Matrix metalloproteinase-9-mediated tissue injury overrides the protective effect of matrix metalloproteinase-2 during colitis

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Garg P, Vijay-Kumar M, Wang L, Gewirtz AT, Merlin D, Sitaraman SV. Matrix metalloproteinase-9-mediated tissue injury overrides the protective effect of matrix metalloproteinase-2 during colitis. Am J Physiol Gastrointest Liver Physiol 296: G175–G184, 2009; doi:10.1152/ajpgi.90454.2008.—Matrix metalloproteinases (MMPs) play an important role in pathogenesis of inflammatory bowel disease (IBD). Two known gelatinases, MMP-2 and MMP-9, are upregulated during IBD. Epithelial-derived MMP-9 is an important mediator of tissue injury in colitis, whereas MMP-2 protects against tissue damage and maintains gut barrier function. It has been suggested that developing strategies to block MMP-9 activity in the gut might be of benefit to IBD. However, given that MMP-2 and MMP-9 are structurally similar, such approaches would also likely inhibit MMP-2. Thus, to gain insight into outcome of inhibiting both MMP-2 and MMP-9, MMP-2−/−/MMP-9−/− double knockout mice (dKO) lacking both MMP-2 and MMP-9 were used in this study. Three models of murine colitis were used: dextran sodium sulfate (DSS), Salmonella typhimurium (S.T.), and trinitrobenzene sulfonic acid (TNBS). Our data demonstrate that both MMP-2 and MMP-9 activities are highly upregulated in wild-type (WT) mice treated with DSS, S.T., or TNBS whereas dKO mice were resistant to the development of colitis. WT mice had extensive inflammation and tissue damage compared with dKO mice as suggested by histological assessment and myeloperoxidase activity. In conclusion, these results suggest an overriding role of MMP-9 in mediating tissue injury compared with the protective role of MMP-2 in development of colitis. Thus inhibition of MMP-9 may be beneficial in treatment of colitis even if resulting in inhibition of MMP-2.

Matrix metalloproteinase-9-mediated tissue injury overrides the protective effect of matrix metalloproteinase-2 during colitis. Am J Physiol Gastrointest Liver Physiol 296: G175–G184, 2009; doi:10.1152/ajpgi.90454.2008. — Matrix metalloproteinases (MMPs), which include the collagensases, stromelysins, gelatinases, and membrane-type MMPs (MT-MMP), are a family of structurally related zinc-dependent proteases that are involved in cellular infiltration, cytokine activation, cell migration, tissue damage, remodeling, and repair (3, 36). MMPs are central to the regulation of extracellular matrix turnover, having the ability to cleave majority of extracellular matrix proteins. Importantly, dysregulated expression of MMPs has been shown to have a pathogenic role in a number of diseases including arthritis, atherosclerosis, myocardial infarction, colorectal cancer, tumor invasion, and inflammatory bowel disease (IBD) (8, 18, 26, 54). MMP-2 and MMP-9 are the two MMPs referred to as gelatinases. MMP-2 and MMP-9 differ from other MMPs in terms of their structure as well as substrate specificity (39, 47). Although they have the basic structure of MMPs: a prodomain, a thiol group, a signal peptide, and a catalytic domain with Zn at its active center (41), both MMP-2 and MMP-9 have additional fibronectin repeats in the catalytic domain that distinguishes them from other MMPs. The matrix substrates of MMP-9 and MMP-2 are identical (type IV collagen, gelatin). Although MMP-9 is absent from most adult tissues, MMP-2 is constitutively expressed in almost all tissues. Several studies have shown that MMP-2 and MMP-9 are highly expressed in IBD inflamed colonic mucosa of IBD patients and are associated with disease activity (1, 20, 46, 55).

