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Increased Macrophage Infiltration and Fractalkine Expression in Cisplatin-Induced Acute Renal Failure in Mice

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ABSTRACT

Inflammatory mechanisms contribute to cisplatin-induced acute renal failure (CisARF). Our first aim was to determine renal macrophage infiltration in CisARF. A more than 2-fold increase in CD11b-positive macrophages in the kidney on day 2 preceded the increase in blood urea nitrogen (BUN) and serum creatinine (SCr). Our next aim was to determine the chemoattractant for macrophage infiltration in CisARF. Fractalkine (CX_3CL1) is expressed on activated endothelial cells and is a potent chemoattractant for macrophages that express its receptor (CX_3CR1). Immunoblotting showed that whole-kidney CX_3CL1 expression on days 1, 2, and 3 after cisplatin administration was increased. On immunofluorescence, the intensity of renal endothelial staining of CX_3CL1 in blood vessels was significantly increased on day 2. Circulating von Willebrand factor (vWF), a measure of systemic endothelial injury, was increased on day 2. Next we determined whether macrophages played an injurious role in CisARF. Macrophages were depleted with injections of liposome-encapsulated clodronate (LEC). LEC resulted in a decrease in renal CD11b-positive macrophages on day 3. However, LEC-treated mice were not protected from CisARF on day 3. To determine the role of CX_3CR1, both a specific anti-CX_3CR1 antibody and $CX_3CR1^{-/-}$ mice were used. Administration of the CX_3CR1 antibody and $CX_3CR1^{-/-}$ mice was not protected against CisARF. In summary, in CisARF, macrophage infiltration in the kidney, CX_3CL1 expression in whole kidney and blood vessels, and the increase in circulating vWF precede BUN and SCr increase. However, inhibition of macrophage infiltration in the kidney or CX_3CR1 blockade is not sufficient to prevent CisARF.

The chemotherapeutic drug, cisplatin, is a common cause of acute renal failure (ARF) (Edelstein and Schrier, 2007; Safirstein, 2007). ARF is a life-threatening illness that continues to have a high mortality rate of 50 to 80% in an intensive care unit setting (Edelstein and Schrier, 2007). Thus, a better understanding of the pathogenesis of cisplatin-induced ARF (CisARF) is needed to allow interventions that would prevent the need for hemodialysis, shorten the course of ARF, and improve survival in cancer patients.

The role of inflammation in ARF has been described previously (Bonventre and Zuk, 2004; Friedewald and Rabb, 2004; Devarajan, 2006), with involvement of leukocytes, adhesion molecules, and endothelial injury. Inhibition of leukocyte adhesion with antibodies against intercellular adhesion molecule-1 (ICAM-1) reduces cisplatin-induced renal injury (Kelly et al., 1999). T-cell-deficient mice are protected against CisARF (Liu et al., 2006). However, the specific type of leukocyte that mediates CisARF remains unclear.

CisARF is associated with increased renal myeloperoxidase, which is produced by neutrophils and macrophages (Faubel et al., 2004; Liu et al., 2006). However, antibody depletion of neutrophils failed to confer protection against CisARF (Faubel et al., 2007). In vitro studies show that cisplatin (Cis) administration increases macrophage-mediated cytotoxicity against neoplastic cells (Sodhi et al., 1990). In addition, macrophage renal infiltration was recently shown to occur late in the course of CisARF (Lee et al., 2006). However, an injurious role of macrophages in CisARF, as determined by macrophage depletion studies, has not been demonstrated.

In a rat model of cisplatin nephrotoxicity, damage to glomerular capillaries, including endothelial cells, has been described previously (Kohn et al., 2002). Caspases and calpain

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ABBREVIATIONS: ARF, acute renal failure; CisARF, cisplatin-induced acute renal failure; ICAM-1, intercellular adhesion molecule-1; Cis, cisplatin; CX_3CL1, fractalkine; CX_3CR1, CX_3CL1 receptor; BUN, blood urea nitrogen; SCr, serum creatinine; VEGFR, vascular endothelial growth factor receptor; vWF, von Willebrand factor; LEC, liposome-encapsulated clodronate; HPF, high-powered field; TNF, tumor necrosis factor; NK, natural killer; WT, wild type; Veh, vehicle treated; Lipo, liposome; Ab, antibody.

are independent mediators of cisplatin-induced endothelial cell necrosis in vitro (Dursun et al., 2006). Fractalkine (CX_3CL1) is expressed by injured endothelium and functions as both a potent chemoattractant and adhesion molecule for CX_3CL1 receptor (CX_3CR1) expressing inflammatory cells, which include monocytes and/or macrophages (Umehara et al., 2001). The presence of CX_3CL1 expression on injured endothelium in CisARF and the effect of CX_3CR1 inhibition in CisARF are not known.

On this background, we developed the hypothesis that increased CX_3CL1 expression caused by endothelial injury results in macrophage infiltration in the kidney in CisARF and that macrophage depletion and/or CX_3CR1 inhibition is protective against CisARF. The aim of the study was to measure macrophage infiltration, endothelial injury, and CX_3CL1 expression and determine whether macrophage depletion and/or CX_3CR1 inhibition are protective against CisARF.

