Macrophages mediate lung inflammation in a mouse model of ischemic acute kidney injury

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ACUTE KIDNEY INJURY (AKI) is a common complication in hospitalized patients that is associated with increased mortality (3, 9, 24, 25, 35). The increased mortality of AKI may be explained, in part, by the development of pulmonary complications such as respiratory failure and acute lung injury (ALI) (8, 29). For example, patients with AKI are twice as likely to need mechanical ventilation as patients without AKI (30, 44) and patients with AKI have an impaired ability to wean from mechanical ventilation (43).

We recently demonstrated that the proinflammatory cytokines IL-6 and IL-8 are increased in the serum of patients with AKI and predict prolonged mechanical ventilation (26). In mice, we demonstrated that IL-6 and CXCL1 (also known as KC and a murine analog of IL-8) are increased in the serum after AKI (18). Furthermore, inhibition of IL-6 utilizing IL-6-deficient mice and IL-6 antibodies improved lung injury after ischemic AKI and bilateral nephrectomy; improvement in lung injury was associated with a reduction in lung CXCL1, neutrophil infiltration, and pulmonary capillary leak (22). Together, these data suggest that IL-6 contributes to AKI-mediated lung injury via upregulation of lung CXCL1.

The cellular source of serum IL-6 in AKI is unknown. Mononuclear phagocytes, known as monocytes in circulation and macrophages in tissues, are sources of cytokines in inflammatory conditions. With regard to lung injury, alveolar macrophage production of IL-8 may mediate ALI (5, 28, 45). In the present study, therefore, we hypothesized that serum IL-6 produced by circulating monocytes would stimulate alveolar macrophage production of CXCL1, leading to neutrophil recruitment and lung injury after AKI. To determine whether mononuclear phagocytes are a source of serum IL-6 in AKI, systemic mononuclear phagocytes were depleted by diphtheria toxin (DT) injection to CD11b-DTR transgenic mice. To specifically determine the role of alveolar macrophages in AKI-mediated lung injury, liposome-encapsulated clodronate (LEC) was administered intratracheally to deplete alveolar, but not systemic, mononuclear phagocytes (42).

METHODS

Animals. Eight- to 12-wk-old CD11b-DTR transgenic mice on a BALB/c background, BALB/c wild-type mice, and wild-type C57BL6 mice that weighed 20–25 g were used. Mice were maintained on a standard diet, and water was freely available. All experiments were conducted with adherence to the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The animal protocol was approved by the Animal Care and Use Committee of the University of Colorado at Denver.

Surgical protocol. Mice were anesthetized with intraperitoneal avertin (2,2′-tribromoethanol; Aldrich, Milwaukee, WI) and exposed to either sham operation or ischemic AKI. In each group, a midline incision was made, and both renal pedicles were exposed. In the AKI group, both renal pedicles were clamped for 22 min. After clamp removal, kidneys were observed for blood reperfusion, as noted by a return to their original color. The abdomen was closed in one layer. Sham surgery consisted of the same procedure, except no clamps were applied. CD11b-DTR mice received either 25 ng/g body wt DT or vehicle (sterile saline) via tail vein injection 18 h before sham...
operation or ischemic AKI. To deplete alveolar macrophages specifically, C57BL6 mice received either 10 μg LEC (Encapsula Nano-Sciences, Nashville, TN) or empty liposomes via intratracheal (IT) injection 24 h before sham operation or ischemic AKI.

Tissue collection. Animals were euthanized by anesthetic overdose using intraperitoneal pentobarbital sodium, after which blood, bronchoalveolar lavage (BAL) fluid, and organs were collected.

Serum. Blood was collected at time of death via cardiac puncture and serum was collected as previously described (18, 22).

Serum creatinine and blood urea nitrogen measurement. Serum creatinine and blood urea nitrogen (BUN) were measured using the appropriate QuantiChrom assay kit (BioAssay Systems, Hayward, CA). The detection limit for IL-6 is 1.3 pg/ml.

Lung CXCL1. Lung CXCL1 was determined on whole lung homogenates by ELISA (R&D Systems, Minneapolis, MN). The detection limit for CXCL1 is 2.0 pg/ml. Tissue was prepared as previously described (18, 22).

BAL. After cardiac puncture, the trachea was dissected and cannulated with a 20-gauge catheter. Lungs were lavaged with 1 ml of 1 mM EDTA in PBS five times. Protein content of BAL fluid was determined for the first 2 ml, following centrifugation at 3,000 g at 4°C for 5 min.

