Acute Renal Failure after Bilateral Nephrectomy Is Associated with Cytokine-Mediated Pulmonary Injury

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Clinical studies demonstrate that acute renal failure (ARF) is associated with increased mortality, which may be due to pulmonary complications. ARF may affect the lung *via* increased renal production or impaired clearance of mediators of lung injury, such as proinflammatory cytokines. Bilateral nephrectomy is a method to examine directly the deleterious systemic effects of absent renal clearance in ARF without the confounding effects that are associated with ischemia-reperfusion injury (*e.g.*, ischemic ARF) or systemic toxicity (*e.g.*, cisplatin-induced ARF). This study contrasts the effects of ischemic ARF and bilateral nephrectomy on serum cytokines and lung injury. It demonstrates that the acute absence of kidney function after both ischemic ARF and bilateral nephrectomy is associated with an increase in multiple serum cytokines, including IL-6 and IL-1β, and that the cytokine profiles were distinct. Lung injury after ischemic ARF and bilateral nephrectomy was similar and was characterized by pulmonary vascular congestion and neutrophil infiltration. For investigation of the role of proinflammatory cytokines in pulmonary injury after ARF, the anti-inflammatory cytokine IL-10 was administered before bilateral nephrectomy. IL-10 treatment improved pulmonary architecture and was associated with a reduction in inflammatory markers, including bronchoalveolar lavage fluid total protein, pulmonary myeloperoxidase activity (a biochemical marker of neutrophils), and the chemokine macrophage inflammatory protein 2. These data demonstrate for the first time that the acute absence of kidney function results in pulmonary injury independent of renal ischemia and highlight the critical role of the kidney in the maintenance of serum cytokine balance and pulmonary homeostasis.

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Acute renal failure (ARF) is a common complication that occurs in approximately 5% of hospitalized patients and up to 30% of patients in intensive care units (1). When controlling for severity of illness, the development of ARF confers an independent, excess risk for mortality (2–6) regardless of whether the ARF is mild (2,3,5) or requires renal replacement therapy (7,8). For example, ARF from contrast nephropathy confers a 5.5-fold increase in mortality (2), whereas the mortality of critically ill patients with ARF can approach 90% (9). In addition, the acuity of renal failure is important: Critically ill patients who have ARF that does not require dialysis have a higher mortality than critically ill patients who have ESRD and are on dialysis (10). These and other well-controlled studies have shattered the previously held notion that patients die with ARF, not from ARF.

Currently, no specific therapy improves patient outcome in ARF. Pulmonary complications, particularly acute lung injury

(ALI) and respiratory failure, commonly are associated with ARF and may contribute to mortality (11). The current paradigm suggests that lung injury in ARF predominantly is a consequence of impaired fluid excretion that causes volume overload, increased capillary hydrostatic pressure, and pulmonary edema (12,13). Alternatively, increased renal production, reduced clearance, or impaired metabolism of mediators of lung injury may occur in ARF. Cytokines such as IL-1β and TNF-α are known mediators of pulmonary injury in other settings (14,15). The role of the kidney in affecting cytokines and other potential mediators of lung injury in ARF is relatively unexplored.

Pulmonary injury has been examined after *ischemic* ARF and is characterized by noncardiogenic edema and neutrophil infiltration (16,17). Because ischemia accounts for only half of ARF that is seen in hospitalized patients, data that have been accumulated in ischemic ARF models cannot be generalized to all patients with ARF. In addition, it is widely known that remote ischemia-reperfusion injury (*e.g.*, liver, gut, hind limb) causes pulmonary injury (18–22). Thus, the contribution of renal failure itself on lung injury may not be determined specifically in models of ischemic ARF. In this regard, bilateral nephrectomy is a model of ARF that may be used to examine the deleterious systemic effects of ARF without the confounding effects of

ischemia-reperfusion injury or systemic toxicity (*e.g.*, cisplatin-induced ARF).

In this study, we sought to dissociate the effects of renal ischemia from renal failure to determine the unique role of the kidney and ARF in influencing serum cytokines and pulmonary homeostasis. To this end, we developed models of renal ischemia without ARF (unilateral renal ischemia), renal ischemia with ARF (bilateral ischemia), and ARF without renal ischemia (bilateral nephrectomy). We examined a time course of potential mediators of lung injury after unilateral ischemia, ischemic ARF, and bilateral nephrectomy and found that multiple serum cytokines were increased after ischemic ARF and bilateral nephrectomy. Using these data, we examined the efficacy of a potential intervention, IL-10 administration, to alter lung injury in ARF. Our results demonstrate that the kidney is a key regulator of systemic cytokines that mediate pulmonary injury in ARF.

