
To monitor the effect of lack of MMP-2 on the healing phase of colitis, we observed mice for 1 more additional wk after DSS administration during which time animals were given water. All of the mice given DSS, as evidenced by a clinical disease activity score of 7.9 ± 1 and 11.1 ± 0.5, respectively (mean ± SE, 6 mice/group; p < 0.01; Fig. 3A). Almost all of the MMP-2−/− mice developed diarrhea after day 3. These mice were hemo-occult positive starting from day 2 and exhibited frank bleeding on day 5 after DSS administration. DSS-induced colitis is characterized by the presence of inflammation of the colon manifested by crypt destruction, mucosal damage, epithelial erosions, and infiltration of inflammatory cells into the mucosal tissue. Tissues collected from WT and MMP-2−/− mice exposed to DSS were examined histologically and compared with those from normal controls. As shown in Fig. 3, B and C, a mean histological score of 9.7 ± 0.7 was observed in MMP-2−/− mice given DSS compared with a mean score of 6.8 ± 0.6 in WT mice given DSS (mean ± SE, 6 mice/group; p < 0.01 compared with MMP-2−/− mice). Interestingly, ulceration was significantly increased in MMP-2−/− mice. The ulcers were not only more in number, but involved larger surface area compared with WT mice (Fig. 3C). Histological signs of inflammation were not detected in the water control groups (data not shown). Thus, the data obtained corroborated the results obtained from clinical analysis and confirmed the deleterious effect of targeted MMP-2 deletion toward the development of colitis. These data also suggest a potential role of MMP-2 as a protective MMP against colitis.

To monitor the effect of lack of MMP-2 on the healing phase of colitis, we observed mice for 1 more additional wk after DSS treatment, during which time animals were given water. All of the MMP-2−/− mice died during this period, while only 25% of the WT mice died. Fig. 4A shows the percentile of change in body weight during healing phase and indicates that change in body weight of MMP-2−/− was 2-fold more than WTs. Fig. 4B shows the survival curve of WT and MMP-2−/− mice, indicating higher mortality among WTs compared with knockouts during recovery phase. These data implicate the importance of MMP-2 in intestinal recovery and healing following acute colitis.

**MMP-2 mice exhibit increased susceptibility to Salmonella-induced colitis**

As an alternate model of colitis, we used oral infection with S.T., during which S.T. is administered after pretreatment of mice with DSS. We observed that MMP-2−/− mice developed signs of colitis within 6 days after the administration of DSS. However, colitis was significantly worse in MMP-2−/− mice given DSS, as evidenced by a clinical disease activity score of 7.9 ± 1 and 11.1 ± 0.5, respectively (mean ± SE, 6 mice/group; p < 0.01; Fig. 3A). Almost all of the MMP-2−/− mice developed diarrhea after day 3. These mice were hemo-occult positive starting from day 2 and exhibited frank bleeding on day 5 after DSS administration. DSS-induced colitis is characterized by the presence of inflammation of the colon manifested by crypt destruction, mucosal damage, epithelial erosions, and infiltration of inflammatory cells into the mucosal tissue. Tissues collected from WT and MMP-2−/− mice exposed to DSS were examined histologically and compared with those from normal controls. As shown in Fig. 3, B and C, a mean histological score of 9.7 ± 0.7 was observed in MMP-2−/− mice given DSS compared with a mean score of 6.8 ± 0.6 in WT mice given DSS (mean ± SE, 6 mice/group; p < 0.01 compared with MMP-2−/− mice). Interestingly, ulceration was significantly increased in MMP-2−/− mice. The ulcers were not only more in number, but involved larger surface area compared with WT mice (Fig. 3C). Histological signs of inflammation were not detected in the water control groups (data not shown). Thus, the data obtained corroborated the results obtained from clinical analysis and confirmed the deleterious effect of targeted MMP-2 deletion toward the development of colitis. These data also suggest a potential role of MMP-2 as a protective MMP against colitis.

