Matrix metalloproteinase 9-induced increase in intestinal epithelial tight junction permeability contributes to the severity of experimental DSS colitis

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Submitted 30 July 2015; accepted in final form 7 October 2015

Nighot P, Al-Sadi R, Rawat M, Guo S, Watterson DM, Ma T. Matrix metalloproteinase 9-induced increase in intestinal epithelial tight junction permeability contributes to the severity of experimental DSS colitis. Am J Physiol Gastrointest Liver Physiol 309: G988–G997, 2015. First published October 29, 2015; doi:10.1152/ajpgi.00256.2015.—Recent studies have implicated a pathogenic role for matrix metalloproteinases 9 (MMP-9) in inflammatory bowel disease. Although loss of epithelial barrier function has been shown to be a key pathogenic factor for the development of intestinal inflammation, the role of MMP-9 in intestinal barrier function remains unclear. The aim of this study was to investigate the role of MMP-9 in intestinal barrier function and intestinal inflammation. Wild-type (WT) and MMP-9−/− mice were subjected to experimental dextran sodium sulfate (DSS) colitis by administration of 3% DSS in drinking water for 7 days. The mouse colonic permeability was measured in vivo by recycling perfusion of the entire colon using fluorescently labeled dextran. The DSS-induced increase in the colonic permeability was accompanied by an increase in intestinal epithelial cell MMP-9 expression in WT mice. The DSS-induced increase in intestinal permeability and the severity of DSS colitis was found to be attenuated in MMP-9−/− mice. The colonic protein expression of myosin light chain kinase (MLCK) and phospho-MLC was found to be significantly increased after DSS administration in WT mice but not in MMP-9−/− mice. The DSS-induced increase in colonic permeability and colonic inflammation was attenuated in MLCK−/− mice and MLCK inhibitor ML-7-treated WT mice. The DSS-induced increase in colonic surface epithelial cell MLCK mRNA was abolished in MMP-9−/− mice. Lastly, increased MMP-9 protein expression was detected within the colonic surface epithelial cells in ulcerative colitis cases. These data suggest a role of MMP-9 in modulation of colonic epithelial permeability and inflammation via MLCK.

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ileus model of enterocolitis. These studies provided compelling evidence that the increased intestinal expression of MMP-9 is an important pathogenic factor in the development of intestinal inflammation in animal models of IBD. The mechanistic link between MMP-9 and intestinal inflammation is, however, not yet clearly known. In the Caco-2 intestinal cell line, MMP-9 has been shown to inhibit cell adhesion and wound repair (8) while in transgenic mice, expression of MMP-9 in the intestinal epithelium is associated with an increase in the proinflammatory chemokine Kc and increased inflammation during DSS and Salmonella typhimurium-induced enterocolitis (19). The role of MMP-9 in mediating intestinal epithelial permeability, which is an integral factor for development of IBD, has not been previously studied. Thus the aim of this study was to investigate the role of MMP-9 in intestinal epithelial permeability using a chemically induced DSS colitis model. Our results show that the MMP-9-induced increase in myosin light chain kinase (MLCK) expression and increase in intestinal permeability play a key pathogenic role in DSS-induced colitis.

MATERIALS AND METHODS

Experimental animals and induction of colitis. Studies were approved by the University of New Mexico Institutional Animal Care and Use Committee. MMP-9 null (MMP-9−/−) and wild-type (WT) (MMP-9+/+) mice (both of C57BL/6 background) of 9 wk of age were obtained from The Jackson Laboratory (Bar Harbor, Maine). Generation of MLCK−/− mice is described previously (53). Mice received 3% DSS (molecular mass: 36,000–50,000 Da; MP Biomedicals, Santa Ana, CA) in autoclaved drinking water for 7 days (56). The body weights of mice were monitored daily, and the disease activity index and histological grading of colitis lesions were carried out, as described previously (56). For pharmacological inhibition of MLCK, the MLCK inhibitor ML-7 (12764; Sigma) was administered intraperitoneally at the rate of 2 mg/kg body wt, twice a day. To study the role of MMP-9 in inflammatory chemokine Kc and increased inflammation during DSS and Salmonella typhimurium-induced enterocolitis (19). The role of MMP-9 in mediating intestinal epithelial permeability, which is an integral factor for development of IBD, has not been previously studied. Thus the aim of this study was to investigate the role of MMP-9 in intestinal epithelial permeability using a chemically induced DSS colitis model. Our results show that the MMP-9-induced increase in myosin light chain kinase (MLCK) expression and increase in intestinal permeability play a key pathogenic role in DSS-induced colitis.