Total alveolar macrophage assessment. The BAL fluid cell pellet was resuspended in 1 ml PBS and total alveolar macrophages were determined. Ten microliters of the sample were placed on a hemacytometer and total cell number was determined. Additionally, 100 μl of the BAL sample were centrifuged with a Cytofuge (IRIS International) at 300 rpm onto microscope slides. Cells were fixed and stained using a histological stain similar to Wright-Giemsa stain utilizing Protocol HEMA 3 stain set according to the manufacturer’s directions. Alveolar macrophages were counted and the percent of alveolar macrophages relative to other cells was determined. Total alveolar macrophages contained in the BAL fluid sample were then calculated.

Lung myeloperoxidase activity. One quarter of the lung tissue was homogenized in 1 ml of cold hexadecyltrimethylammonium bromide buffer (50 mM KPO4 and 0.5% hexadecyltrimethylammonium bromide; pH 6.0), sonicated on ice for 10 s, and centrifuged at 14,000 g at 4°C for 30 min. Twenty microliters of supernatant were transferred into a 96-well plate, and 200 μl of 37°C O-dianisidine hydrochloride solution (16.7 mg O-dianisidine, 100 ml: 90% water and 10% 50 mM KPO4 + 0.0005% H2O2) were added immediately before the optical density was read at 450 nm, and again 30 s later (Benchmark microplate reader; Bio-Rad).

Lung histology. After cardiac puncture, the right hilum was tied off and the right lobes were removed, rinsed with PBS, and snap-frozen in liquid nitrogen. The left lung was expanded with 0.5% low-melting agarose at a constant pressure of 25 cmH2O, allowing for homogeneous expansion of lung parenchyma as described previously (19). The lung was removed, fixed in 10% formalin, and paraffin-embedded 4-μm sections were stained with hemotoxylin and eosin according to standard protocols.

Lung Evans blue dye content. Lung content of Evans blue dye (EBD), a marker of lung vascular permeability and lung capillary leak, was determined as previously described (22). Briefly, a total of 250 μl of EBD (5 mg/ml) was injected via tail vein 1 h before the animal was euthanized. Lungs were perfused with 5 ml PBS via the
right ventricle to remove EBD within the vasculature, excised, weighed, and homogenized in 2 ml formamide. The homogenate was incubated in a 37°C water bath overnight and then centrifuged at 10,000 g for 30 min. The optical density of supernatant was determined at 620 nm, and EBD concentration was calculated against a standard curve (mg EBD/g lung tissue). Lung EBD accumulation is a well-established measurement of noncardiogenic pulmonary edema. Because the lung endothelial barrier is normally impermeable to albumin and EBD binds albumin, the accumulation of EBD in lung tissue indicates the severity of endothelial injury (i.e., noncardiogenic pulmonary edema).

Flow cytometry on BAL fluid cells. After determining the cell count, BAL fluid cells were reconstituted with 100 μl PBS containing 1% BSA and stained with antibodies against the following surface molecules for 30 min at 4°C in the dark: CD11c-PECy7, CD11b-PE, F4/80-APC (eBiosciences), CD45-V500, and Ly6G-APCCy7 (BD Biosciences). Cells were washed three times in PBS containing 1% BSA and fixed in 200 μl of 1% paraformaldehyde (Sigma). Multiparameter flow cytometry was performed using a BD FACSCanto instrument (BD Biosciences) and analyzed using FacsDiva software (BD Biosciences). Alveolar macrophages are CD45, CD11c, and F4/80 positive and CD11b negative. Ly6G was included as a neutrophil marker to exclude cells from analysis.

Flow cytometry on lung digestion. The lung parenchyma was minced into 1-mm³ pieces and processed by enzymatic digestion: 2 mg/ml collagenase (Roche). The suspension was incubated at 37°C on a rotary shaker for 30 min. The lung was triturated using an 18-gauge needle and filtered through a 70-μm nylon cell strainer (BD Falcon).
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before being washed in serum containing RPMI-1640 medium (GIBCO). Cells were treated with ACK red blood cell lysis buffer (Quality Biological). Cells were washed three times in PBS containing 1% BSA, stained, and fixed as explained for BAL fluid cells. Interstitial macrophages are CD45, F480 positive, CD11b positive, and Ly6G negative.