We and others have demonstrated that MMP-9 activity and protein expression is absent from normal colonic mucosa but is upregulated during experimental colitis in response to luminal toxin (dextran sodium sulfate, DSS) as well as bacteria Salmonella enterica serovar Typhimurium (S.T.) (4, 14, 19, 43). MMP-9−/− mice exposed to DSS or S.T. had dramatically reduced inflammation and mucosal injury and showed protection against acute colitis. Similar to MMP-9, MMP-2 protein expression and activity is highly upregulated during DSS- and S.T.-induced colitis (10). In contrast to MMP-9, MMP-2 served to protect from experimental colitis induced by chemicals (DSS) or bacteria (S.T.). MMP-2−/− mice were highly sensitive to experimental colitis, resulting in a significant increase in inflammation and mortality. Bone marrow chimera studies showed that epithelial, but not immune cell-derived, MMP-2 and MMP-9 mediated the effects on the development of colonic inflammation (4, 10). Together, these studies show that MMP-2 and MMP-9 have contrasting roles in the development of colitis despite structural similarities and upregulation of both during colitis. That MMP-9 drives colitis makes it a logical therapeutic target for IBD treatment. However, an in vivo dosing regimen capable of maintaining inhibition of MMP-9 would likely also block MMP-2 since the proteases have structurally similar active sites. Thus the aim of the present study is to determine the outcome of absence of both MMP-9 and MMP-2 in colitis using mice that are deficient in both MMP-2 and MMP-9.

MATERIALS AND METHODS

Experimental animals. The Animal Care Committee of Emory University, Atlanta approved all procedures performed on animals, which were in accordance with the “Guide for the Care and Use of Laboratory Animals” [DHEW Publication No. (NIH) 85-23, Revised 1996, Office of Science and Health Reports, DRR/NIH, Bethesda, MD 20205]. MMP-2−/− mice (C57/B6 background) were a kind gift from Dr. Lynn Matrisian (Vanderbilt University) (10, 17). MMP-9−/− mice were highly sensitive to experimental colitis, resulting in a significant increase in inflammation and mortality. Bone marrow chimera studies showed that epithelial, but not immune cell-derived, MMP-2 and MMP-9 mediated the effects on the development of colonic inflammation (4, 10). Together, these studies show that MMP-2 and MMP-9 have contrasting roles in the development of colitis despite structural similarities and upregulation of both during colitis. That MMP-9 drives colitis makes it a logical therapeutic target for IBD treatment. However, an in vivo dosing regimen capable of maintaining inhibition of MMP-9 would likely also block MMP-2 since the proteases have structurally similar active sites. Thus the aim of the present study is to determine the outcome of absence of both MMP-9 and MMP-2 in colitis using mice that are deficient in both MMP-2 and MMP-9.

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mice were purchased from Jackson Laboratories (Bar Harbor, ME) and bred at our facility (4). The homozygous MMP-2−/− mice of C57/B6 background with disruption of the MMP-2 gene (10, 17, 40) were backcrossed with the homozygous MMP-9−/− mice of C57/B6 background with disruption of the MMP-9 gene to generate homozygous MMP-2−/−/MMP-9−/− double knockout mice (dKO). Genotyping was done by PCR analysis using genomic DNA isolated from a small piece of tail. For MMP-2 wild-type (WT) mice, we used the primers 5'-CAACGATGGGACGACATG-3' and 5'-GC-GGAGACTTGATCATGG-3', and for MMP-2−/− we used the primers 5'-GACCCCGAGCAACAGATC-3' and 5'-CAAGAGGGGATAGAAGG-3'. For MMP-9 wild types, we used 5'-GCATCTG-TGTACCGCTATGG-3' and 5'-TGTTGATGTATGATGTTG GCCC-3' primers. For the MNP-9−/−, we used 5'-ATGATTGAA ACAAGATG-3' and 5'-TCGTCGACAATCATCGTACGAC-3' primers. The lack of MMP-2 and MMP-9 protein was confirmed by Western blot analysis. WT and MMP-2−/−/MMP-9−/− dKO littersmates used in the study were around 8 wk old at the beginning of the experimental protocol and were maintained on a 12:12-h dark-light cycle with free access to pelleted nonpurified diet and tap water under conditions of controlled temperatures (25 ± 2°C).