RESULTS

DSS-induced epithelial permeability is attenuated in MMP-9−/− mice. Elevated MMP-9 intestinal tissue levels in IBD patients and animal models of intestinal inflammation have been reported to closely correlate with the disease activity and degree of inflammation. Consistent with previous reports (13, 31), we observed increased levels of MMP-9 in murine DSS colitis tissue by Western blot (Fig. 1A). Furthermore, in confocal immunofluorescence, MMP-9 immunostaining was detected within the epithelial cells in DSS colitis and not control colon (Fig. 1B). Paracellular permeability and epithelial TJ barrier is a critical component of clinical as well as experimental colitis. However, the role of MMP-9 in intestinal permeability is not known. Thus we investigated colonic paracellular permeability in DSS colitis, using in vivo method of whole length colonic recycling perfusion with Texas red-labeled dextran (10 kDa) as a macromolecular marker. We found that baseline colonic dextran flux was not significantly different in WT and MMP-9−/− mice. DSS colitis led to a substantial

AJP-Gastrointest Liver Physiol • doi:10.1152/ajpgi.00256.2015 • www.ajpgi.org
increase in dextran flux in the colon of WT DSS-treated mice. This DSS-induced increase in dextran flux was greatly attenuated in MMP-9−/− mice colon (Fig. 1C). As an alternative method to assess colonic permeability, TER was measured by mounting mouse colonic tissues on Ussing chambers. Induction of DSS colitis led to a 25% reduction in the average colonic TER in WT mice (41.28 ± 0.46 and 33 ± 0.25 Ω·cm² colonic TER in control and DSS mice, respectively; Fig. 1D). This DSS-induced reduction in TER was not evident in MMP-9−/− mice (44.23 ± 1.16 and 42.46 ± 0.84 Ω·cm² colonic TER in control and DSS mice, respectively). Thus the DSS-induced increase in intestinal permeability was found to be significantly attenuated in MMP-9−/− mice, suggesting an important role of MMP-9 in DSS-induced increase in intestinal permeability in DSS colitis. We also found that exogenous MMP-9 alone also caused an increase in colonic dextran 10-kDa flux in a dose-dependent manner (Fig. 1E). The MMP-9-injected mice did not show any symptoms of colitis, histological changes of inflammation in colon, or elevated levels of proinflammatory cytokines TNF-α and IL-1β mRNA in the colonic tissue (data not shown), indicating that the MMP-9-mediated increase in colonic permeability did not result from colonic inflammation.

Severity of DSS colitis is reduced in MMP-9−/− mice. Attenuation of the DSS-induced colitis in MMP-9-deficient mice has been reported previously (44). To validate these findings, we compared the induction of experimental colitis in WT and MMP-9−/− mice; we found that the severity of experimental colitis after 7 days of oral administration of 3% DSS was significantly lower in MMP-9−/− mice compared with the WT mice. The loss of body weight and the disease activity index (56) were significantly attenuated in MMP-9−/− mice compared with WT mice (data not shown). Furthermore, the DSS-treated MMP-9−/− mice had much less histopathological changes compared with WT DSS mice in terms of colonic mucosal erosion, neutrophilic and mononuclear infiltration in the lamina propria, loss of crypts, and edema in muscularis layer of the colon (histological score: 2.55 ± 0.15 and 1.56 ± 0.17 for WT and MMP-9−/− mice, respectively, on a scale of 0–4; *P < 0.01). Thus the reduction of clinical severity and inflammation in DSS-induced colitis in MMP-9−/− mice was found to be accompanied by reduction in DSS-induced colonic permeability in MMP-9−/− mice (Fig. 1, C and D).

TJ protein analysis in DSS colitis in MMP-9−/− mice. As intestinal permeability is regulated by interepithelial apical TJ complex, we next studied the protein level of select TJ proteins. The total protein level of occludin in the colon was not affected in DSS colitis in WT mice (Fig. 2, A and B). In MMP-9−/− mice, the occludin level was found to be increased in DSS colitis compared with control tissue. DSS colitis did not significantly affect protein levels of Claudin-1 and Claudin-3 in WT and MMP-9−/− mice. DSS colitis also caused an increase in Claudin-2 and decrease in Claudin-5 protein levels to a similar extent in WT and MMP-9−/− mice.