Flow cytometry on blood. Approximately 250 µl of whole blood were mixed with 50 µl EDTA to prevent clotting. Two hundred fifty microliters of this mixture were then added to 5-ml ACK lysis buffer (Quality Biological) to lyse red blood cells and incubated for 5 min. Five milliliters of RPMI-1640 medium (Sigma) were added to each sample to dilute the ACK lysis buffer. Samples were spun down at 1,500 rpm for 5 min. Cells were reconstituted with 5 ml RPMI-1640 medium and spun down again at 1,500 rpm. This process was repeated and cells were reconstituted with 100 µl 1% BSA in PBS. White blood cells were stained with the same antibodies used for BAL fluid cell staining. Blood monocytes are CD45 positive, F480 positive, CD11b positive, CD11c negative, and Ly6G negative.

RESULTS

Blood flow cytometry to assess monocyte depletion in CD11b-DTR transgenic mice with ischemic AKI. To confirm that injection of DT to CD11b-DTR transgenic mice resulted in a reduction in circulating monocytes, blood flow cytometry was performed. Mice were injected with DT or vehicle 18 h before induction of AKI; DT-injected mice had complete depletion of blood monocytes (CD45+, F4/80+, CD11b+, CD11c−, and Ly-6G−; Fig. 1).

Circulating neutrophils are decreased with DT injection to CD11b-DTR transgenic mice. Since neutrophils also express CD11b, flow cytometry of blood confirmed a reduction in neutrophils after DT injection to CD11b-DTR transgenic mice, but not vehicle injection. Specifically, Ly6G-positive cells were 53% in vehicle-injected compared with 43% in DT-injected (P < 0.01, n = 3).

Serum creatinine, BUN, and serum IL-6 in CD11b-DTR transgenic mice. As shown in Fig. 2, A and B, serum creatinine and BUN were similar in vehicle-injected and DT-injected (systemic mononuclear phagocyte-depleted) CD11b-DTR transgenic mice 4 h after AKI. Serum creatinine and BUN were also determined 24 h after AKI; serum creatinine was 0.9 ± 0.2 in vehicle and 0.9 ± 0.3 in DT injection (P = NS, n = 9); BUN was 80 ± 15 in vehicle-injected and 74 ± 8 in DT-injected (P = NS, n = 9). Thus, renal function is not affected by mononuclear phagocyte depletion in the CD11b-DTR transgenic model.

Unexpectedly, serum IL-6 was significantly increased in DT-injected CD11b-DTR transgenic mice (systemic mononuclear phagocyte-depleted) with AKI vs. vehicle-injected CD11b-DTR transgenic mice with AKI. Serum IL-6 (pg/ml) was 1,160 ± 130 in vehicle-injected AKI and was 3,700 ± 700 in DT-injected AKI (P < 0.01, n = 7–9; Fig. 2C).

Lung CXCL1, lung myeloperoxidase activity, and lung neutrophil content in CD11b-DTR transgenic mice. To determine whether systemic mononuclear phagocyte depletion reduced lung inflammation, lung CXCL1, lung myeloperoxidase (MPO) activity, and lung neutrophils by flow cytometry were determined 4 h after sham operation or AKI in vehicle-injected and DT-injected (mononuclear phagocyte-depleted) CD11b-DTR transgenic mice. CXCL1 is a potent neutrophil chemokine and MPO activity is a widely used marker of neutrophil infiltration. We previously demonstrated that lung MPO activity correlates with lung neutrophil infiltration as judged by histology (22).

As expected, lung CXCL1, lung MPO activity, and lung neutrophils by flow cytometry were all significantly lower in the systemic mononuclear phagocyte-depleted mice with AKI. Lung histology in systemic mononuclear phagocyte-depleted mice with AKI also demonstrated less cellular/neutrophil in-
Specifically, lung CXCL1 (pg/mg) was 704 ± 58 in sham-operated, vehicle-injected vs. 324 ± 52 in sham-operated, DT-injected (P < 0.01, n = 4) and was 1,299 ± 57 in vehicle-injected AKI vs. 859 ± 51 in DT-injected AKI (P < 0.001, n = 17–18; Fig. 3A). Lung MPO (Δ/min/mg) activity was 1.13 ± 0.11 in sham-operated, vehicle-injected and was 0.48 ± 0.07 in sham-operated, vehicle-injected (P < 0.01, n = 4) and was 2.27 ± 0.17 in vehicle-injected AKI and was 1.04 ± 0.01 in DT-injected AKI (P < 0.001, n = 15–18; Fig. 3B). Finally, lung neutrophils as assessed by flow cytometry confirmed the reduction in lung MPO activity were (%) 36 ± 10 in vehicle-injected AKI vs. 12 ± 3 in DT-injected AKI (P < 0.05, n = 5). In normal mice, without AKI or other injury, lung neutrophil content was 4.8 ± 0.5% (n = 11), suggesting that the reduction in lung neutrophils with DT injection was due to reduced lung neutrophil recruitment vs. a reduction in baseline lung neutrophils.

