CXCR2 knockout mice are protected against DSS-colitis-induced acute kidney injury and inflammation

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Ranganathan P, Jayakumar C, Manicassamy S, Ramesh G. CXCR2 knockout mice are protected against DSS-colitis-induced acute kidney injury and inflammation, Am J Physiol Renal Physiol 305: F1422–F1427, 2013. First published August 28, 2013; doi:10.1152/ajprenal.00319.2013.—Organ cross talk exists in many diseases of the human and animal models of human diseases. A recent study demonstrated that inflammatory mediators can cause acute kidney injury and neutrophil infiltration in a mouse model of dextran sodium sulfate (DSS)-colitis. However, the chemokines and their receptors that may mediate distant organ effects in colitis are unknown. We hypothesized that keratinocyte chemoattractant (KC)/IL-8 receptor chemokine (C-X-C motif) ligand 2 (CXCL2) mediates DSS-colitis-induced acute kidney injury. Consistent with our hypothesis, wild-type (WT) mice developed severe colitis with DSS treatment, which was associated with inflammatory cytokine and chemokine expression and neutrophil infiltration in the colon. DSS-colitis in WT was accompanied by acute kidney injury and enhanced expression of inflammatory cytokines in the kidney. However, CXCR2 knockout mice were protected against DSS-colitis as well as acute kidney injury. Moreover, the expression of cytokines and chemokines and neutrophil infiltration was blunted in CXCR2 knockout mice in the colon and kidney. Administration of recombinant KC exacerbated DSS-colitis-induced acute kidney injury. Our results suggest that KC/IL-8 and its receptor CXCR2 are critical and major mediators of organ cross talk in DSS colitis and neutralization of CXCR2 will help to reduce the incidence of acute kidney injury due to ulcerative colitis and Crohn’s disease in humans.

OVER A MILLION PEOPLE SUFFER from chronic diseases of the gastrointestinal tract such as Crohn’s disease and ulcerative colitis (UC; Ref. 5). Disease progression is persistent with the eventual outcome being surgical intervention for a majority of patients (27). Other organ involvement and complications are common in patients with inflammatory bowel disease (IBD). Renal or urinary complications occur in 4–23% of patients, often in those with severe, long-standing disease. The most common manifestations are kidney stones, enterovesical fistulas, and ureteral obstruction (22). Renal tubular injury was also seen in many IBD patients (8). A recent study also showed the presence of an anti-brush border antibody in these IBD patients that cross-reacts to kidney proximal tubular brush border membrane antigen. There is also an antigenic relationship among human kidney, colon, and the common antigen of Enterobacteriaceae (23, 26). An antigenic relation was also shown in an animal model of UC (11). In rats, the use of hyperosmolar enemas can induce nephritis through massive acidosis and electrolyte disturbances with hypocalcemia and hypernatremia. Inflammatory changes also occur in kidneys of mutant mice with interleukin-2 (IL-2) receptor gamma chain deficiency (13). A description of tubulointerstitial disease unrelated to the consumption of nephrotoxic agents in such patients is not extensive. However, the presence of tubulointerstitial disease in IBD patients is increasingly appreciated even before the start of nephrotoxic drugs such as aminosalicylate (8, 12, 14, 15). Moreover, it is now even recommended for clinicians to look for the presence of tubulointerstitial disease and renal failure in IBD patients (14). Recent studies from our laboratory have shown that inflammation is the key mediator of acute kidney injury in the mouse model of acute colitis. Overexpression of anti-inflammatory molecules such as netrin-1 in the colon and kidney suppressed colitis-induced acute kidney injury and associated inflammation (24). Neutrophil infiltration into colon epithelium is the key feature of acute colitis, and genetic deletion of neutrophils and chemokine keratinocyte chemoattractant (KC)/chemokine (C-X-C motif) ligand 1 (CXCL1) or its receptors protected colon against dextran sodium sulfate (DSS)-induced colitis in mice (3, 4, 7). However, whether colitis-induced acute kidney injury is also mediated by KC receptor is unknown.