Materials and Methods

Animals

Eight- to 10-wk-old C57BL/6 mice (Jackson Laboratories, Bar Harbor, ME) that weighed 20 to 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 Health Sciences Center.

Surgical Protocol

Surgical procedures were performed on four groups of mice: (*1*) Sham operation, (*2*) unilateral renal ischemia, (*3*) ischemic ARF, and (*4*) bilateral nephrectomy. For all procedures, mice were anesthetized with IP Avertin (2,2,2-tribromoethanol; Aldrich, Milwaukee, WI), a midline incision was made, and the renal pedicles were identified. In the ischemic ARF group, both renal pedicles were clamped for 22 min (23,24). In the unilateral ischemia model, the left renal pedicle was clamped for 22 min. After clamp removal, kidneys were observed for restoration of blood flow by the return to their original color. The abdomen was closed in one layer. Sham surgery consisted of the same procedure except that clamps were not applied. In the bilateral nephrectomy model, both renal pedicles were tied off with suture, then cut distal to the suture. The ureters were pinched off with forceps, and the kidneys were removed. Mice were weighed before and after procedures.

Collection and Preparation of Serum Samples

When the mice were killed, blood was obtained *via* cardiac puncture 1, 2, 4, 8, and 24 h after the procedure. For establishment of baseline cytokine values, blood was collected from mice that did not have any type of surgical procedure.

For ensuring uniformity, all samples were processed identically. Blood was allowed to clot at room temperature for 2 h, then centrifuged at $3000 \times g$ for 10 min. Serum was collected and centrifuged a second time at $3000 \times g$ for 1 min to ensure elimination of red blood cells. Samples with notable hemolysis were discarded.

Blood Urea Nitrogen and Serum Creatinine Measurement

Blood urea nitrogen (BUN) and serum creatinine were measured using a BUN and a creatinine autoanalyzer (Beckman Instruments, Fullerton, CA).

Flow Cytometry Determination for Serum Cytokines

Serum IL-1α, IL-2, IL-4, IFN-γ, macrophage inflammatory protein (MIP)-1α, TNF-α, IL-1β, IL-3, IL-17, IL-5, G-CSF, GM-CSF, IL-12 (p40), IL-12 (p70), IL-10, IL-6, and keratinocyte-derived chemokine (KC) were measured using a bead-based multiplex cytokine kit (Bio-Rad, Hercules, CA) in conjunction with flow-based protein detection and the Luminex LabMAP multiplex system (Luminex, Austin, TX) according to the manufacturer's directions. The detection limit for each cytokine was 1.95 pg/ml.

PCR and Tissue Processing for KC

Kidneys were collected after sham operation and unilateral ischemia (ischemic kidney) 1 h after the procedure and stored at −80°C until use. RNA was isolated using TRIzol reagent (Life Technologies BRL, Grand Island, NY); cDNA was generated from 1 μg of RNA using MuLV Reverse Transcriptase (Applied Biosystems, Foster City, CA). Primers were designed using Beacon Designer software (PREMIER Biosoft International, Palo Alto, CA), and quantitative real-time PCR was performed (forward primer 5'-GGC TGG GAT TCA CCT CAA GAA C-3', reverse primer 5'-TGT GGC TAT GAC TTC GGT TTG G-3'). Samples were prepared using iQ Sybergreen Supermix (Bio-Rad) and the iCycler iQ detection system (Bio-Rad). Reactions were performed in triplicate. Expression data were normalized to cyclophilin (forward primer 5'-TGG AGA GCA CCA AGA CAG ACA-3', reverse primer 5'-TGC CGG AGT CGA CAA TGA T-3').

One half of the kidney was homogenized in 500 μl of cell lysis buffer (Bio-Rad) that contained 0.4% 500 mM PMSF and 1% protease inhibitor cocktail (Sigma, St. Louis, MO). Samples were sonicated (model VC500; Sonics & Materials, Danbury, CT) on ice using five 5-s pulses then centrifuged at $4500 \times g$ for 15 min at 4°C. Supernatants were analyzed for protein content using a Bio-Rad DC protein assay kit with BSA as standards. KC was determined on the supernatant using a bead-based single-plex cytokine kit for KC (Bio-Rad) as described previously for serum samples.