Lung capillary leak assessment by BAL fluid protein and lung EBD in CD11b DTR transgenic mice. Since neutrophils are known to be injurious in lung injury and contribute to vascular leak, we sought to determine whether the decrease in lung inflammation with systemic mononuclear phagocyte depletion also improved capillary leak. Using BAL fluid protein content and the EBD method, we determined that lung capillary leak was unexpectedly increased in mice with AKI and systemic mononuclear phagocyte depletion (DT-injected CD11b-DTR transgenic mice).

Specifically, 4 h after AKI, BAL fluid protein (μg/μl) was 110 ± 6 in vehicle-injected AKI vs. 149 ± 17 in DT-injected AKI (P < 0.05, n = 3–7; Fig. 4A). One hour after intravenous injection, lung EBD content (μg/g) was 94 ± 7 in vehicle-injected AKI vs. 143 ± 17 in DT-injected AKI (P < 0.05, n = 4–5; Fig. 4B).

Serum creatinine, BUN, serum IL-6, lung CXCL1, and lung MPO activity after DT injection to wild-type mice. Because serum IL-6 was increased in DT-injected CD11b-DTR transgenic mice with AKI, we questioned whether DT injection in the setting of AKI might be proinflammatory in and of itself. Therefore, DT vs. vehicle was injected in wild-type BALB/c mice with sham operation or ischemic AKI and serum creatinine, BUN, serum IL-6, lung CXCL1, and lung MPO activity were determined.

Serum creatinine and BUN were not different between vehicle-injected and DT-injected wild-type mice after either sham operation or AKI. Serum IL-6 was significantly increased in DT-injected wild-type mice with AKI vs. vehicle-injected wild-type mice with AKI. Lung CXCL1 and lung MPO activity were similarly increased in DT-injected wild-type mice with AKI vs. vehicle-injected wild-type mice with AKI. These data demonstrate that DT injection in the setting of AKI results in an increased proinflammatory response (Fig. 5, A–E).

Lung interstitial and alveolar macrophage counts in CD11b-DTR transgenic mice with AKI. Although DT injection results in an increased systemic proinflammatory response as evidenced by increased serum IL-6, systemic mononuclear phagocyte-depleted mice with AKI utilizing the CD11b-DTR trans-
genic mice were still protected against lung inflammation. Since resident lung interstitial and alveolar macrophages are important factors that initiate lung inflammation in response to circulating proinflammatory cytokines, we hypothesized that lung interstitial or alveolar macrophage depletion in the CD11b-DTR transgenic mice might be responsible for the protection against lung inflammation. Therefore, we examined whether lung interstitial or alveolar macrophages were reduced in the CD11b-DTR transgenic mice.

As shown in Fig. 6, lung interstitial, but not alveolar, macrophage counts were reduced as determined by flow cytometry of lung digestions and BAL fluid, respectively.

Alveolar macrophage counts in BAL fluid by flow cytometry in mice administered IT LEC with AKI. To assess whether alveolar macrophages are important in lung inflammation after AKI, alveolar macrophages were depleted by administering LEC intratracheally. To confirm that alveolar macrophages were reduced in this model, BAL fluid cells were assessed for alveolar macrophages by flow cytometry and were significantly reduced in intratracheally administered LEC mice with AKI (Fig. 7B). Lung interstitial macrophages were not affected by IT LEC administration (Fig. 7A).

Blood monocytes and neutrophils in mice administered IT LEC with AKI. Blood monocytes were assessed by flow cytometry after IT LEC administration and were not reduced. Four hours after AKI, blood monocytes were 3.7% after IT vehicle and were 4.4% after IT LEC (Fig. 8). Four hours after AKI, splenic macrophages were 3.1% after IT vehicle and were 3.2% after IT LEC. We also examined circulating neutrophils via flow cytometry after IT LEC and confirmed that these were also not reduced. Four hours after ischemic AKI, circulating neutrophils were 70% in IT vehicle and were 75% in IT LEC (n = 3, P = NS).