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streptomycin. In this model, S.T. induces clinical and histological features of enterocolitis predominantly involving the caecum (24, 30). We chose this model because it recapitulates some aspects of clinical and histological human infection as well as acute flares of IBD, wherein mucosal-pathogen interaction is thought to play an important role in the pathogenesis. The characteristic histological feature of gut-restricted S.T. enteritis includes neutrophil infiltration of the intestinal mucosa, the hallmark of infectious colitis, as well as acute flares of IBD. Other histological features of S.T. colitis include epithelial ulceration, edema, and induction of ICAM-1. WT and MMP-2−/− mice were pretreated with streptomycin and then administered S.T. Mice were sacrificed at 24 and 48 h after the administration of S.T., and colonic tissue was removed, weighed with the contents, and photographed. The caecum was processed for histology and MPO activity. The caeca of all the mice infected with S.T. appeared pale and shriveled to a small size and were filled with purulent exudates. MMP-2 activity was significantly up-regulated in WT mice treated with S.T. (Fig. 5A, lanes 3 and 4) similar to that seen in WT mice during DSS colitis. MMP-2 activity was not detected in the MMP-2−/− mice with (Fig. 5A, lanes 7 and 8) or without (Fig. 5A, lanes 5 and 6) treatment of S.T. MPO activity reflected the clinical observation (Fig. 5B). MMP-2−/− mice showed significantly increased MPO activ-

![FIGURE 3.](image)

**FIGURE 3.** MMP-2−/− develop severe DSS-induced colitis. Eight- to 10-wk-old WT C57BL/6 and MMP-2−/− mice were weighed and given water or DSS (3% w/v) for 6 days. Mice were euthanized, and colon was processed for histology. Disease severity was assessed and expressed in terms of clinical activity score in A. Clinical score was calculated using parameters of stool consistency, weight loss, and fecal blood. Each bar represents mean ± SE; *, p < 0.02. B, mean histological score; *, p < 0.02 and #, p < 0.05 compared with WT. C, representative sections from two individual experiments. n = 5 for DSS group, and n = 4 for control group. Colon specimens from descending colon were fixed in formalin, and histological score was calculated on H&E sections based on three variables: inflammatory cells, crypt damage, and ulcers. Both WT and MMP-2−/− mice developed inflammatory infiltration and crypt damage. However, MMP-2−/− mice showed extensive ulceration compared with WT mice.

![FIGURE 4.](image)

**FIGURE 4.** MMP-2−/− mice exhibit impaired recovery from DSS-induced colitis. WT and MMP-2−/− mice were given water or DSS for 6 days, as described in Fig. 3 (n = 4 per group). After 6 days, mice were given water and followed for weight loss (A) and mortality (B) during recovery phase. Each bar represents mean ± SE; #, p < 0.05.

![FIGURE 5.](image)

**FIGURE 5.** MMP-2−/− mice showed increased susceptibility to Salmonella-induced colitis. WT and MMP-2−/− mice (C57BL/6) were pretreated with streptomycin before the administration of S.T. and sacrificed 48 h after the administration of S.T. The caecum and colon were harvested. Western blot analysis (A) of protein from the caecum of WT given water (lanes 1 and 2) or S.T. (lanes 3 and 4) and MMP-2−/− given water (lanes 5 and 6) or S.T. (lanes 7 and 8) probed with anti-MMP-2 was done. Each lane shows protein (40 μg/lane) from an individual mouse with or without S.T. treatment. β-actin served as the loading control. Colon tissues were snap frozen in liquid nitrogen, and MPO activity was measured (B) as an index of neutrophil infiltration into the injured tissue. WT mice are represented by ■, and MMP-2−/− mice by □. Each bar represents mean ± SE; n = 3 animals for each group. *, p < 0.005. C, H&E sections based on three variables: inflammatory cells, crypt damage, and ulcers showed that both WT and MMP-2−/− mice developed inflammatory infiltration and crypt damage to S.T. induced colitis though knockouts showed more ulceration compared to WT.
ity compared with WT mice. Clinical symptoms and histological changes appeared at 24 h in WT and MMP-2−/− mice. However, MMP-2−/− mice showed increased severity of inflammation compared with WT mice at 48 h (Fig. 5C) as marked by leukocyte infiltration with loss of crypts as well as ulcerations at 48 h in MMP-2−/− mice treated with S.T. Taken together, these data suggest that MMP-2 is up-regulated during S.T.-induced colitis and MMP-2−/− mice develop severe S.T.-induced colitis compared with WT mice.