Materials and Methods

Cisplatin Administration. Eight- to 10-week-old male C57BL/6 mice weighing 20 to 25 g were used for all of the mouse studies. All experiments were conducted with adherence to the Institute of Laboratory Animal Resources (1996). The animal protocol was approved by the Animal Care and Use Committee of the University of Colorado Health Sciences Center. Mice were maintained on a standard diet, and water was freely available. Mice were housed five per cage under a 12-h light and dark schedule for at least 1 week before cisplatin administration. Six hours before cisplatin administration, food and water were withheld. Cisplatin [*cis*-diamminedichloro-platinum (II); Aldrich Chemical Co., Milwaukee, WI] was freshly prepared the day of administration in sterile normal saline at a concentration of 1 mg/ml. Mice were given either 30 mg/kg b.wt. cisplatin or vehicle (saline) i.p., after which the mice again had free access to food and water (Faubel et al., 2004).

We have described this model of cisplatin-induced ARF in detail elsewhere (Faubel et al., 2004). In brief, after cisplatin injection, blood urea nitrogen (BUN) and serum creatinine (SCr) were normal on day 1 and slightly increased on day 2. On day 3 after cisplatin injection, renal dysfunction, renal neutrophil infiltration, and acute tubular necrosis scores were severe. Under anesthesia with 2,2,2-tribromoethanol (Avertin; Aldrich Chemical Co.), kidneys were removed, and blood samples were collected via cardiac puncture on days 1, 2, and 3 after cisplatin administration. BUN and SCr were measured using an Astra Autoanalyzer (Beckman Instruments Inc., Fullerton, CA).

Endothelial Cells in Culture. MS1 (MILE SVEN 1) mouse endothelial pancreatic islet cells were purchased from American Type Culture Collection (ATCC catalog number CRL-2279; Manassas, VA). The line retains the properties of endothelial cells, including uptake of acetylated low-density lipoprotein and expression of both factor VIII-related antigen and vascular endothelial growth factor receptor (VEGFR). The expression of VEGFR-1 and VEGFR-2 in MS1 microvascular endothelial cells (data not shown) was confirmed by Western blot analysis. Cells were grown in Dulbecco's modified Eagle's medium. Experiments were performed when the plates became at least 80% confluent. The cells were treated with cisplatin (50 μM) for 24 h as we have described previously (Dursun et al., 2006).

von Willebrand Factor Assay. von Willebrand factor (vWF) was measured in duplicate in plasma using a commercially available Asserachrom enzyme-linked immunosorbent assay kit from Diagnostica Stago (Parsippany, NJ) (Hubbard et al., 2001).

Immunoblot Analysis. Whole kidney was homogenized in radioimmunoprecipitation assay buffer plus proteinase inhibitors and immunoblotted as described previously (Dursun et al., 2006). A polyclonal anti-rat CX_3CL1 antibody produced in goats that recognizes full-length mouse CX_3CL1 (100 kDa) was used (catalog number AF-537; R&D Systems, Inc., Morrisville, NC). Recombinant mouse CX_3CL1 that lacks 57 carboxyl-terminal amino acids (85 kDa) was used as a positive control. Images for densitometry were analyzed using 1D Image Software (Kodak Digital Science, Rochester, NY). A rabbit polyclonal antibody raised against amino acids 76 to 375 of actin of human origin (34 kDa) was used as a loading control (catalog number sc-10731; Santa Cruz Biotechnology, Santa Cruz, CA).

Immunofluorescence Studies. Kidney tissues were embedded in OCT, snap-frozen in liquid nitrogen, and stored at −80°C until sectioning. Five-micrometer cryostat sections were fixed in 70% acetone/30% methanol and prepared for immunofluorescence studies as described previously (Dursun et al., 2006). The following primary antibodies were used: 1) CX_3CL1 (same antibody as described for immunoblot) and 2) a rat anti-mouse CD11b monoclonal antibody (catalog number MCA74; Serotec, Oxford, UK). The intensity of endothelial staining of CX_3CL1 in blood vessels was scored as follows: 0 = no staining; 1+ = mild staining; 2+ = moderate staining; and 3+ = severe staining. More than five blood vessels were counted in each kidney by a blinded observer, and the mean intensity score was determined for each kidney.

CX_3CR1 Inhibition. Twenty-five micrograms of a polyclonal rabbit anti-rat CX_3CR1 antibody (1 mg/ml) (Torrey Pines Biolabs, Inc., Houston, TX) or vehicle (rabbit serum) was injected either 1 h or 1 day after cisplatin administration. The same dosage and route of administration of the CX_3CR1 antibody have been demonstrated to ameliorate cardiac allograft rejection (Robinson et al., 2000) and crescentic glomerulonephritis (Feng et al., 1999) in mice.

CX_3CR1 Knockout Mice. Male CX_3CR1 knockout mice on the C57BL/6 background were purchased from Taconic Farms (Germantown, NY). Animals were age-matched with control C57BL/6 animals aged 8 to 10 weeks.

Macrophage Depletion. Empty liposomes (vehicle) and liposome-encapsulated clodronate (LEC) were prepared as previously described in detail (Van Rooijen and Sanders, 1994). Clodronate (clodronic acid) [(dichloromethylene) bisphosphonic acid] ($CH_4Cl_2O_6P_2$) was obtained from Roche Diagnostics GmbH (Mannheim, Germany). In brief, macrophages phagocytose the liposomes, resulting in the release of clodronate into the cytoplasm and death of the macrophage. Empty liposomes, not containing clodronate and prepared under exactly the same conditions as the LEC, were used as a control. The LEC suspension or empty liposomes was injected at 100 μl per 10 g b.wt. via the tail vein 2 days before and 1 day after cisplatin injection.