Serum creatinine, BUN, and IL-6 after IT LEC. Serum creatinine, BUN, and serum IL-6 were similar in IT vehicle and IT LEC-treated mice 4 h after AKI (Fig. 9, A–C). Serum creatinine remained elevated after 24 h and was 1.5 ± 0.6 in IT vehicle and 2.0 ± 0.3 in IT LEC (P = NS, n = 4). Thus, renal function and serum IL-6 are not affected by alveolar macrophage depletion with IT LEC.

Lung CXCL1, lung myeloperoxidase activity, and lung histology after IT LEC. To determine whether alveolar macrophage depletion reduced lung inflammation, lung CXCL1 and lung myeloperoxidase activity (MPO) were determined at 4 h after AKI. As
expected, both lung CXCL1 and lung MPO activity were lower in mice with alveolar macrophage depletion and AKI. Lung histology demonstrated less cellular/neutrophil infiltration in mice with alveolar macrophage depletion and AKI vs. mice with normal alveolar mononuclear phagocyte content and AKI (Fig. 10C). Specifically, in mice with AKI, lung CXCL1 (pg/mg) was 601 ± 49 in IT vehicle and was 443 ± 33 in IT LEC (P < 0.05, n = 10–13; Fig. 10A); lung MPO activity (Δ/min/mg) was 1.02 ± 0.06 in IT vehicle and was 0.85 ± 0.05 in IT LEC (P < 0.05, n = 13–14; Fig. 10B).

Fig. 7. Lung interstitial and alveolar macrophages in wild-type mice administered intratracheal (IT) liposome-encapsulated clodronate (LEC) with AKI. Lung interstitial macrophages and alveolar macrophages were determined in lung digests and BAL fluid, respectively, by flow cytometry in IT vehicle-treated (Veh) or IT LEC-treated wild-type C57Bl/6 mice 4 h after ischemic AKI. The percent (A) and total number (B) of interstitial macrophages were similar in IT Veh- and IT LEC-treated mice (n = 10). The percent (C) and total number (D) of alveolar macrophages were reduced in IT LEC-treated mice (n = 10).

Fig. 8. Blood monocytes in wild-type mice administered IT LEC with AKI. Blood monocytes were assessed 4 h after AKI in mice treated with IT vehicle or IT LEC. Blood monocytes as assessed by flow cytometry are similar in IT vehicle (3.7%) and IT LEC (4.4%); thus, IT LEC does not affect circulating monocytes (P = NS, n = 5).
Lung capillary leak assessment by BAL fluid protein and lung EBD after IT LEC. To determine whether the decrease in lung inflammation with alveolar macrophage depletion improved capillary leak, BAL fluid protein and lung EBD content were determined. Similar to systemic mononuclear phagocyte depletion in the CD11b-DTR transgenic mice with AKI, lung capillary leak as determined by both parameters was unexpectedly increased in mice after IT LEC with AKI.

Specifically, 4 h after AKI, BAL fluid protein (μg/μl) was 106 ± 11 in IT vehicle-treated AKI vs. 174 ± 25 in IT LEC-treated AKI (P < 0.05, n = 3–7; Fig. 11A). One hour after intravenous injection, lung EBD content (μg/g) was 141 ± 8 in IT vehicle-treated AKI vs. 223 ± 32 in IT LEC-treated AKI (P < 0.05, n = 4–5; Fig. 11B).