**MMP-2 localizes to epithelial cells and immune cells**

We next determined the localization of MMP-2 in normal and inflamed colon. Immunofluorescence of colon sections performed with affinity-purified MMP-2 Ab directed against the catalytic domain of mouse MMP-2 confirmed the presence of this enzyme mainly in epithelial cells (Fig. 6A). There was no staining in the lamina propria immune cells (Fig. 6A, boxed). In mice fed with DSS, MMP-2 staining was strongly induced and was seen in the crypt epithelial cells (Fig. 6B). Strong MMP-2 staining was also detected in the infiltrating immune cells (Fig. 6C). Control section stained with isotype Ab (anti-rabbit IgG) instead of primary Ab showed no staining. There was no staining visualized in MMP-2−/− mice (Fig. 6D). Thus, the expression of MMP-2 is induced in mice exposed to DSS and is localized to epithelial and immune cells.

**Mucosa-derived MMP-2 mediates its protective effect**

To understand the role of MMP-2 in the pathogenesis of colitis, we first determined the relative contribution of mucosal vs immune cell-derived MMP-2 in the development of colitis. To explore this question, we generated bone marrow chimeras of WT or MMP-2 mice exposed to DSS and is localized to epithelial and immune cells.

**Severe colitis in MMP-2−/− mice is not due to a compensatory increase in MMP-9**

We recently demonstrated that MMP-9 is up-regulated during experimental colitis and mucosa-derived MMP-2 mediates tissue damage during colitis. MMP-9−/− mice are protected from DSS- and S.T.-induced colitis (24). To verify whether a compensatory increase in MMP-9 among MMP-2−/− mice is contributing to the severity of colitis seen in these mice, we performed Western blot in the colonic tissue lysates of WT and MMP-2−/− mice administered water or DSS. Fig. 8, A and B, shows a representative Western blot of WT and MMP-2−/−, respectively. In contrast to WT mouse fed water (Fig. 8A, lanes 2–4), colonic extracts from WT mice given DSS (Fig. 8A, lanes 5 and 6) showed increased MMP-9 expression. Interestingly, MMP-9 expression was not increased in MMP-2−/− mice fed with water (Fig. 8B, lanes 2–4) and administration of DSS increased the expression of MMP-9 (Fig. 8B, lanes 5 and 6) similar to WT mice given DSS. These data suggest that a compensatory increase in MMP-9 does not play a role in the severity of colitis seen with the deletion of MMP-2.

**MMP-2−/− mice exhibit compromised epithelial barrier function**

Because our data show that epithelial-derived MMP-2 plays a protective role in the development of colitis, we hypothesized that MMP-2 may play a role in epithelial barrier function and barrier function may be decreased in MMP-2−/− mice, rendering them susceptible to injury and inflammation. Loss of barrier function provided by epithelial cells is thought to be the initial initiating event.
event that underlies injury and inflammation in many intestinal disorders, including IBD (37, 38). Such barrier defects result in the migration of antigenic material, previously confined to the intestinal lumen, into the submucosa, exposing lamina propria immune cells to naive Ags, eliciting inflammatory response and epithelial

FIGURE 8. Severe colitis in MMP-2−/− mice is not due to a compensatory increase in MMP-9 expression. WT (A) and MMP-2−/− (B) mice (C57BL/6) were given water or 3% DSS (w/v) for 6 days, at which time mice were euthanized. Colon was harvested and processed for Western blot. Representative Western blot of protein from the colon of WT and MMP-2−/− mice given water (lanes 2–4) or DSS (lanes 5 and 6) probed with anti-MMP-9 is shown in A and B, respectively. Purified MMP-9 (4 ng/lane) was loaded in lane 1 in both A and B. Each lane shows protein (40 μg/lane) from an individual mouse with or without DSS treatment. β-actin served as the loading control.