**Induction of DSS colitis.** Colitis was induced in two groups of age- and sex-matched male and female WT and MMP-2−/−/MMP-9−/− dKO littersmates by oral administration of DSS (ICN Biomedicals, Aurora, OH) at 3% (wt/vol) in tap water ad libitum for 7 days. Age-matched male and female WT and MMP-2−/−/MMP-9−/− dKO littersmates receiving tap water served as control. Mice were observed daily and evaluated for changes in body weight and the development of clinical symptoms. N = 6 mice/group.

**S. T. infection.** Gut-restricted S. T. infection was induced as described previously (2, 4). To prepare S. T. inocula, bacteria (S. T. SL3201) were grown overnight at 37°C in 10 ml of Luria-Bertani broth in a 20-ml container with shaking (150 rpm) and were then used to inoculate fresh medium (1:100) and were grown under the same conditions for 2–3 h until an optical density at 550 nm of 0.35–0.6 was reached. Bacterial cultures were then diluted in normal saline, and the colony-forming units were enumerated by plating a dilution series on nutrient agar (45). Briefly, for inflammation, rare inflammatory cells in the lamina propria were counted as 0, increased numbers of granulocytes in the lamina propria as 1, and confluent of inflammatory cells extending into the submucosa as 2; a score of 3 was given for transmural extension of the infiltrate. For crypt damage, intact crypt was scored 0, loss of one-third basal was counted as 1, loss of two-thirds basal was counted as 2, entire crypt loss was scored 3, change of epithelial surface with erosion was scored 4, and a score of 5 was given for confluent erosion. For evaluation of ulcers, an absence of ulcer was scored 0, one or two foci of ulcerations were scored as 1, three or foci of ulcerations were scored as 2, and confluent/extensive ulceration was scored 3. These values were added to give a total histological score of 11.

**MPO activity in the colon.** Neutrophil infiltration into colon was quantified by measuring myeloperoxidase (MPO) activity as described previously (4, 11). Briefly, a portion of colon was homogenized in 1:20 (wt/vol) of 50 mM phosphate buffer (pH 6.0) containing 0.5% hexadecyltrimethyl ammonium bromide (Sigma, St. Louis, MO) on ice by use of a Polytron homogenizer. The homogenate was sonicated for 10 s, freeze-thawed three times, and centrifuged at 14,000 rpm for 15 min. The supernatant (1 μl) was added to 1 mg/ml of o-diaminobenzidine hydrochloride (Sigma) and 0.005% 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 of peroxide per minute at 25°C. The results were expressed as absorbance per gram of tissue.

**Measurement of cytokines.** Proinflammatory cytokines were measured by ELISA in medium collected from colonic organ culture. Colitis was induced by DSS in two groups of age- and sex-matched male and female WT and MMP-2−/−/MMP-9−/− dKO littersmates. Age-matched male and female WT and MMP-2−/−/MMP-9−/− dKO

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Western blot analysis as described in METHODS. Western blot lysates were prepared from the colon mucosal stripping for colitis. Mice were euthanized after 7 days of DSS and protein