DISCUSSION

ALI and its more severe form, acute respiratory distress syndrome, are common and occur in ~10% of patients who are admitted to the intensive care unit (7, 15). Numerous direct and indirect causes of ALI have been identified. Direct etiologies of ALI directly affect the lungs and include pneumonia and aspiration of gastric contents; indirect etiologies of ALI are due to systemic injury such as pancreatitis, sepsis, or trauma (45). The common link among the disparate causes of ALI is the role that proinflammatory cytokines play in mediating lung injury. In animal models, cytokines mediate lung injury via the up-regulation of pulmonary adhesion molecules and chemokines causing neutrophil infiltration and subsequent tissue damage (1, 5, 6, 10, 11, 36, 39, 40, 46, 47). Alveolar macrophage production of the chemokine IL-8 (analogous to murine CXCL1), in particular, appears to play a key pathogenic role (13). Other lung mononuclear phagocyte populations including marginated monocytes and lung interstitial mononuclear phagocytes may also play a role in CXCL1 production and subsequent lung injury (34). Although AKI is recognized as a proinflammatory state that is associated with increased incidence of respiratory failure requiring mechanical ventilation (16, 41), AKI is currently not widely viewed as a cause of ALI. In the present study, we demonstrate that either systemic mononuclear phagocyte depletion or alveolar macrophage depletion reduces CXCL1 production and lung inflammation after AKI in mice. Thus, AKI activates pathways in the lung known to be important in the pathogenesis of ALI, supporting the notion that AKI may be a potential cause of ALI in patients.

In this report, we first sought to examine the role of systemic mononuclear phagocyte depletion on IL-6 production and lung injury. Mononuclear phagocytes, known as monocytes in circulation and macrophages in tissues, are sources of cytokines in inflammatory conditions. We previously demonstrated that serum IL-6 increases by 2 h after AKI in animal models of AKI (18, 22) as well as in patients with AKI (26). Furthermore, we demonstrated that strategies to inhibit IL-6 reduce lung injury after AKI (22). The source and pulmonary target of IL-6 in AKI are unclear. Increased renal production of IL-6 occurs early after AKI, suggesting that the kidney is the source of increased serum IL-6; however, additional data suggest that macrophages recruited to the kidney are the source of renal IL-6 production in AKI (20). Macrophages have also been implicated in AKI-mediated lung injury as administration of CNI-1493 in rats with AKI resulted in reduced lung injury as demonstrated by improved pulmonary vascular leak (23). CNI-1493 is a macrophage pacificant that interferes with macrophage production of proinflammatory cytokines such as TNF-α and IL-6. Although administration of CNI-1493 was found to lower lung capillary leak, it did not protect against kidney injury; the mechanism of the beneficial effect of CNI-1493 was not determined and serum cytokines, lung chemokines, and lung neutrophil infiltration were not examined (Fig. 11).

Therefore, to determine whether systemic mononuclear phagocytes were a source of circulating IL-6, we utilized CD11b-DTR transgenic mice. Selective depletion of mononuclear phagocytes in mice that are transgenic for CD11b-DTR may be achieved with DT injection (14). The transgene was created with insertion of the DT receptor under the CD11b promoter, thereby creating a conditional ablation system. Surprisingly, we found that serum IL-6 was increased in the CD11b-DTR model of mononuclear phagocyte depletion demonstrating that IL-6 production can occur in AKI even in the absence of mononuclear phagocytes. In this regard, we demonstrated that both renal and extrarenal production of IL-6 increases after ischemic AKI, suggesting that multiple cell types might increase IL-6 production after AKI in addition to mononuclear phagocytes (2). Although serum IL-6 was increased in the CD11b-DTR model of systemic macrophage
depletion, lung inflammation was reduced as judged by lower lung CXCL1 levels and reduced lung MPO activity. Previous studies demonstrated that mononuclear phagocyte depletion by other methods protects against renal injury; however, renal function was similar in mononuclear phagocyte-depleted AKI vs. AKI without mononuclear phagocyte depletion in the CD11b-DTR model.

We sought to determine why serum IL-6 was higher in macrophage-depleted mice utilizing the CD11b-DTR method. We hypothesized that DT injection might be inflammatory itself and thus might be a confounding factor. Indeed, DT injection to wild-type mice with AKI resulted in a dramatic rise in serum IL-6 compared with vehicle-injected mice with AKI. Interestingly, DT injection in the absence of AKI did not result in an increase in serum IL-6.

Utilization of this transgenic conditional ablation strategy to deplete certain cells has become a commonly used tool to study the effects of macrophages (CD11b-DTR), dendritic cells (CD11c-DTR), and other cell types in rodent models of a wide variety of diseases including renal transplantation (37), chronic glomerular injury (38), noninflammatory lung injury (32), and wound healing (31). It has been presumed that the injection of DT is without systemic consequences. Our data demonstrate that in the setting of AKI, injection of DT is proinflammatory and may have other deleterious systemic effects. Furthermore, CD11b-DTR mice injected with DT were not protected against renal failure, although mononuclear phagocyte depletion utilizing other methods is protective (12). The role of various leukocytes such as mononuclear phagocytes and dendritic cells in the pathogenesis of ischemic AKI (12) is an active area of interest and the use of the DTR transgenic approach has been anticipated to further examine the contributing roles of macrophages and dendritic cells in AKI (21). However, due to the confounding factors identified in our study, we suggest that the
CD11b-DTR transgenic approach is limited in its utility to study the pathogenesis or systemic effects of AKI due to the proinflammatory effect of DT injection in the setting of AKI.