Statistical Analysis. Non-normally distributed data were analyzed by the nonparametric unpaired Mann-Whitney test. Multiple group comparisons were performed using analysis of variance with post-test according to Newman-Keuls. A P value of <0.05 was considered statistically significant. Values are expressed as means ± S.E.

Results

Immunofluorescence Staining for CD11b-Positive Macrophages. The CisARF mice had a greater number of CD11b-staining macrophages than vehicle-treated mice beginning on day 2 after cisplatin administration. The number of CD11b-positive cells per high-powered field (HPF) (400×) in the outer stripe of the outer medulla was 4.3 ± 1.0 in vehicle-treated mice, 5.5 ± 0.8 on day 1 after cisplatin in wild-type mice, 13.2 ± 1.9 on day 2 after cisplatin in wild-type mice ($P < 0.05$ versus vehicle, $n = 11$), 13.1 ± 1.8 on day 3 after cisplatin in wild-type mice ($P < 0.05$ versus vehicle, $n = 14$), and 3.3 ± 0.4 on day 3 after cisplatin in $CX3CR1^{-/-}$ mice ($P < 0.05$ versus day 3 after cisplatin in wild-type mice, $n = 4$) (Fig. 1A). Representative pictures of the immunofluorescence staining for CD11b-positive cells in the kidney in cisplatin-induced ARF are demonstrated in Fig. 1, B to F.

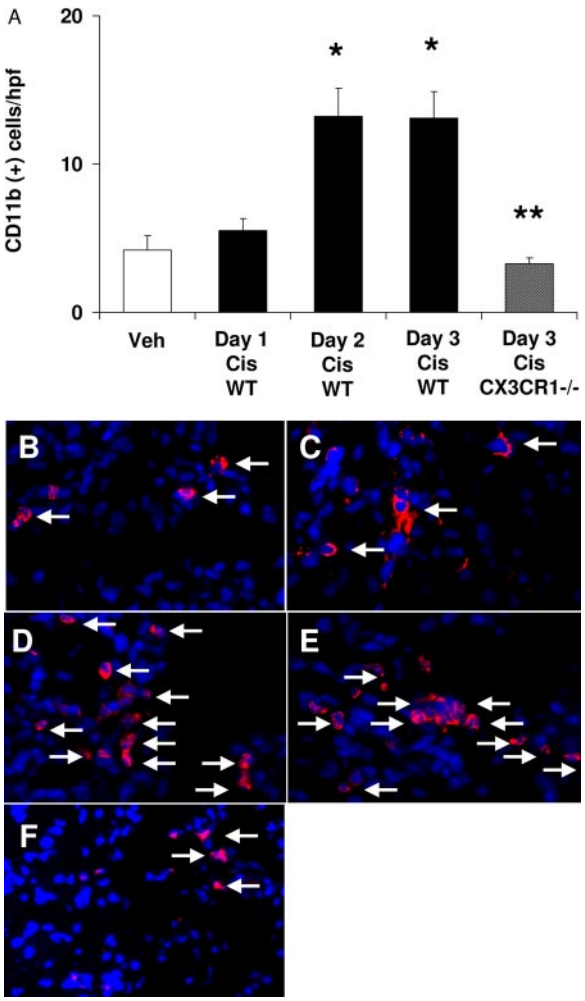

Fig. 1. Immunofluorescence staining for CD11b. A, the CisARF (Cis) mice had a greater number of CD11b staining macrophages than vehicle-treated (Veh) mice beginning on day 2 after cisplatin administration (*, $P < 0.05$ versus vehicle). The number of CD11b staining macrophages on day 3 after cisplatin administration was significantly reduced in $CX_3CR1^{-/-}$ mice compared with wild-type (WT) mice (**, $P < 0.05$ versus WT day 3). Representative pictures of the immunofluorescence staining for CD11b-positive cells (arrows) in the kidney in vehicle-treated (B), day 1 in WT (C), day 2 in WT (D), day 3 in WT (E), and day 3 in $CX_3CR1^{-/-}$ (F) after cisplatin administration are shown.