B6 MMP-2

MMP-2 in the pathogenesis of colitis, we created C57/B6 MMP-2−/− mice. To investigate the relative role of MMP-9 and MMP-2, we created C57/B6 MMP-9−/− dKO mice with targeted deletion of MMP-2 and MMP-9 (4, 17). These mice exhibit a normal phenotype. We administered 3% DSS in drinking water to age-matched mice and sexmatched C57/B6 WT and homozygous MMP-2−/−/MMP-9−/− dKO mice. Both WT and MMP-2−/−/MMP-9−/− dKO mice exposed to DSS for 7 days developed signs of colitis. Mice were euthanized after 7 days of DSS and protein lysates were prepared from the colon mucosal stripping for Western blot analysis as described in METHODS. Western blot analysis showed that MMP-2−/−/MMP-9−/− dKO mice with or without the treatment of DSS lacked MMP-9 and MMP-2 protein expression (Fig. 1, A and B, lanes 5–8). WT mice given water showed no MMP-9 protein expression but constitutive MMP-2 protein expression (Fig. 1, A and B, respectively, lanes 1–2). WT mice treated with DSS showed increased MMP-9 and MMP-2 protein expression compared with WT control mice (Fig. 1, A and B, respectively, lanes 3–4). Densitometric analysis shown by the graphs revealed a 3.67 ± 0.22-fold increase in MMP-9 protein expression and a 3.87 ± 0.63-fold increase in MMP-2 protein expression in WT mice treated with DSS compared with WT mice treated with water (means ± SE, 6 mice per group; P < 0.05) (Fig. 1, A and B, respectively). We next assessed the clinical signs of disease according to a previously described grading system (4, 5). Almost all the WT mice developed diarrhea after day 5. These mice were hemorrhagic positive starting from day 4 and exhibited frank bleeding on day 7 of DSS administration. Interestingly, MMP-2−/−/MMP-9−/− dKO mice were protected from DSS-induced colitis. Clinical score, based on the three parameters of weight loss, stool consistency, and fecal blood, was 4.6 ± 1.2 in MMP-2−/−/MMP-9−/− dKO mice compared with WT mice having a score of 9.7 ± 0.2 as shown in Fig. 2A (means ± SE, 6 mice per group; P value is <0.05). 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−/−/MMP-9−/− dKO mice exposed to DSS were examined histologically and compared with those from normal controls. Figure 2B shows a mean histological score of 4 ± 1.7 in MMP-2−/−/MMP-9−/− among dKO mice given DSS compared with a mean histological score of 7.4 ± 0.6 for WT mice given DSS (means ± SE, 6 mice per group; P < 0.05). Interestingly, MMP-2−/−/MMP-9−/− dKO mice treated with DSS showed less inflammation and colonic damage compared with WT mice treated with DSS.

**RESULTS**

MMP-2−/−/MMP-9−/− dKO mice are resistant to DSS-induced colitis. To investigate the relative role of MMP-9 and MMP-2 in the pathogenesis of colitis, we created C57/B6 MMP-2−/−/MMP-9−/− dKO mice with targeted deletion of MMP-2 and MMP-9 (4, 17). These mice exhibit a normal phenotype. We administered 3% DSS in drinking water to age-matched mice and sexmatched C57/B6 WT and homozygous MMP-2−/−/MMP-9−/− dKO mice. Both WT and MMP-2−/−/MMP-9−/− dKO mice exposed to DSS for 7 days developed signs of colitis. Mice were euthanized after 7 days of DSS and protein lysates were prepared from the colon mucosal stripping for Western blot analysis as described in METHODS. Western blot analysis showed that MMP-2−/−/MMP-9−/− dKO mice with or without the treatment of DSS lacked MMP-9 and MMP-2 protein expression (Fig. 1, A and B, lanes 5–8). WT mice given water showed no MMP-9 protein expression but constitutive MMP-2 protein expression (Fig. 1, A and B, respectively, lanes 1–2). WT mice treated with DSS showed increased MMP-9 and MMP-2 protein expression compared with WT control mice (Fig. 1, A and B, respectively, lanes 3–4). Densitometric analysis shown by the graphs revealed a 3.67 ± 0.22-fold increase in MMP-9 protein expression and a 3.87 ± 0.63-fold increase in MMP-2 protein expression in WT mice treated with DSS compared with WT mice treated with water (means ± SE, 6 mice per group; P < 0.05) (Fig. 1, A and B, respectively). We next assessed the clinical signs of disease according to a previously described grading system (4, 5). Almost all the WT mice developed diarrhea after day 5. These mice were hemorrhagic positive starting from day 4 and exhibited frank bleeding on day 7 of DSS administration. Interestingly, MMP-2−/−/MMP-9−/− dKO mice were protected from DSS-induced colitis. Clinical score, based on the three parameters of weight loss, stool consistency, and fecal blood, was 4.6 ± 1.2 in MMP-2−/−/MMP-9−/− dKO mice compared with WT mice having a score of 9.7 ± 0.2 as shown in Fig. 2A (means ± SE, 6 mice per group; P value is <0.05). 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−/−/MMP-9−/− dKO mice exposed to DSS were examined histologically and compared with those from normal controls. Figure 2B shows a mean histological score of 4 ± 1.7 in MMP-2−/−/MMP-9−/− among dKO mice given DSS compared with a mean histological score of 7.4 ± 0.6 for WT mice given DSS (means ± SE, 6 mice per group; P < 0.05). Interestingly, MMP-2−/−/MMP-9−/− dKO mice treated with DSS showed less inflammation and colonic damage compared with WT mice treated with DSS.