MLCK and phospho-MLC protein analysis in DSS colitis in MMP-9−/− mice. Intestinal barrier function or epithelial permeability is also known to be regulated by MLCK via MLC phosphorylation (45, 51, 65). We examined MLCK protein levels in colonic tissue from WT and MMP-9−/− mice after DSS administration. DSS colitis caused a significant increase in the MLCK protein level in the colon of WT but not MMP-9−/− mice, as seen in Western blots (Fig. 3A). Next, we investigated phosphorylation of MLC in DSS colitis tissue. We found comparable levels of total MLC in control and DSS colon of WT and MMP-9−/− mice (Fig. 3B). However, protein
expression of phospho-MLC was found to be upregulated in the DSS colon of WT but not MMP-9−/− mice. In confocal immunofluorescence examination, DSS administration resulted in a marked increase in phospho-MLC staining on the colonic apical membrane of WT but not MMP-9−/− mice (Fig. 3C).

Role of MLCK in DSS colitis. The above results suggested that MMP-9 modulates both intestinal permeability and severity of inflammation through an increase in MLCK expression. To further clarify the role of MLCK in DSS colitis, we induced DSS colitis in MLCK−/− mice in which the long MLCK isoform that is normally expressed in intestine is selectively deleted but the production of the short MLCK isoform expressed in smooth muscles is retained (53). We observed that the severity of experimental colitis after 7 days of oral administration of 3% DSS was significantly lower in MLCK−/− mice compared with the WT mice. The loss of body weight (Fig. 4A), the disease activity index (56) (Fig. 4B), and the histological inflammatory changes (Fig. 4, C and D) were also significantly attenuated in MLCK−/− mice compared with WT mice. Additionally, in vivo colonic perfusion studies revealed that the DSS-induced increase in dextran flux in the colon of WT mice was significantly prevented in MLCK−/− mice (Fig. 4E).

Fig. 2. Effect of DSS colitis on various tight junction proteins. A: DSS colitis caused an increase in occludin protein level in MMP-9−/− mice, as assessed by Western blot analysis. DSS colitis also caused an increase in claudin-2 and decrease in claudin-5 protein levels to a similar extent in WT and MMP-9−/− mice but did not significantly affect claudin-1, and -3. Representation of more than 3 blots in each group. B: densitometry analysis for occludin protein levels.

Fig. 3. Myosin light chain kinase (MLCK) and phospho-MLC protein analysis in DSS colitis in MMP-9−/− mice. A: in Western blot analysis, MLCK protein expression was found to significantly increased in WT DSS mice colon but not MMP-9−/− DSS mice colon. The bar graph at bottom represents densitometry of MLCK protein bands from more than 3 samples, as normalized to β-actin protein bands of respective samples. Data are means ± SE. *P < 0.01 vs. all other groups. B: DSS colitis caused an increase in phosphorylated MLC (pMLC) protein level in WT but not in MMP-9−/− mice, as assessed by Western blot analysis. Levels of MLC are shown as control (representative of n = 4). C: in confocal immunofluorescence, increased staining of pMLC (red) was seen on apical membrane in DSS colitis tissue of WT but not MMP-9−/− mice. Actin is stained in green and nuclei in blue color. Red bar = 10 μM.
As an alternative to above studies in MLCK\textsuperscript{−/−} mice, we also examined the effect of the MLCK inhibitor ML-7 on DSS-induced colitis in WT mice. We found that the severity of experimental colitis after 7 days of oral administration of 3% DSS was significantly attenuated in mice concurrently treated with ML-7 compared with control DSS mice. The loss of body weight (Fig. 5A), the disease activity index (56) (Fig. 5B), and the histological inflammatory changes (Fig. 5, C and D) were significantly attenuated in mice treated with the ML-7 compared with control DSS mice. Also, in vivo colonic perfusion studies revealed that ML-7 treatment significantly reduced the DSS-induced increase in colonic dextran flux (Fig. 5E). These data, together with the findings in MLCK\textsuperscript{−/−} mice, clearly demonstrate the central role of MLCK in mediating the DSS-induced increase in intestinal permeability and colonic inflammation.

**MLCK mRNA expression in MMP-9\textsuperscript{−/−} DSS colitis.** To further corroborate the relevance of the role of intestinal epithelial MMP-9 and MLCK in mediating the increase in colonic permeability, we examined MLCK mRNA expression in colonic surface epithelial cells by capturing a pure population of colonic epithelial cells using laser capture microdissection. We found that DSS administration resulted in a significant increase in MLCK mRNA expression in the pure population of colonic surface epithelial cells (Fig. 6, A and B). In MMP-9\textsuperscript{−/−} mice, however, DSS did not cause an increase in MLCK mRNA expression in colonic surface epithelial cells (Fig. 6B). These results further support the role of MMP-9 in mediating...
the DSS-induced increase in MLCK expression, intestinal permeability, and intestinal inflammation.