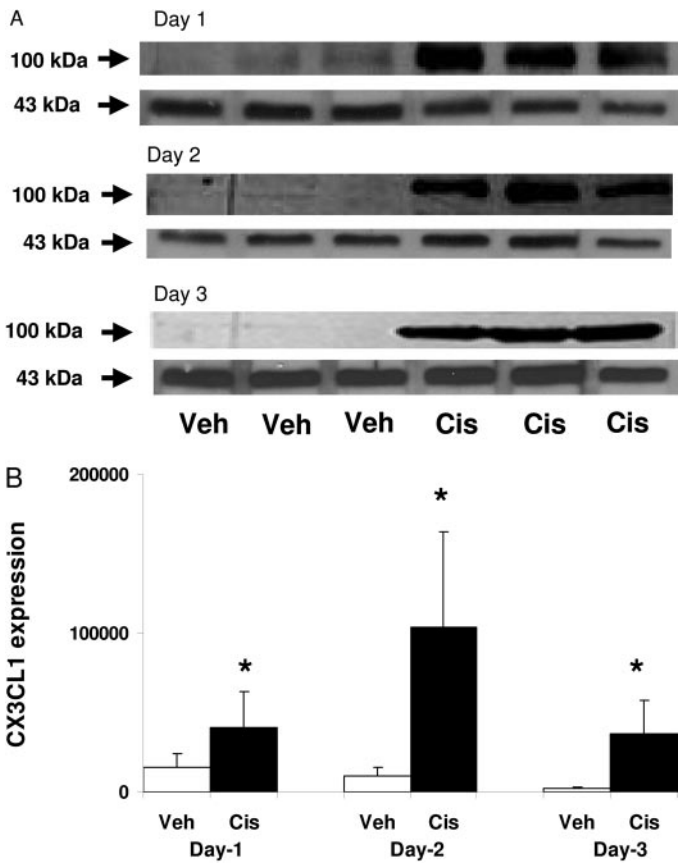

Fig. 2. CX_3CL1 protein expression. A, the expression of CX_3CL1 in whole kidney was virtually absent in Veh mice and significantly increased on days 1, 2, and 3 after cisplatin-administration. Densitometric analysis for CX_3CL1 protein expression is demonstrated in B (*, $P < 0.01$ versus vehicle). Actin (43 kDa) was used as a loading control.

Immunoblotting of CX_3CL1. The protein expression of CX_3CL1 in whole kidney was increased on days 1, 2, and 3 after cisplatin administration (Fig. 2A). Densitometric analysis is demonstrated in Fig. 2B.

Immunofluorescence Staining for CX_3CL1. CX_3CL1 is predominantly expressed in endothelial cells. To confirm the endothelial location of CX_3CL1 staining in the kidney, immunofluorescence was performed. The intensity of endothelial staining was determined as outlined under *Materials and Methods*. The endothelial staining score was 1.2 ± 0.2 in vehicle-treated mice and 2.1 ± 0.2 on day 2 after cisplatin-administration ($P < 0.05$, $n = 4$ per group; Fig. 3A). Representative pictures of the endothelial staining in vehicle and cisplatin-treated mice are shown in Fig. 3, B and C.

Endothelial Cells. To confirm that cisplatin has a direct effect on endothelium, mouse microvascular endothelial cells (MS1 cells) were treated with cisplatin (as described under *Materials and Methods*) and then immunoblotted for CX_3CL1. There was an up-regulation of CX_3CL1 protein expression in cells incubated with cisplatin (50 μM for 24 h) compared with vehicle-treated cells (Fig. 4).

vWF Assay in Plasma. In previous studies, circulating vWF was increased 24 h after ischemic renal perfusion in mice (Sutton et al., 2003). Circulating vWF was measured as a marker of endothelial injury in mice with CisARF. vWF in plasma was increased on day 2 after cisplatin administration, demonstrating the presence of systemic endothelial cell injury. vWF (percentage of calibrator) was zero in vehicle-treated and 7.4 ± 5.6 in cisplatin (not significant versus vehicle) on day 1, 4.8 ± 4 in vehicle-treated and 40.1 ± 13.5 in cisplatin-induced ARF on day 2 ($n = 4$, $P < 0.05$ versus vehicle), and 4.2 ± 4 in vehicle-treated and 9.1 ± 8.2 in cisplatin ARF on day 3 (not significant versus vehicle) (Fig. 5).

Effects of Macrophage Depletion. In separate experiments, the effect of macrophage depletion using LEC on SCr and BUN was determined. Macrophage depletion had no significant protective effects as determined by SCr and BUN in CisARF. The day 3 serum creatinine was 2.0 ± 0.1 mg/dl in cisplatin plus vehicle and 1.8 ± 0.3 mg/dl in cisplatin plus LEC ($P > 0.05$, $n = 12$) (Fig. 6A). The day 3 BUN was 175 ± 8 mg/dl in cisplatin plus vehicle and 177 ± 24 mg/dl in cisplatin plus LEC ($P > 0.05$, $n = 12$) (Fig. 6B).

Administration of LEC resulted in a 70 to 80% reduction in macrophages in the spleen (data not shown). The effect of LEC on macrophages in the kidney was examined. The num-