**Fig. 1.** Matrix metalloproteinase (MMP)-2 and MMP-9 protein expression is upregulated during dextran sodium sulfate (DSS) colitis. Mice were given water or 3% DSS (wt/vol) for 7 days, after which time mice were euthanized. Colon was harvested and processed for Western blot. A and B: representative Western blots of protein from the colon of wild-type (WT) mice given water (lanes 1–2) or DSS (lanes 3–4) and MMP-2−/−/MMP-9−/− double knockout (dKO) mice given water (lanes 5–6) or DSS (lanes 7–8). Blots were probed with anti-MMP-9 (1:1,000) or anti-MMP-2 (7.5 μg/ml) (Abcam, Cambridge, MA). Western blot was quantified by scanning densitometry and represented as fold increase of MMP-9/β-tubulin (A) and MMP-2/α-tubulin (B) in WT mice treated with DSS compared with WT mice treated with water in adjacent graphs. Values are representative of 3 individual experiments, means ± SE; n = 6.

**RESULTS**

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WT mice had extensive ulceration with almost complete loss of crypt architecture and increased infiltration of neutrophils. The ulcers were not only greater in number but involved larger surface area compared with MMP-2−/−/MMP-9−/− dKO mice (Fig. 2C). Histological signs of inflammation were not detected in the water control groups (data not shown). To confirm the histological finding of granulocyte infiltration, the myeloperoxidase assay was performed. MMP-2−/−/MMP-9−/− dKO
mice showed significantly less MPO activity of 708.85 ± 143.93 units/g tissue wt compared with WT mice having MPO activity of 1,267.05 ± 198.85 units/g tissue wt (means ± SE, 6 mice per group; \( P < 0.05 \)) (Fig. 2D). Thus the data obtained supported the results obtained from clinical analysis and confirmed the resistance toward the development of colitis as a synergistic effect of targeted MMP-9 and MMP-2 deletion.

We next measured IL-6 and IL-8 (KC) in the serum of WT and MMP-2−/−/MMP-9−/− dKO mice given DSS or water. Proinflammatory cytokine (IL-6) and chemokine (KC) were measured by ELISA after inducing colitis by DSS in two groups of age- and sex-matched male and female WT and MMP-2−/−/MMP-9−/− litters. Age-matched male and female WT and MMP-2−/−/MMP-9−/− littersmates receiving tap water served as control. Figure 2E shows that there was significantly decreased (\( P < 0.05 \)) expression of IL-6 among MMP-2−/−/MMP-9−/− dKO mice (7.4 ± 2.6) compared with WT mice (43.5 ± 1.6) treated with DSS. Similarly, Fig. 2E shows decreased expression (\( P < 0.005 \)) of chemokine KC among MMP-2−/−/MMP-9−/− dKO mice (56.67 ± 13.4) compared with WT mice (130.75 ± 10.8) in the DSS-treated group.