FIGURE 9. MMP-2−/− mice exhibit compromised epithelial barrier function. WT and MMP-2−/− mice (C57BL/6) were gavaged FITC-dextran (m.w. 4000), as described in Materials and Methods. Serum was obtained retro-orbitally and processed for fluorescence. Data are represented as mg FITC/mg protein/hour. Each bar represents mean ± SE. n = 10; p < 0.01.
injury that characterize these diseases (37, 38). To test our hypothes-
us, we studied barrier function in WT and MMP-2−/− mice using a
FITC-labeled dextran method, as described in Materials and
Methods. Mice were administered FITC-dextran by gavage, and
fluorescence was quantitated in the serum at 4 and 24 h after the
administration of FITC-dextran. As shown in Fig. 9, WT mice
showed an FITC-dextran of 1.05 ± 0.1 mg of FITC/µg protein/h.
In comparison, there was ~2-fold increase in FITC-dextran levels
in MMP-2−/− mice (1.8 ± 0.03 mg of FITC/µg protein/h), sug-
gestg decreased barrier function in these mice.

Discussion
In this study, we demonstrate that although MMP-2 and MMP-9
are both gelatinases that originate from similar epithelial sources
as well as share structural and substrate similarities, they have
opposing effects on the development of acute colitis. Consistent
with other studies, we show that MMP-2, like MMP-9, is up-reg-
ulated during colitis (4, 16, 24, 39). Using two models of acute
colitis, chemical- and bacteria-induced colitis, we demonstrate that
MMP-2−/− mice are highly susceptible to the development of co-
litis compared with their WT counterparts. These results are in
contrast to the effect of MMP-9 on colitis; MMP-9−/− mice are
protected from the development of chemical- or bacteria-induced co-
litis. These results were surprising given that MMP-2 and MMP-9
belong to the family of gelatinases that share structural and matrix
substrate similarities. Both MMP-2 and MMP-9 possess the fi-
bronectin repeat characteristic of the gelatinases. However,
MMP-2 lacks the collagen V-like domain present in MMP-9, po-
tentially changing their nonmatrix substrate specificity. For example,
McQuibban et al. (40) reported contrasting effect of MMP-2 and
MMP-9 on MCP-3. MMP-2 degrades MCP-3, while MMP-9 has no
effect on the protein. The authors extrapolated the difference in
MCP-3 degradation between MMP-2 and MMP-9 to the colla-
gen V domain. The effect of MMP-2 on MCP-3 degradation and
possibly degradation of other chemoattractants is thought to un-
derlie the protective effect of MMP-2 on inflammatory arthritis,
myocardial damage, and corneal injury (11, 41–49). In addition to
degradation of chemoattractants or chemokines, a role for MMP-2
in tissue remodeling has been shown in wounded corneal epithelial
cells. In this study, MMP-9 impaired remodeling, while MMP-2
was required for proper tissue remodeling and wound healing.
These authors further showed that fibroblast-derived MMP-2 was
required for stromal remodeling that is integral to corneal wound
healing. Along with the observations that myofibroblasts are an
important source of MMP-2 during intestinal inflammation (19)
and are integral to epithelial wound healing, it is likely that
MMP-2 may play a role in tissue remodeling that is required for
proper wound healing (50, 51).