We next sought to explain why, even with increased serum IL-6, lung CXCL1 and neutrophil infiltration were improved in the CD11b-DTR transgenic mice with AKI. Since alveolar macrophages can mediate inflammation on other models of lung injury, we questioned whether a reduction in alveolar macrophages in the CD11b-DTR mice might be the explanation for reduced lung inflammation in the setting of AKI; however, alveolar macrophages were similar in vehicle-injected and DT-injected CD11b-DTR mice with AKI. Recently, the role of lung interstitial and lung-margined mononuclear phagocytes in CXCL1 production in ALI has been highlighted, thus, we examined interstitial mononuclear phagocytes and found that they were reduced. Thus, reduction of circulating (and thus lung margined) as well as lung interstitial mononuclear phagocytes in the CD11b-DTR model resulted in a dramatic reduction in lung CXCL1 and lung neutrophil infiltration.

Our data suggest that the reduction in lung neutrophils in the CD11b-DTR model is primarily due to a reduction in lung neutrophil recruitment. Although circulating neutrophils were modestly reduced (blood leukocytes were 53% neutrophils in vehicle vs. 43% in DT, a 9% reduction), lung neutrophils were reduced from 36% in vehicle to 12% in DT, a 67% reduction. Neutrophils are only 5% of lung leukocytes. Since lung CXCL1, a major chemoattractant for neutrophils, was also reduced in the CD11b-DTR model, these data suggest that lung neutrophil recruitment was inhibited and that the reduction in blood or systemic neutrophils per se had less of an effect.

To specifically examine the role of alveolar macrophages in AKI-mediated lung injury, we depleted alveolar macrophages utilizing IT administration of LEC. Intratracheally administered LEC is a well-established method of alveolar macrophage depletion that specifically eliminates alveolar macrophages, but not interstitial lung macrophages or systemic mononuclear phagocytes (42). Indeed, in our study, IT LEC in the setting of ischemic AKI reduced alveolar macrophages, but not systemic or lung interstitial mononuclear phagocytes, and both renal function and serum IL-6 were similar to IT vehicle injection. As expected, lung inflammation and CXCL1 production were both reduced. It is interesting to note that systemic and lung interstitial mononuclear phagocyte depletion (CD11b-DTR model) had a similar effect on lung CXCL1 and lung neutrophils as alveolar macrophage depletion (IT LEC model), suggesting similar and redundant roles of these mononuclear phagocyte populations in lung inflammation.

An unexpected finding of our study was that despite a reduction in lung CXCL1 and lung MPO activity, lung capillary leak was increased in AKI with both models of macrophage depletion (CD11b-DTR and IT LEC). In models of direct lung injury (e.g., ventilator-induced lung injury or IT instillation of endotoxin), many (17, 27), but not all (4, 33, 42), studies have demonstrated that alveolar macrophage depletion results in improved inflammation as well as improved lung capillary leak. The role of systemic and alveolar macrophages in indirect models of lung injury is less well-studied and the protective vs. harmful role of systemic and alveolar macrophages remains unresolved; in general however, lung inflammation and lung capillary leak trend together. Thus, although the inflammatory pathway regarding lung injury after AKI may be similar to other experimental models of lung injury, AKI may have a unique effect on lung capillary leak. Our data suggest that both systemic (circulating and lung interstitial) and alveolar macrophages play a protective role in maintenance of alveolar membrane integrity to prevent lung capillary leak in AKI and that further exploration of this protective factor is warranted.

In summary, our data demonstrate that systemic and alveolar macrophages play a role in AKI-mediated lung inflammation via CXCL1 production and neutrophil infiltration. Although animal and patient data are accumulating to suggest that AKI may lead to ALI, currently, AKI is not generally considered to be a cause of ALI. In the present study, we demonstrate that AKI initiates a similar paradigm of injury mechanisms that
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REFERENCES