Here, we report for the first time that genetic deletion of KC receptor CXC chemokine receptor 2 (CXCR2) suppressed DSS-induced colitis, inflammation, and acute kidney injury. Our study suggests that organ cross talk in DSS-induced colitis can be effectively treated with the anti-CXCR2-based approach.

MATERIALS AND METHODS

DSS-colitis. The Institutional Animal Care and Use Committee of the Georgia Regents University approved all of the protocols and procedures using animals (approval number-2011-0348). Eight-week-old wild-type (WT) and CXCR2 knockout mice were purchased from Jackson Laboratories and used in DSS studies. DSS (3.5%; MP Biomedicals) was added in drinking water. Control animals were fed with regular drinking water. DSS was removed after 8 days, and the animals were fed with normal water. Animals were killed on day 2 after removal of DSS. To determine whether exogenous administration of CXCR2 ligand KC exacerbates acute kidney injury, some DSS-treated mice were injected with vehicle (0.1% BSA in PBS) or 100 ng KC per mice per day intraperitoneally starting from day 6 until the animals were killed on day 10.

Quantification of mRNA by real-time RT-PCR. Real-time RT-PCR was performed in an Applied Biosystems 7700 Sequence Detection System (Foster City, CA). Then, 1.5 μg total RNA was reverse transcribed in a reaction volume of 20 μl using Omniscript RT kit and random primers. The product was diluted to a volume of 150 μl, and 6-μl aliquots were used as templates for amplification using the SYBR Green PCR amplification reagent (Qiagen) and gene-specific primers. The primer sets used were as follows: mouse TNF-α (for-
ward: GCATGATCCGCGACGTGGAA; reverse: AGATCCATGC- 
CGTTGGCCAG), monocyte chemoattractant protein-1 (MCP-1; for-
ward: ATGCAGGTCCCTGTCATG; reverse: GCTTGAGGTGGTT-
GTGGA), ICAM-1 (forward: AGATCACATTCACGGTGCTG; re-
verse: CTTCAGAGGCAGGAAACAGG), IL-6 (forward: GATGC-
TACCAAACTGGATATAATC; reverse: GGTCCTTAGCCACTC-
TTCTGTG), and cyclooxygenase-2 (COX-2; forward: GCGACAT-
ACTCAAGCAGGAGCA; reverse: AGTGGTAACCGCTCAGGT-
GTTG). The amount of RNA was normalized to the
/H9252-actin signal
amplified in a separate reaction (forward primer: AGAGGGAAATCGT-
GCGTGAC; reverse: CAATAGTGATGACCTGGCCGT).

Serum cytokine measurement. Serum cytokines and chemokines
were measured using ELISA kit from eBiosciences.

Renal function. Renal function was assessed by measurements of
serum creatinine (cat no: DZ072B; Diazyme Laboratories).

Histology and immunostaining. Kidney tissue was fixed in buffered
10% formalin for 12 h and then embedded in paraffin wax. For
assessment of injury, 5-μm sections were stained with periodic
acid-Schiff followed by hematoxylin. To quantify leukocyte infil-
tration, sections were stained with rat anti-mouse neutrophil antibody or
anti-mouse macrophage antibody (1:200 dilution; Abcam, Cambridge,
MA) followed by goat anti-rat biotin conjugate. Color was developed
after incubation with ABC reagent (Vector Laboratories). Stained
sections were photographed, and five ×40 fields of neutrophils were
examined for quantification of leukocytes.

Samples of proximal colon were fixed in 10% buffered formalin
and stained with hematoxylin and eosin. The histological examination
was performed in a blinded fashion using a scoring system previously
validated and described (6). Three independent parameters were
measured: severity of inflammation (0–3: none, slight, moderate,
severe); depth of injury (0–3: none, mucosal, mucosal and submucu-
osal, transmural); crypt damage (0–4: none, basal 1/3 damaged, basal
2/3 damaged, only surface epithelium intact, entire crypt and epithel-
ium lost); and percentage of the involved area (0–4: 0, 1–10, 10–25,
25–50, and 50–100%). All scores on the individual parameters to-
gether could result in a total score ranging from 0 to 14.