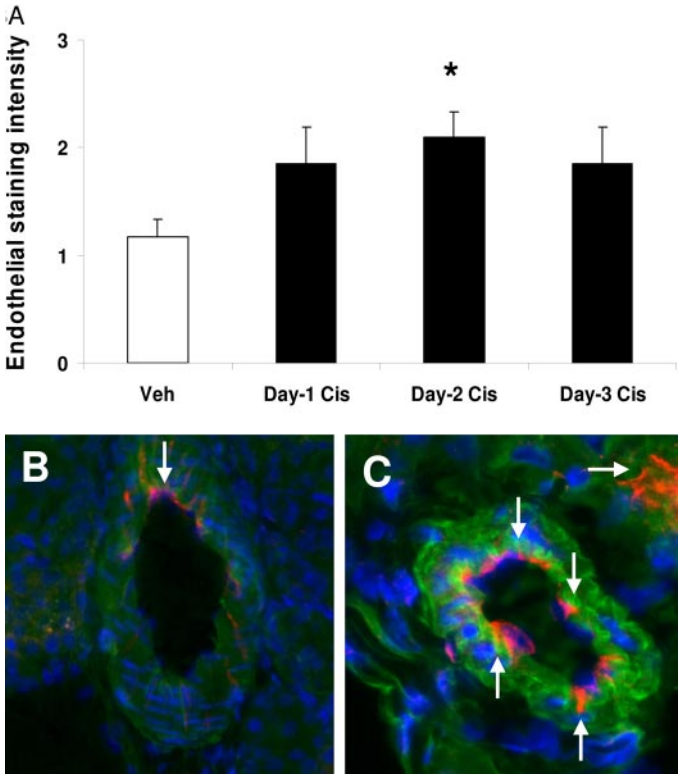

Fig. 3. Immunofluorescence staining for CX_3CL1. The intensity of endothelial staining was determined as outlined under *Materials and Methods*. A, the endothelial intensity staining score was significantly higher at day 2 after cisplatin administration (*, $P < 0.05$ versus vehicle, day 1 and day 3). Representative pictures of the endothelial staining (orange/red, arrows) in vehicle-treated (B) and CisARF (C) are shown.

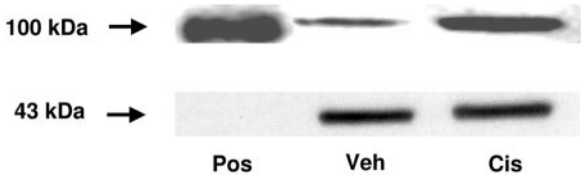

Fig. 4. Endothelial cells. Mouse microvascular endothelial cells (MS1 cells) were treated with Cis (as described under *Materials and Methods*) and then immunoblotted for CX_3CL1. There was an up-regulation of CX_3CL1 protein expression (100 kDa) in cells incubated with Cis (50 μM for 24 h) compared with Veh cells. Recombinant mouse fractalkine that lacks 57 carboxyl-terminal amino acids (85 kDa) was used as a positive control (Pos). A representative immunoblot of at least three separate experiments is shown. Actin (43 kDa) was used as a loading control.

ber of CD11b-positive macrophages was decreased by LEC. The number of CD11b-positive cells (per HPF) on day 3 was 6.1 ± 2.7 in cisplatin plus vehicle (empty liposomes) and 2.4 ± 1.4 in cisplatin plus LEC ($P < 0.01$ versus vehicle).

Effects of CX_3CR1 Inhibition. Administration of the anti-CX_3CR1 antibody (25 μg i.p. 1 h after administration of cisplatin) was not sufficient to prevent the rise in serum creatinine or BUN. Serum creatinine on day 3 was 2.2 ± 0.1 mg/dl in cisplatin-induced ARF plus vehicle and 1.9 ± 0.2 mg/dl in cisplatin-induced ARF plus anti-CX_3CR1 antibody ($P > 0.05$, $n = 8$) (Fig. 7A). BUN on day 3 was 225 ± 26 mg/dl in cisplatin-induced ARF plus vehicle and 233 ± 8.3 mg/dl in cisplatin-induced ARF plus anti-CX_3CR1 antibody ($P > 0.05$, $n = 8$) (Fig. 7B). Administration of the anti-CX_3CR1 antibody (25 μg i.p.) 1 day after the administration of cisplatin also

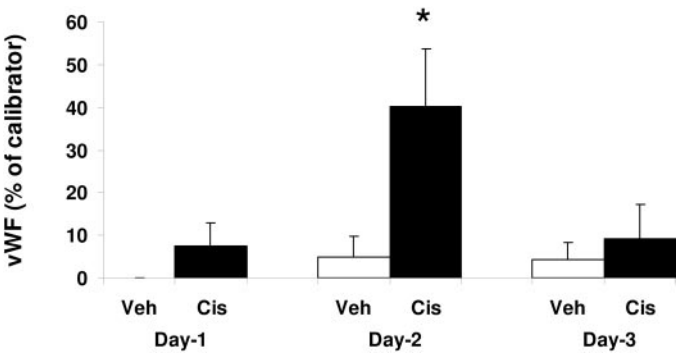

Fig. 5. vWF assay in plasma. Circulating vWF was measured as a marker of endothelial injury. vWF in plasma was increased on day 2 after cisplatin administration, demonstrating the presence of systemic endothelial cell injury (*, $P < 0.05$ versus Veh).