**MMP-9 immunolocalization in UC.** To extrapolate the relevance of our experimental findings in an animal model of colitis to UC in human patients, we examined human colonic biopsies for protein levels and immunolocalization of MMP-9. In Western blot analysis, the total protein level of MMP-9 in colonic tissue was found to be markedly increased in UC patients (Fig. 7A). In confocal immunofluorescence examination, sparse staining for MMP-9 was observed in the colonic epithelium of healthy control patients. However, in UC, the colonic epithelium showed strong staining for MMP-9 (Fig. 7B). These data also support a potential role of MMP-9 in the increase in MLCK and intestinal permeability in UC patients (7).

**DISCUSSION**

The focus of the present study was to investigate the role of MMP-9 in intestinal permeability and intestinal inflammation in DSS-induced colitis. We investigated the role of MMP-9-mediated colonic permeability in DSS-induced colitis using in
vivo colonic reperfusion technique. In agreement with others and our previous reports (38, 46, 61), the DSS administration significantly increased colonic dextran flux in WT mice. The DSS-induced increase in colonic permeability was associated with a marked increase in MMP-9 expression in intestinal epithelial cells in WT mice; the DSS-induced increase in colonic permeability was significantly attenuated in MMP-9−/− mice. Alternatively, we measured TER of colonic tissues, ex vivo, on Ussing chambers. Consistent with the in vivo colonic perfusion studies, DSS caused a drop in TER and the decrease in TER was found to be attenuated in MMP-9−/− mice. These data suggested that MMP-9 was required for the DSS-induced increase in colonic permeability. We also observed that the severity of DSS colitis is decreased in MMP-9−/− mice. The loss of body weight, clinical disease activity score, and colonic histological score of inflammation were significantly attenuated in MMP-9−/− mice following DSS administration. Several studies have shown epithelial TJ barrier function to correlate with occludin localization and expression (2, 29, 57, 64). Occludin has been shown to be cleaved and degraded by MMP-9, particularly in regards to blood-brain-barrier (6, 20, 26, 60). Total expression of claudin-1 and -3 was not affected in DSS colitis in WT or MMP-9−/− mice. Alternatively, the pore-forming TJ protein claudin-2 was found to be upregulated to similar extent in colon of both WT and MMP-9−/− mice, following DSS administration. Upregulation of claudin-2 has been reported in several animal models of intestinal inflammation (42), IBD (40, 54), and colonic cancer (15). Consistent with previous reports (33, 59), claudin-5 levels were found to be decreased in DSS colitis in our studies.

Previous studies from our laboratory and others have shown the central role of MLCK in the regulation of tight junction permeability in intestinal epithelium (23, 50, 63). Thus we also studied the role of MLCK in MMP-9-mediated colonic permeability. We observed that DSS treatment led to an increased expression of MLCK protein in colonic tissue of WT but not MMP-9−/− mice. As a consequence, increased expression of pMLC was found in colonic tissue of WT but not MMP-9−/− mice by Western blot analysis and confocal immunofluorescence. MLCK catalyzes the phosphorylation of MLC, which,

Fig. 6. Colonic epithelial MLCK mRNA expression in MMP-9−/− DSS colitis. A pure population of colonic surface epithelial cells was collected using laser capture microdissection (LCM). A: before and after LCM image of colonic mucosa where arrows indicate the epithelial cells removed by LCM. B: DSS colitis caused a significant increase in colonic epithelial cell MLCK mRNA level in WT but not MMP-9−/− mice, as measured by real-time PCR (means ± SE; n = 3; *P < 0.001 vs. all other groups). The MLCK mRNA expression was normalized to GAPDH mRNA expression in respective samples.