Myeloperoxidase assay. Kidney neutrophil sequestration was quan-
tified using a fluorescence based myeloperoxidase assay kit (cat. no.
K745-100; BioVision, Milpitas, CA). In short, animals were eutha-
nized and kidney were perfused with 5 ml of PBS through the right
ventricle. Kidney was excised, homogenized in 4 vol of assay buffer,
and centrifuged (13,000 g, 10 min) to remove insoluble material. Fifty
microliters of the sample were used for the assay. Enzyme activity
was calculated from the standard curve. Enzyme activity was normal-
ized initially per mg of protein, and then fold increase over control
samples was calculated.

Statistical methods. All assays were performed in duplicate or
triplicate. The data are reported as means ± SE. Statistical signifi-
cance was assessed by an unpaired, two-tailed Student t-test for single
comparison or ANOVA for multiple comparisons.

Fig. 1. Disease activity in mice with chemokine (C-X-C
motif) ligand 2 (CXCL2) gene deletion during dextran
sodium sulfate (DSS)-colitis. Gender-, age-, and weight-
matched mice with CXCR2 gene deletion [knockout (KO)]
and their wild type (WT) were exposed to DSS (3.5%) for
8 days, followed by death on day 10 and harvesting of the
whole colon by blunt dissection. A: daily weight measure-
ments were obtained for each group of mice. *P < 0.05, as
measured by ANOVA. *P < 0.05 vs. other groups. #P <
0.05 vs. water-treated groups. B: spleen weight at harvest
was measured for each mouse and is displayed as means ±
SE. *P < 0.05 vs. other groups. D: at harvest, colon weight
was measured for each mouse and is displayed as the
means ± SE. *P < 0.01 vs. other groups. E: at harvest,
colon length was measured for each mouse and is displayed
as the means ± SE. *P < 0.05 vs. other groups; n = 8.
RESULTS

CXCR2 knockout mice are protected against DSS-induced colitis. Previous studies indicate that mice with CXCR2 inhibition or gene deletion experienced a blunted inflammatory response in models of acute colitis (3, 4, 7). Consistent with those studies, we observed that CXCR2 knockout mice experienced blunted disease severity compared with WT controls as measured by weight loss, colon and spleen weight, and histologic damage (Figs. 1 and Fig. 2, A and B). Thus we conclude that CXCR2 play a critical role in the development of DSS-colitis.