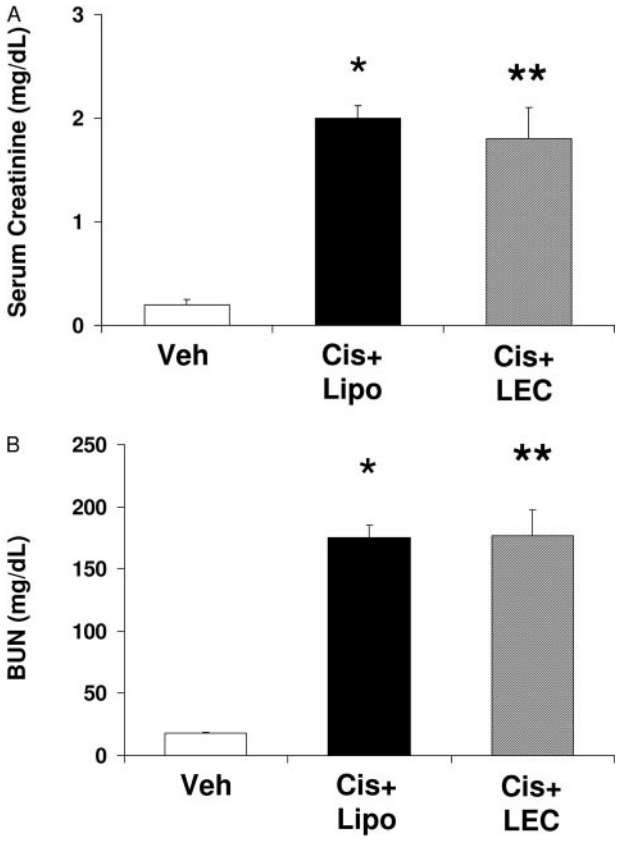

Fig. 6. Macrophage depletion using LEC. Macrophage depletion using LEC was not sufficient to prevent CisARF on day 3 after cisplatin administration, as determined by serum creatinine (A) and BUN (B) [*, $P < 0.05$ versus Veh; **, $P > 0.05$ versus Cis+Lipo (liposomes)]. Empty Lipo was used as the vehicle. Macrophage depletion by LEC was confirmed by flow cytometry analysis of the spleen and immunofluorescent staining of kidney sections (see *Results*).

had no protective effect on serum creatinine. Serum creatinine on day 3 was 2.4 ± 0.1 mg/dl in cisplatin-induced ARF plus vehicle and 2.8 ± 0.3 mg/dl in cisplatin-induced ARF plus anti-CX_3CR1 antibody ($P > 0.05$, $n = 8$).

CX_3CR1 knockout mice ($CX_3CR1^{-/-}$) were then used to examine the effects of constitutive CX_3CR1 deficiency in CisARF. Likewise, $CX_3CR1^{-/-}$ mice were not protected from CisARF as determined by serum creatinine and BUN. Serum creatinine on day 3 was 2.3 ± 0.3 mg/dl in wild-type mice

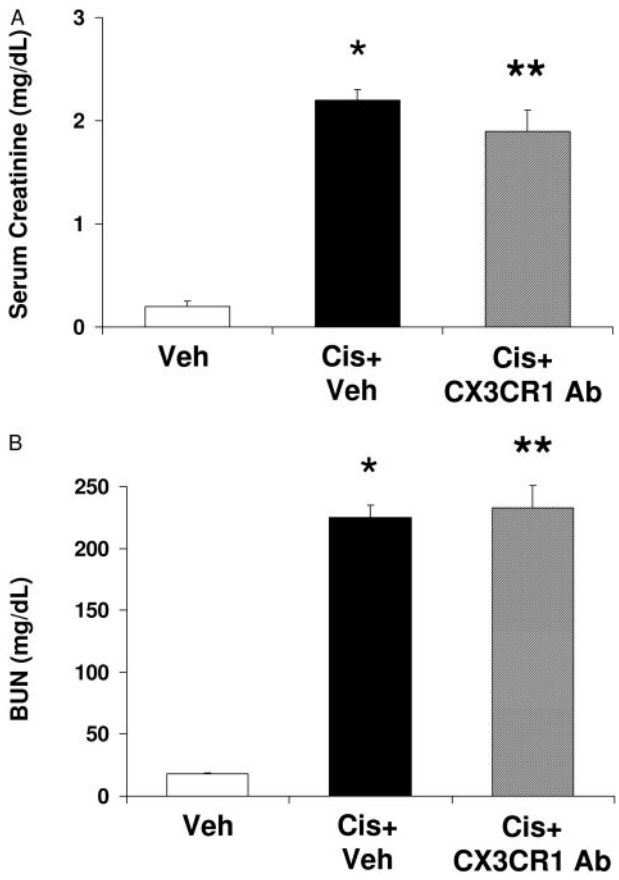

Fig. 7. Anti-CX_3CR1 antibody (CX_3CR1 Ab). Administration of CX_3CR1 Ab (25 μg i.p. 1 h after administration of cisplatin) was not sufficient to prevent CisARF on day 3 after cisplatin administration, as determined by serum creatinine (A) and BUN (B) (*, $P < 0.05$ versus Veh; **, $P > 0.05$ versus Cis+Lipo). In addition, administration of the anti-CX_3CR1 Ab 1 day after administration of cisplatin also had no protective effect on serum creatinine and BUN (see *Results*).