MMP-2−/−/MMP-9−/− dKO mice are resistant to salmonella-induced colitis. The bacteria-induced colitis model was used as a second model to confirm the data obtained by the DSS-induced colitis model. S.T. strain SL3201 was used for this model and was administered orally after pretreatment of mice with streptomycin. In this model, S.T. induces clinical and histological features of enterocolitis predominantly involving the cecum (2, 4). We chose this model since it recapitulates several aspects of clinical and histological human S.T. infection as well as acute flares of IBD, wherein mucosa-pathogen interaction is thought to play an important role in the pathogenesis of inflammation. 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. Mice were euthanized at 48 h after the administration of S.T. and colonic tissue was removed, weighed with the contents, and photographed. Western blot analysis of cecum tissues showed that there was no protein expression of MMP-9 and MMP-2 among the MMP-2−/−/MMP-9−/− dKO mice (Fig. 3, A and B, respectively, lanes 7–10) with or without the treatment of S.T. WT mice without the treatment of S.T. showed no protein expression of MMP-9 (Fig. 3A, lanes 1–2) and showed constitutive protein expression of MMP-2 (Fig. 3B, lanes 1–2). In contrast, WT mice infected with S.T. showed increased protein expression of MMP-9 and MMP-2 (Fig. 3, A and B, respectively, lanes 3–6). Densitometric analysis shown by the graphs revealed 16.17 ± 0.12-fold increase in MMP-9 protein expression and a 4.65 ± 1.04-fold increase in MMP-2 protein expression in WT mice treated with S.T. compared with WT mice treated without S.T. (means ± SE, 6 mice per group; \( P < 0.05 \)) (Fig. 3, A and B, respectively). The cecum was processed for histology and MPO activity. MMP-2−/−/MMP-9−/− dKO mice showed relatively normal cecae and significantly reduced inflammatory infiltrates. In contrast, cecae of all the WT mice infected with S.T. appeared pale and shriveled to a small size and were filled with purulent exudates. This clinical observation was reflected by histological score (Fig. 3C) and leukocyte infiltration with loss of crypts as well as ulcerations (Fig. 3D).

Figure 3C shows a mean histological score of 3.34 ± 0.34 in MMP-2−/−/MMP-9−/− dKO mice treated with S.T. compared with a mean histological score of 7.0 ± 0.01 for WT mice treated with S.T. (means ± SE, 6 mice per group; \( P < 0.05 \)). MMP-2−/−/MMP-9−/− dKO mice treated with S.T. showed less inflammation and damage compared with WT mice treated with S.T. (Fig. 3D). MMP-2−/−/MMP-9−/− dKO mice had reduced MPO activity of 136.86 ± 0.01 units/g tissue wt compared with WT mice 2,148.48 ± 0.15 units/g tissue wt (means ± SE, 6 mice per group; \( P < 0.05 \)) (Fig. 3E). Taken together, these data suggest that MMP-9−/−/MMP-2−/− dKO mice are protected from S.T.-induced colitis.