CXCR2 gene deletion suppresses inflammation of the colon during colitis. Recent studies demonstrated that KC/IL-8 via CXCR2 mediates neutrophil infiltration into tissue such as lung and kidney during acute inflammation (4, 7, 19, 25). Inappropriate neutrophil accumulation within the lamina propria (LP) is a key feature of early and active UC (6, 7, 16). DSS-colitis in WT was associated with rapid neutrophil influx into the LP, closely modeling the human disease (Fig. 2, A and C). However, CXCR2 knockout mice showed very few neutrophils in the LP. Additionally, expression levels of the proinflammatory cytokines (TNFα, IL-1β, and IL-6) and chemokines (IP-10, MCP-1, KC, CXCL2, and CXCL3) and proinflammatory enzyme COX-2 (Fig. 3, A and B) were significantly enhanced in WT mice following DSS treatment confirming the exaggerated inflammatory response observed in these mice. These changes were minimal in CXCR2 knockout mice (Fig. 3, A and B). Consistent with increased expression of inflammatory cyto-
Kines and chemokines in the colon, serum levels of IL-6 (Fig. 3C) and KC (Fig. 3D) also significantly increased in WT mice treated with DSS compared with water-treated WT mice. With genetic deletion of CXCR2, the increase in IL-6 and KC in the circulation is completely blunted with DSS treatment. Experimental colitis induces acute kidney injury, but genetic deletion of CXCR2 protected mice from DSS-colitis-induced acute kidney injury. Human and mouse studies indicate the involvement of nonintestinal organs in UC (14, 15, 23, 24). Therefore, we determined whether DSS-colitis-induced acute kidney injury is suppressed in CXCR2 knockout mice. DSS treatment of WT mice caused acute kidney injury as seen by increased serum creatinine (Fig. 4). Genetic deletion of CXCR2 protected kidney from DSS-induced acute kidney injury. Renal dysfunction was associated with structural changes in the kidney such as increased plugging of red blood cells in the blood vessel of WT mice treated with DSS compared with CXCR2 knockout mice or water-treated WT and CXCR2 mice kidney. Neutrophil infiltration as determined by myeloperoxidase activity (Fig. 4C) was also completely blunted in CXCR2 knockout mice kidney compared with WT mice kidney. Additionally, expression levels of the proinflammatory cytokines TNFα, IP-10, KC, and MCP-1 and proinflammatory enzyme COX-2 (Fig. 5) were significantly enhanced in WT mouse kidney following DSS treatment confirming the exaggerated inflammatory response observed in these mice. These changes were minimal in netrin-1 transgenic mouse kidney. In contrast to KC/CXCL1 expression, the expression of CXCL2 is not altered whereas CXCL3 expression is downregulated in response to DSS treatment in WT mice. CXCR2 gene deletion caused a significant downregulation of CXCL2 in response to DSS treatment whereas CXCL3 expression was not changed compared with WT DSS-treated animal kidney.

Administration of recombinant KC exacerbated colitis-induced acute kidney injury. To determine directly if KC is a critical mediator of colitis-induced acute kidney injury, we administered KC as described in MATERIALS AND METHODS. Kidney function was monitored by measuring serum creatinine. Colitis was monitored by recording body weight. As shown in Fig. 6, DSS treatment causes significant reduction in body weight and administration of KC on day 6 onwards did

Fig. 4. CXCR2 gene deletion protects kidney from DSS-induced dysfunction. A: serum creatinine was measured at 10 days after DSS treatment in WT and CXCR2 KO mice. Values are means ± SE. *P < 0.01 vs. all other groups. #P < 0.05 vs. water-treated groups; n = 6. B: histological changes was assessed in periodic acid-Schiff (PAS)-stained kidney section. DSS treatment induced increase red blood cells plugging in vessel, which was blunted in CXCR2 KO mice. Scale bar: = 100 μM. C: neutrophil infiltration was quantified by measuring myeloperoxidase activity in the kidney. DSS induced a significant increase in myeloperoxidase activity in WT mice kidney which was blunted in CXCR2 KO mice kidney treated with DSS. *P < 0.05 vs. all other groups; n = 6.
not alter changes in body weight. However, DSS-colitis-induced acute kidney injury was exacerbated with KC administration (Fig. 6B). Consistent with kidney dysfunction, kidney tissue sections also showed damage in the tubules like dilation, necrosis, and red blood cell plugging in arteries with KC treatment compared with vehicle-treated kidney. These data suggest that KC is a critical mediator of kidney dysfunction in acute colitis.