Serum and Urine IL-6

Mice received 250 ng of recombinant murine IL-6 (R&D Systems, Minneapolis, MN) in 100 μl of sterile saline or vehicle (saline) by tail-vein injection. At 1 h, blood was obtained by cardiac puncture and urine was collected *via* bladder aspiration. Baseline serum and urine samples were obtained. IL-6 was determined by ELISA (R&D Systems). The detection limit is 1.3 pg/ml.

Tissue Processing of Lungs for Histology and Morphologic Assessment

After bronchoalveolar lavage (BAL), 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 cmH$_2$O, allowing for homogeneous expansion of lung parenchyma as described previously (25). The lung was removed, fixed in 10% formalin, and paraffin-embedded. Five-micrometer sections then were stained with either hematoxylin and eosin or trichrome according to standard protocols. Morphologic evaluation was performed in a blinded manner.

MIP-2

Frozen lung was homogenized on ice in 500 μl of PBS that contained 1% protease inhibitor cocktail (Sigma, St. Louis, MO). A total of 125 μl of 5% triton X in PBS was added, and samples were vortexed, frozen and thawed, and incubated on ice for 20 min. Samples were centrifuged at 4°C at $14,000 \times g$ for 15 min. Protein content and MIP-2 were

determined on the supernatant. MIP-2 was determined by ELISA (R&D Systems); the detection limit is 7.8 to 500 pg/ml.

BAL

After pentobarbital anesthesia, the trachea was dissected and cannulated with a 20-G catheter. Lungs were lavaged with 1 ml of PBS twice. Samples with <80% return were discarded. BAL fluid was centrifuged at $14,000 \times g$ and 4°C for 5 min, and protein content was determined on the supernatant.

Myeloperoxidase Activity

One fourth of lung was homogenized in 1 ml of cold hexdecyltrimethylammonium bromide buffer (50 mM KPO_4 and 0.5% hexdecyltrimethylammonium bromide [pH 6.0]), sonicated on ice for 10 s, and centrifuged at $14,000 \times g$ for 30 min at 4°C. Twenty microliters of supernatant was 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 KPO_4 buffer + 0.0005% H_2O_2) was added immediately before the optical density was read at 450 nm and again 30 s later (Benchmark microplate reader; BioRad).

IL-10 Administration

Mice received 1 μg of recombinant murine IL-10 (Peprotech, Rocky Hill, NJ) in 100 μl of sterile saline or vehicle (saline) *via* tail-vein injection after anesthesia before bilateral nephrectomy.

Statistical Analyses

All values are expressed as mean ± SE. Data were analyzed by one-way ANOVA comparing the four conditions (sham operation, unilateral ischemia, bilateral ischemia [ischemic ARF], and bilateral nephrectomy) at each time point. For example, the 1-h values for sham operation, unilateral ischemia, ischemic ARF, and bilateral nephrec-

tomy were compared with each other. When significant F statistic from ANOVA existed, this test was followed by Dunnett *post hoc* multiple comparison procedure with sham operation as the control group. Dunnett is the multiple comparison procedure of choice in this setting to account for multiple group comparisons. $P < 0.05$ was considered statistically significant.

Results

Survival, Time Course of ARF, and Weight

All mice survived until they were killed. Serum creatinine and BUN were determined at baseline and at 1, 2, 4, 8, and 24 h after sham operation, unilateral ischemia, ischemic ARF, and bilateral nephrectomy. Serum creatinine and BUN did not change after sham operation or unilateral ischemia. Remarkably, serum creatinine and BUN increased at the same rate after ischemic ARF and bilateral nephrectomy (Figure 1, A and B).

For assessment volume status, weight was determined before and 24 h after procedure (Figure 1C). All experimental groups lost weight, suggesting that these mice were not volume overloaded. Combined two-kidney weight was 0.27 ± 0.01 g ($n = 11$), accounting for approximately 1% of total body weight; therefore, kidney removal accounts for only a portion of the 6% weight loss after bilateral nephrectomy.