MMP-2−/−/MMP-9−/− dKO mice are resistant to TNBS-induced colitis. TNBS-induced colitis was used as a third model to support the data from DSS-induced colitis model and S.T.-induced colitis model. Colitis was induced by use of TNBS (150 mg/kg body wt) as described in methods. Western blot analysis showed that MMP-2−/−/MMP-9−/− dKO mice with or without the treatment of TNBS lacked MMP-9 and MMP-2 protein expression (Fig. 4, A and B, lanes 7–10). WT mice treated with vehicle showed no expression of MMP-9 protein but demonstrated constitutive expression of MMP-2 (Fig. 4, A and B, respectively, lanes 1–5). Both MMP-2 and MMP-9 expression increased with TNBS treatment (Fig. 4, A and B, respectively, lanes 4–6). Densitometric analysis revealed a 13.45 ± 0.3-fold increase in MMP-9 protein expression and a 3.19 ± 0.2-fold increase in MMP-2 protein expression in WT mice treated with TNBS compared with WT mice treated with vehicle. (means ± SE 6 mice per group; \( P < 0.005 \)) (Fig. 4, A and B, respectively). MMP-2−/−/MMP-9−/− dKO mice developed significantly less inflammation compared with WT mice that received TNBS. Tissues collected from MMP-2−/−/MMP-9−/− dKO mice and WT mice exposed to TNBS were examined histologically and compared with those from controls. MMP-2−/−/MMP-9−/− dKO mice showed less inflammation with less damage to crypt architecture compared with WT mice (Fig. 4C). There was also some infiltration of neutrophils in controls, likely due to ethanol, which damages the mucosal barrier (Fig. 4C). The histological findings were supported by the MPO assay. MMP-2−/−/MMP-9−/− dKO mice showed significantly less MPO activity of 708.85 ± 143.93 units/g tissue wt compared with WT mice having MPO activity of 1,267.05 ± 198.85 units/g tissue wt (means ± SE, 6 mice per group; \( P < 0.05 \)) (Fig. 2D). Thus the data obtained supported the results obtained from clinical analysis and confirmed the resistance toward the development of colitis as a synergistic effect of targeted MMP-9 and MMP-2 deletion.
mice had significantly reduced MPO (325.11 ± 58.1 units/g tissue wt) compared with WT mice administered TNBS 646.1 ± 106.1 units/g tissue wt (MPO units means ± SE; 10 mice per group; P < 0.05) (Fig. 4D). Thus these data corroborated the results obtained from the DSS-induced colitis model and the S.T.-induced colitis model and confirmed the resistance of MMP-9--/MMP-2-- dKO mice to the development of colitis.
MMP-2-/-/MMP-9-/- dKO mice are resistant to trinitrobenzene sulfonic acid (TNBS)-induced colitis. WT C57/B6 and MMP-2-/-/MMP-9-/- dKO mice 8 wk old were weighed and treated with or without TNBS for 48 h. Mice were euthanized, and colon was processed for Western blot and histology. A and B: representative Western blots of protein from the colon of WT mice given vehicle (lanes 1–3) or TNBS (lanes 4–6) and MMP-2-/-/MMP-9-/- dKO mice given vehicle (lanes 7–8) or TNBS (lanes 9–10). Western blot was quantified by scanning densitometry and represented in adjacent graphs as the fold increase of MMP-9/β-tubulin (A) or MMP-2/α-tubulin (B) in WT mice treated with TNBS compared with WT mice treated with vehicle. Values are representative of 3 individual experiments, means ± SE; n = 10. Disease severity was assessed and expressed in terms of histological score. C: representative photomicrographs from 2 individual experiments; n = 10 for TNBS group and n = 10 for control group with magnifications ×4, ×10, and ×40. Colon were snap frozen in liquid nitrogen and myeloperoxidase activity was measured (D) as an index of neutrophil infiltration into the injured tissue. Each bar represents mean ± SE; n = 10 animals for each group. *P < 0.05.
DISCUSSION

This study examined the phenotype of mice lacking both MMP-9 and MMP-2 in three distinct models of experimental colitis (DSS, S.T., and TNBS). We observed that such MMP-9−/−/MMP-2−/− dKO mice were protected from the development of colitis measured by clinical, histological, and biochemical parameters. Thus, if specific inhibition of MMP-9 proves unfeasible, inhibition of both MMP-9 and MMP-2 may still be therapeutically useful. Although developing pharmacological approaches that can effectively achieve potent block of these MMPs in the gut may be difficult, our results support that, at least if successful, the approach would have therapeutic potential.

Our previous data showed that MMP-2 deficiency led to barrier dysfunction and dramatically increased susceptibility to colitis (10). Furthermore, overexpression of MMP-2 in cultured intestinal epithelial cells demonstrated increased barrier protection measured by decreased translocation of fluorescently labeled dextran as well as transepithelial resistance (unpublished data). Conversely, MMP-2−/− mice exhibited decreased barrier function as measured by translocation of fluorescently labeled dextran. Together these data suggested that MMP-2 expression during colitis served to protect from the development of inflammatory response (10) likely through its effect on the epithelial barrier. In contrast, we and others have previously demonstrated that the absence of MMP-9 attenuated the development of inflammatory response to colitogenic agents (4, 32, 43). We demonstrated that overexpression of MMP-9 impaired wound healing in cultured intestinal epithelial cells in vitro (4) and modulated colonic epithelial differentiation (10), suggesting that MMP-9 mediates inflammatory response and/or tissue damage. Our study with MMP-2−/−/MMP-9−/− dKO mice with chemical/and bacterial models of colitis showed that unlike MMP-2−/− mice, MMP-2−/−/MMP-9−/− dKO mice mimic MMP-9−/− mice during colitis. Our data implicate that the tissue damaging effect of MMP-9 overrides the barrier protective role of MMP-2 during colitis.