Fig. 7. Protein expression and immunolocalization of MMP-9 protein in ulcerative colitis (UC). A: in Western blot analysis, MMP-9 protein levels were significantly elevated in UC colonic tissue. Representation of n = 4. B: sparse staining for MMP-9 (red) was detected in normal human colon but marked staining was observed in the colonic epithelium of ulcerative colitis patient. Nuclei are stained in blue. White bar = 10 μM.
in turn, leads to the activation of myosin-Mg\(^{2+}\)-ATPase and subsequent contraction of perijunctional acto-myosin ring and mechanical opening of the TJ barrier (24). Our data suggested that MMP-9 modulates the DSS-induced increase in colonic permeability by inducing an increase in MLCK expression. The involvement of MLCK in DSS-induced increase in colonic permeability was demonstrated by using MLCK\(^{-/-}\) mice and a pharmacologic inhibitor of MLCK, ML-7. The DSS-induced increase in colonic permeability and severity of DSS colitis, in terms of loss of body weights, disease activity index, and histological inflammatory changes, were significantly attenuated in MLCK\(^{-/-}\) mice or following treatment with ML-7. The role of MLCK in intestinal TJ permeability is very well established in previous studies from our laboratory and others (1, 10, 12, 14, 23, 25, 36, 49, 55). The role of MLCK in DSS colitis, however, has not been studied extensively. In one study, MLCK inhibitor ML-7 significantly ameliorated intestinal mucosal permeability and the severity of DSS-induced colitis (21). In another recent study, however, long MLCK\(^{-/-}\) mice when exposed to DSS had no significant differences in terms of disease activity and inflammation, compared with WT mice (48). This disparity between the latter study and our present study could be in part due to the differences in mouse strain used and local experimental conditions including host microbiome or other factors that affect DSS colitis development.

Considering the above findings on the role of MMP-9 in modulation of epithelial permeability via MLCK, it was important to investigate if MMP-9 directly affects expression of MLCK in the colonic epithelial cells. Indeed, in pure colonic surface epithelial cells collected by laser capture, we were able to show that MLCK mRNA upregulation after DSS colitis in WT mice is abolished in MMP-9\(^{-/-}\) DSS mice. The association between MLCK and actin rearrangement with MMP-9 expression has been described in previous studies in various cell types such as human head and neck squamous cell carcinoma (47), osteoclasts (43), and human malignant glioma cells (9). In patients with active UC, increased MLCK immunofluorescence staining has been demonstrated in the colonic surface epithelium (7). Our results including the MMP-9 immunolocalization in colonic surface epithelium in DSS colitis (Fig. 1) and in the colonic epithelial lining of UC patients (Fig. 7) indicate that under inflammatory conditions, MMP-9 regulates MLCK expression, which, in turn, regulates the epithelial barrier function.

In summary, we have shown that the DSS-induced increase in colonic permeability is associated with an increase in MMP-9 and MLCK expression and the increase in colonic permeability is attenuated in the absence of MMP-9. Furthermore, the DSS-induced increase in MLCK protein expression and subsequent increase in MLCK activity as measured by pMLC level were also inhibited in MMP-9\(^{-/-}\) mice. In complementary studies, we showed that MLCK\(^{-/-}\) mice as well as mice treated with the MLCK inhibitor ML-7 have attenuation of DSS-induced colonic permeability. Consistent with modulated colonic permeability, the clinical severity and degree of colonic inflammation were significantly reduced in MMP-9\(^{-/-}\), MLCK\(^{-/-}\), and ML-7-treated mice compared with control DSS mice, suggesting a cause-and-effect relationship between MLCK expression and increase in colonic permeability and inflammation. Although the mechanistic link between MMP-9 and MLCK needs to be investigated further, this study for the first time reports an important role of MMP-9 in the modulation of intestinal permeability.

ACKNOWLEDGMENTS

We thank the Clinical and Translational Science Center Laboratory and the Fluorescence Microscopy Shared Resource, Health Sciences Center, University of New Mexico for excellent technical assistance.

GRANTS

This research work has been supported in part by a Veterans Affairs (VA) Merit Review grant from the VA Research Service and National Institute of Diabetes and Digestive and Kidney Diseases Grants R01-DK-64165-01 (to T. Ma) and K01-DK-100562-01 (to P. Nighot).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: P.K.N. conception and design of research; P.K.N., R.A.-S., M.R., S.G., and D.M.W. performed experiments; P.K.N. analyzed data; P.K.N., D.M.W., and T.Y.M. interpreted results of experiments; P.K.N. prepared figures; P.K.N. drafted manuscript; T.Y.M. edited and revised manuscript; T.Y.M. approved final version of manuscript.

REFERENCES

12. Feighery LM, Cochrane SW, Quinn T, Baird AW, O’Toole D, Owens SE, O’Donoghue D, Mrsny RJ, Brayden DJ. Myosin light chain kinase...


48. Turner JR, Rill BK, Carlson SL, Carmes D, Kerner R, Mrsy RJ, Madara JL. Physiological regulation of epithelial tight junctions is...