Our data show that cellular constituents of intestinal mucosa,
including epithelial cells as well as immune cells, express MMP-2
in response to DSS or S.T. These observations are consistent with
published data that MMP-2 is highly expressed in the intestinal
epithelia during human IBD. However, to our knowledge, the role
of MMP-2 in the pathogenesis of colitis has not been demon-
strated. We demonstrate that MMP-2 may be involved in the reg-
ulation of epithelial barrier function. MMP-2−/− mice exhibited
barrier dysfunction, as evidenced by increased FITC-dextran trans-
location in these mice compared with their WT counterpart. Data
from human and animal studies have demonstrated a central role
for epithelial dysfunction in the pathogenesis of intestinal inflam-
mentation (37, 38). The current paradigm for the pathogenesis of IBD
supported by evidence from human and animal studies as well as
cultured cell models proposes three key components to be neces-
sary for the initiation and progression of disease: 1) disruption of
the epithelial barrier (direct effect on barrier or impaired healing in
response to injury); 2) access of luminal contents to the lumina
propria, that is, immune cells; and 3) an abnormal immune re-
response (37, 38). Epithelial dysfunction is seen in patients much
earlier than histologic or clinical manifestation of IBD. It is
thought that barrier dysfunction allows contents of the intestinal
lumen to mix freely with the contents of the lamina propria, elici-
ting an immune/inflammatory response, which characterizes in-
flammatory diseases of the intestine. Our data show that barrier
integrity is compromised in MMP-2−/− mice, which may possibly
lead to their increased susceptibility to luminal toxins (DSS) or
pathogens (S.T.).

There are several possible mechanisms by which MMP-2
might affect barrier function. One possibility is that MMP-2 can
modulate tight junction directly by associating with tight junction
proteins. A series of studies have shown that MMP-2 intimately
associates with claudins (52–54). For example, it has been demon-
strated that MMP-2 localizes to tight junctions in Madin-
Darby canine kidney cells and ectodomain of claudin-1 inter-
acts with the catalytic domain MMP-2 (52). Claudin family
proteins are the major constituents of tight junction strands,
which are directly involved in paracellular sealing (barrier func-
tion) as well as in membrane domain differentiation (fence func-
tion) in epithelial and endothelial cell (55, 56). Hence, the
association of MMP-2 with claudin may modulate paracellular
permeability. In support of this association, our data show that
overexpression of MMP-2 increases barrier function measured
using FITC translocation (our unpublished data). In this con-
text, it is interesting that MMP-9, unlike MMP-2, has been
associated with increased paracellular permeability by cleaving
extracellular domain of occludin while having no effect on clau-
din (57). Such disparate effect of MMP-2 and MMP-9 on per-
meability may account for their opposite effects on the patho-
genesis of colitis.

Given our previous observation that MMP-9 mediates in-
flammatory response and tissue damage, a possible explanation
for the severe colitis seen in MMP-2−/− mice could be related
to a compensatory increase in MMP-9 in MMP-2−/− mice.
Such a compensatory increase in MMP-9 has been shown to
mediate severe inflammatory response in an autoimmune en-
cephalitis model in MMP-2−/− mice (45). The potential role of
MMP-2 as a modulator of MMP-9 expression via the mem-
brane-type 1-MMP/TIMP-2 complex has been suggested (45,
58). However, we did not observe any increase in MMP-9 ex-
pression or activity in MMP-2−/− mice fed water, and the in-
crease in MMP-9 was modest after DSS colitis compared with
WT mice fed DSS. Thus, expression of MMP-9 may be partially
modulating the development of colitis in MMP-2−/− mice,
while the loss of MMP-2 expression may play a crucial role in
the severity of colitis. However, further studies are required to
delineate the precise mechanism by which MMP-2 modulates
its protective effects.

In summary, we demonstrate that MMP-2, a gelatinase that
shares structural and substrate similarities with MMP-9, plays a
protective role toward the development of acute colitis. We also
show that mucosal MMP-2 contributes to its protective effect, and
immune cell-derived MMP-2 is not required for its migration or
protective effect. Lastly, we show that MMP-2 is required for in-
tact epithelial barrier function. Together, our data suggest that
MMP-2 plays a protective role in the development of murine col-
litis possibly by contributing to barrier function. Furthermore,
a critical balance between the two gelatinases determines the out-
come of inflammatory response during acute colitis. Our data are
relevant in designing gelatinase-based therapeutic strategies for the treatment of intestinal inflammation.

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Disclosures

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References