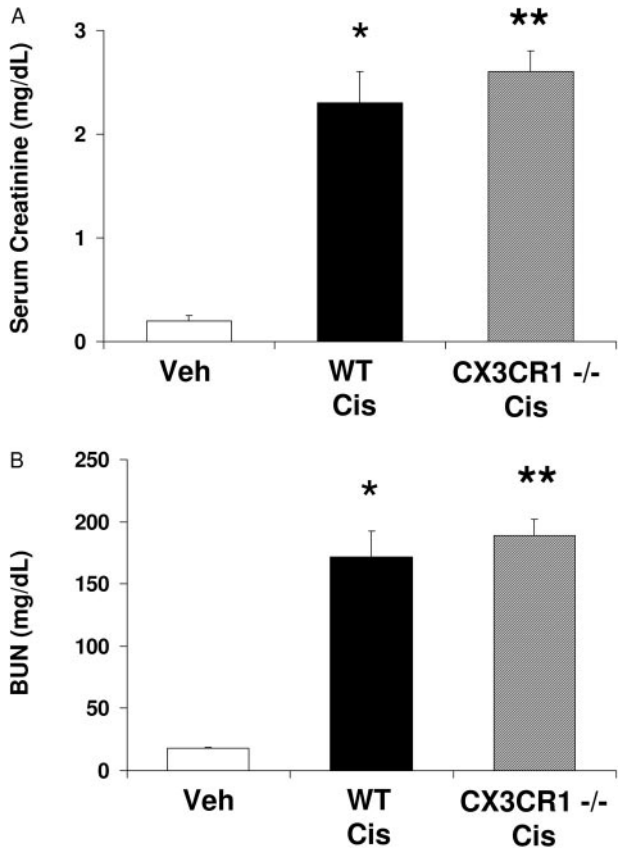

Fig. 8. CX_3CR1 knockout mice ($CX_3CR1^{-/-}$). $CX_3CR1^{-/-}$ mice were not protected from CisARF compared with WT mice, as determined by serum creatinine (A) and BUN (B) (*, $P < 0.05$ versus Veh; **, $P > 0.05$ versus Cis+Lipo).

with CisARF and 2.6 ± 0.2 mg/dl in $CX_3CR1^{-/-}$ mice with CisARF ($P > 0.05$, $n = 20$) (Fig. 8A). Serum BUN on day 3 was 172 ± 24 mg/dl in wild-type mice and 189 ± 13 mg/dl in $CX_3CR1^{-/-}$ mice ($P > 0.05$, $n = 20$) (Fig. 8B).

The effect of CX_3CR1 antibody treatment on the number of CD11b-staining cells was also determined. It is interesting to note that the number of CD11b staining cells was increased rather than decreased by the CX_3CR1 antibody. The number of CD11b-positive cells (per HPF) on day 3 was 6.5 ± 0.1 in cisplatin plus vehicle and 9.3 ± 1.0 in cisplatin plus CX_3CR1 antibody ($P < 0.05$ versus vehicle).

Discussion

There is increasing evidence that CisARF is an inflammatory process (Ramesh and Reeves, 2004; Safirstein, 2007). Early inflammation is largely mediated by the innate immune system, which provides rapid nonadaptive responses against infections and injuries. Innate immune cells include the macrophage, the role of which in ischemic ARF is increasingly appreciated. Macrophage adhesion and infiltration occurred in the outer stripe of the outer medulla as early as 24 h after ischemic reperfusion in the rat (De Greef et al., 2001) and mouse (Furuichi et al., 2003; Day et al., 2005; Jo et al., 2006). In addition, specific macrophage depletion was protective against ischemic ARF in mice (Day et al., 2005; Jo et al., 2006), and gene therapy in rats expressing a nonfunctional amino-terminal truncated monocyte chemoattractant protein-1 reduced macrophage infiltration and acute tubular necrosis (Furuichi et al., 2003). In vitro studies show that cisplatin administration increases macrophage-mediated cytotoxicity against neoplastic cells (Sodhi et al., 1990). Macrophage renal infiltration was recently shown at 72 h after cisplatin administration (Lee et al., 2006). The chronology of macrophage infiltration, however, was not demonstrated. In addition, macrophages are the principal cellular reservoirs of caspase-1, an inflammatory enzyme whose deficiency confers protection in CisARF (Faubel et al., 2004). Therefore, we hypothesized that macrophages may play an injurious role in CisARF. In the present study, we found an increase in renal CD11b-positive macrophages beginning on day 2 after cisplatin administration, which preceded the time of maximal renal dysfunction. We then studied the effects of macrophage depletion. After LEC administration, there was a significant decrease in renal CD11b-positive macrophages on day 3 after cisplatin. This depletion in renal macrophages, however, was not sufficient to protect against CisARF as determined by serum creatinine and BUN. Our data suggest that, although macrophage recruitment occurs early in the course of CisARF, these infiltrating macrophages are not essential for the occurrence of CisARF. However, these findings do not preclude other roles of macrophages in CisARF such as tissue repair.

If macrophage infiltration is not essential for the occurrence of CisARF, the question of which inflammatory cells might play an injurious role in CisARF is raised. CisARF is associated with neutrophil and macrophage infiltration in the kidney, as detected by naphthol AS-D chloroacetate esterase staining, that is attenuated by IL-10 (Deng et al., 2001). Cytokine antagonism against tumor necrosis factor (TNF)-α production and downstream signaling has been shown to be protective in CisARF, with resultant decreases in naphthol AS-D chloroacetate esterase staining (Ramesh and Reeves, 2002, 2003). CisARF is associated with neutrophil infiltration in the kidney (Faubel et al., 2004; Li et al., 2005). Although neutrophil and macrophage infiltration in the kidney increases in CisARF, a specific injurious role would be demonstrated by the protective effect of neutrophil or macrophage depletion. In this regard, we have demonstrated that neutrophil depletion is not protective against CisARF (Faubel et al., 2007), and in the present study, we have shown that macrophage depletion is not protective. It has been recently demonstrated that T-cell-deficient (nu/nu) mice and CD4- or CD8-deficient mice are protected against CisARF, suggesting a role for lymphocytes in CisARF (Liu et al., 2006). In view of the evidence of inflammation in CisARF, the role of lymphocyte subsets [e.g., natural killer (NK) and NKT cells] merits further study in CisARF.