**DISCUSSION**

Clinical and epidemiological evidence suggest that IBD affects other organs including the kidney. Recent animal studies document the role of inflammatory mediators in colon-kidney cross talk in mouse model of DSS-colitis. Therefore, effective control of inflammation may be a good and effective way of treating IBD and associated acute kidney injury. Neutrophil plays a central role in acute inflammation and organ injury (4, 7, 17–19, 25). However, it was not known whether neutrophil chemokine KC/IL-8 receptor mediates neutrophil infiltration and organ cross talk in mouse model of acute colitis. Interestingly, our studies revealed that mice that overexpress genetic deletion of KC receptor CXCR2 showed protection against DSS-induced colitis. DSS colitis in WT mice was associated with robust neutrophil infiltration into the colonic LP and a significant increase in tissue cytokine expression, which was completely suppressed in netrin-1 transgenic mice. In addition, netrin-1 overexpression was associated with attenuated weight loss, improved tissue histology, and diminished colonic inflammation. Colitis was associated with acute kidney injury in WT mice. In addition, DSS-treated WT mice showed increased cytokine in the circulation, histological changes in the kidney, and increased expression of inflammatory cytokines and chemokine expression. These changes in the colon and kidney were blunted in CXCR2 knockout mice suggesting a critical role of KC receptor in mediating inflammation and organ cross talk in the inflammatory model of acute colitis.

Polymorphonuclear neutrophils infiltration is mediated by specific chemoattractants generated in the mucosa (1), where epithelial cells, including in the intestine, have been shown to secrete ELR (Glu-Leu-Arg) motif-containing CXC chemokines (10). ELR+ CXC chemokines in the mouse include CXCL5/LIX and the functional homologs of human CXCL8, CXCL1/KC, and CXCL2/3 (macrophage inflammatory protein-2). Epithelial cell may also express receptors for this family of chemokines (9). The murine ELR+ CXC chemokines bind to CXCR2, a seven-transmembrane-spanning G protein-coupled receptor, which in addition to mediating chemotaxis causes polymorphonuclear neutrophils activation (20). Neutrophil activation and migration releases proinflammatory cytokines such as IFNγ, IL-17, IL-6, and other chemokines (17, 18). Inhibition of neutrophil migration by genetic deletion of CXCR2 receptor or its ligand also suppressed ischemia reperfusion-induced kidney injury (19) as well as acute kidney injury-induced lung injury (2). Consistent with these data our studies show that genetic deletion of CXCR2 protects both colon and kidney by suppressing inflammation and inflammatory mediator production.

CXCR2 receptor is expressed both on renal parenchymal cells as well as neutrophils (17, 28). However, it is not clear whether the neutrophil CXCR2 or renal parenchymal cell expressed receptor-mediated kidney dysfunction. Epithelial cell necrosis was not increased significantly in both WT and CXCR2 knockout mice suggesting that observed dysfunction may be due to activities of receptor in the neutrophils and vascular endothelial cells. Our view was reinforced by recent studies in the lung where the endothelial cell CXCR2 receptor mediates LPS-induced lung injury (25). However, urinary tract infection was not cleared properly if the epithelial CXCR2 receptor is not expressed suggesting the importance of both leukocytes and epithelial cell receptor (28). Recent studies from our laboratory had shown that IL-6 mediated organ cross talk in acute colitis model (24). Consistent with our earlier observation, current study also shows elevated levels of IL-6 in the plasma. IL-6 is known to induce KC expression. KC expression is increased in the kidney suggesting that KC/CXCR2 axis may be downstream of IL-6 (21). Administration of recombinant KC exacerbated kidney dysfunction suggesting that excess stimulation of CXCR2 receptor contributes to acute kidney injury. Our studies also show that in the absence of
CXCR2 signaling its ligand expression also blunted suggesting a positive feedback loop exists between receptor and ligand in colitis model. However, it is also possible that inhibition of ligand expression may due to better preservation of tissue morphology in CXCR2 knockout mice. Further studies may be needed to clarify the receptor-mediated regulation of ligand expression in this model.

In summary, our studies demonstrate for the first time that colon-kidney cross talk in DSS-induced acute colitis is mediated by the KC/IL-8 receptor CXCR2. Genetic deletion of CXCR2 in mice suppressed inflammation and acute kidney injury. Administration of recombinant KC exacerbated acute kidney injury. Therefore, CXCR2 receptor inhibition could be a therapeutic approach for the treatment of colitis-induced acute kidney injury in human.

REFERENCES