Recently it has been found that there are a number of nonmatrix MMP substrates that potently influence cellular functions (30), such as cytokines (16), growth factor receptors (25), and chemokines (31). It is well known that MMP-2 and MMP-9 have similar substrate specificities for matrix protein (28, 41). So the opposite roles of MMP-9 and MMP-2 during colitis or the dominating role of MMP-9 over MMP-2 during colitis may be caused by the differences of nonmatrix substrates. For example, MMP-9 cannot degrade monocyte chemotactant protein-3 (31) and fibroblast growth factor receptor-1 (25). This hypothesis is also supported by the fact that MMP-9 has an additional collagen-V like domain that is highly glycosylated and alters the substrate specificity and confers resistance to degradation (53).

Various studies have examined the effect of MMP-9 and MMP-2 deletion in the development of inflammation in a variety of organs, and for the most part these studies have observed that the effects of MMP-2 and MMP-9 are negated in the dKO mice in that the responses to injury in the dKO were similar to those of WT mice. For example, Itoh et al. (18) have observed that their MMP-2−/−/MMP-9−/− dKO mice showed no significant difference from the WT mice in an arthritis model. In another study, Corry et al. (6) have shown the overlapping and independent roles of MMP-9 and MMP-2 to lung allergic inflammatory cell egression. They observed that allergic lung phenotype of MMP-9−/− mice were similar to WT and didn’t alter by concomitant deletion of MMP-2 gene. In another study, done by Zeisberg et al. (57), observed that ablation of both MMP-9 and MMP-2 did not display significant abnormalities in the kidney. In contrast to these studies, Lambert et al. (23) have shown the synergistic effect of MMP-2 and MMP-9 in promoting the choroidal neovascularization. Their study showed that both MMP-9 and MMP-2 cooperate in the course of experimental choroidal neovascularization. Indeed, choroidal pathological angiogenesis was almost completely prevented in MMP-2−/−/MMP-9−/− dKO mice (23). Our data contrast these studies in demonstrating a nonredundant role for MMP-9 in inflammation and tissue damage.

Synthetic MMP inhibitors containing reactive zinc-chelating groups such as thiol or hydroxamate (51) that inhibit the active enzymatic site common to all MMP have been demonstrated to reduce tissue injury and inflammation in some animal models of IBD (7, 33, 34, 37, 50). For example, an orally active MMP inhibitor, ONO-4817, reduces DSS-induced colitis in mice (37). However, lack of efficacy and/or significant side effects associated with general MMP inhibition observed in clinical trials for cancer have precluded their use in human trials (42). Recently, attempts have been made to synthesize specific gelatinase inhibitors (21). However, because of the structural similarity of the gelatinases, targeted inhibition of a specific gelatinase has not been possible and the gelatinase inhibitors developed thus far do not differentiate between MMP-9 and MMP-2 (21). One potential problem of the nonspecificity of inhibitors will be the inhibition of MMP-2. For example, MMP-2 is required for wound healing in different organs (24, 27, 29, 38, 49, 52, 56). Targeted synthetic peptides also cannot solve the problem completely because of their instability (13, 21, 44). Alternatively, tissue inhibitors of metalloproteinases (TIMP)-1 could be used to inhibit MMP-9 given its ability to form 1:1 specific stoichiometry complex with pro-MMP-9 and inhibit MMP-9 activity (35). However, it also can form such complexes with other nonmembrane-bound active MMPs and inhibits their proteolytic activity, e.g., MMP-3, MT-MMPs, and MMP-19 (15, 22, 48). In addition, independent of its action on MMPs, TIMP-1 has been recently recognized to have other biological activities including cell growth, migration, and apoptosis (48). Therefore, therapeutic application of TIMPs through gene therapy is still in an early stage (9).

In summary, we demonstrate that MMP-9 is indispensable for inflammatory response and tissue damage. Together with our previous work, the data here underscore the overriding role of MMP-9 over protective effect of MMP-2 in mediating tissue damage and the potential therapeutic efficacy of MMP-9 inhibition. Data presented herein suggest that concomitant inhibition of MMP-2 and MMP-9 suppresses inflammation underscoring the potential therapeutic efficacy of gelatinase inhibition in the treatment of IBD.

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