Recent studies suggest that renal parenchymal cells rather than infiltrating immune cells may have an important role in the inflammatory response in CisARF. Cisplatin stimulates renal epithelial cells to produce TNF-α in vitro, suggesting that renal parenchymal cells may be the major source of TNF-α in CisARF (Ramesh and Reeves, 2005). In another study, chimeric mice were created in which the bone marrow was ablated and replaced with donor bone marrow cells from wild-type or TNF-α knockout mice (Zhang et al., 2007). In the study, chimeric mice produced from wild-type recipients were equally susceptible to CisARF, regardless of whether their immune systems were from wild-type or TNF-α-deficient donors. In contrast, chimeras of TNF-α knockout recipients were resistant to CisARF, regardless of the origin of their immune systems. In addition, urinary excretion of several proinflammatory cytokines was lower in the wild-type bone marrow-knockout chimera mouse than in wild-type background mice. These data suggest that CisARF is mediated by TNF-α and other cytokines produced by renal parenchymal cells.

The vascular endothelium may initiate an inflammatory response in CisARF because it is in direct contact with circulating inflammatory cells (Bonventre and Zuk, 2004; Friedewald and Rabb, 2004). It has been demonstrated that endothelial injury and dysfunction can progress to tubular injury in cisplatin-induced ARF (Offerman et al., 1984; Winston and Safirstein, 1985). We have recently demonstrated that cisplatin-induced endothelial cell death in vitro is mediated by caspases and calpain (Dursun et al., 2006). In humans, endothelial injury due to cisplatin can manifest as a thrombotic microangiopathy (Jackson et al., 1984; Weinblatt et al., 1987). vWF is synthesized by endothelial cells, and plasma vWF levels are elevated with endothelial cell injury. In humans, an increase in renal endothelial vWF deposition is associated with increased allograft rejection and renal macrophage infiltration (Ozdemir et al., 2006). In our study, circulating vWF levels were increased at 2 days after cisplatin administration, preceding peak renal dysfunction. This finding supports the hypothesis that endothelial injury and activation may play a role in inflammatory cell recruitment in CisARF.

We next examined the role of CX_3CL1, which is expressed on injured endothelial cells (Umehara et al., 2001). CX_3CL1 acts as both an adhesion molecule and chemokine for NK cells, monocyte/macrophages, and some $CD8^+$ T cells that express CX_3CR1(Umehara et al., 2001). Evidence is accumulating regarding the pathophysiological effects of CX_3CL1 and its ligand CX_3CR1 in the pathogenesis of various inflammatory diseases, such as atherosclerosis (Lesnik et al., 2003), allograft rejection (Haskell et al., 2001), and rheumatoid arthritis (Volin et al., 2001). In renal disease, CX_3CL1 expression is increased in patients with renal tubulointerstitial inflammation with the strongest expression localized to vascular sites near macrophage infiltration (Cockwell et al., 2002). CX_3CR1-positive cells were seen in human kidney biopsy specimens in a variety of conditions, including kidney tumors, renal transplant nephrectomies, renal transplant biopsies, and glomerulonephritis (Segerer et al., 2002). In addition, a recent report demonstrated that crescentic glomerulonephritis in the rat is prevented by CX_3CR1 antibody administration (Feng et al., 1999). In the present study, evidence of endothelial injury (expression of CX_3CL1 and the increase in vWF in the blood) preceded the increase in BUN and serum creatinine. In addition, our in vitro data demonstrating an increase in CX_3CL1 expression in cultured endothelial cells treated with cisplatin support the in vivo evidence that cisplatin increases CX_3CL1 expression on injured endothelial cells. However, we demonstrated that blockade of CX_3CR1, using both a specific antibody and knockout mice, is not sufficient to reduce renal failure in CisARF. These findings suggest that adhesion molecules and chemokines, other than CX_3CL1, may be more important in the pathogenesis of CisARF. In this regard, there is increased expression of keratinocyte-derived chemokine protein (Liu et al., 2006), ICAM-1 (Lee et al., 2006), macrophage inflammatory protein-2 (Ramesh and Reeves, 2002), monocyte chemoattractant protein-1 (Ramesh and Reeves, 2002), vascular cell adhesion molecule-1 (Li et al., 2005), and the chemokine receptors CCR1/CCR5 (Li et al., 2005) in CisARF. In addition, inhibition of ICAM-1 is protective against CisARF, suggesting an injurious role of ICAM-1 (Kelly et al., 1999). ICAM-1 is implicated in neutrophil and monocyte-endothelial cell interaction. However, the lack of a protective effect of neutrophil and macrophage depletion in CisARF suggests that the protective effect of ICAM-1 inhibition may be independent of neutrophils or macrophages. The present study highlights the fact that multiple inflammatory cells, chemokines, and adhesion molecules are increased in CisARF but that inflammatory cell depletion and chemokine or adhesion molecule inhibition studies are necessary to determine the precise injurious role of these cells and molecules.

In summary, in CisARF, macrophage infiltration in the kidney, CX_3CL1 expression in the kidney and blood vessels, and the increase in circulating vWF precede BUN and serum creatinine increase. However, inhibition of macrophage infiltration in the kidney and CX_3CR1 blockade is not sufficient to prevent CisARF.

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