Serum Cytokines after Sham Operation

Serum IL-1α, IL-2, IL-4, IFN-γ, MIP-1α, TNF-α, IL-1β, IL-3, IL-17, IL-5, G-CSF, GM-CSF, IL-12 (p40), IL-12 (p70), IL-10, IL-6, and KC were measured at baseline and at 1, 2, 4, 8, and 24 h after sham operation ($n = 3$ to 10 for each time point and condition). Nine cytokines increased after sham operation com-
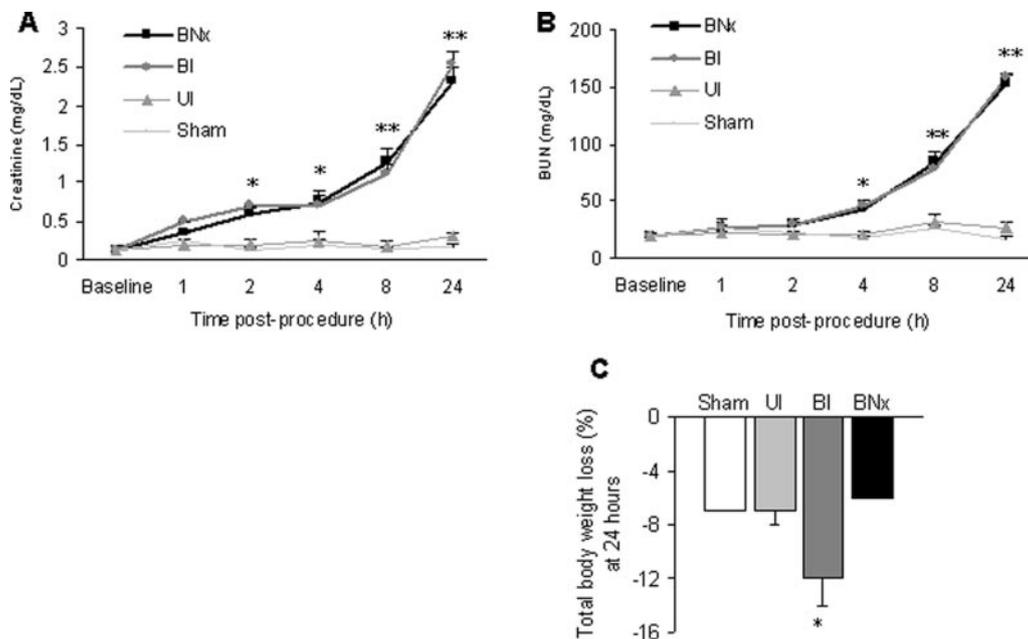

Figure 1. Renal failure and weight loss. (A and B) Serum creatinine (mg/dl; A) and blood urea nitrogen (BUN; mg/dl; B) were determined at baseline and 1, 2, 4, 8, and 24 h after sham operation (sham), unilateral ischemia (UI), ischemic acute renal failure (ARF; bilateral ischemia [BI]), and bilateral nephrectomy (BNx). *$P < 0.01$ for BI and BNx *versus* sham; **$P < 0.001$ for BI and BNx *versus* sham ($n = 3$ to 11). (C) Percentage total body weight loss was determined at 24 h for Sham, UI, BI, and BNx. *$P < 0.05$ *versus* Sham ($n = 3$ to 10). All groups of mice lost weight after surgery.

pared with baseline: KC, GM-CSF, IL-6, IL-10, G-CSF, IL-5, IL-17, IFN-γ, and MIP-1α (Figure 2). Because cytokines increased after sham operation, cytokine values after unilateral ischemia, ischemic ARF, and bilateral nephrectomy were compared with sham operation at the corresponding time point.

Serum Cytokines after Unilateral Ischemia

KC was the only cytokine of the 17 measured that increased significantly after unilateral ischemia at any time point; it increased 1 h after unilateral ischemia (Figure 3A).

Renal KC 1 H after Unilateral Ischemia

For determination of whether the increase in serum KC 1 h after unilateral ischemia was due to increased kidney production, mRNA and protein were determined in ischemic kidneys 1 h after unilateral ischemia; KC mRNA and protein increased after unilateral ischemia (Figure 3, B and C).

Serum Cytokines after Ischemic ARF and Bilateral Nephrectomy

Two cytokines, KC and GM-CSF, increased significantly after ischemic ARF but not bilateral nephrectomy; the increase in KC after bilateral nephrectomy did not reach statistical significance (Figure 4A and B). Three cytokines, IL-6, IL-1β, and IL-12 (p40), increased significantly after both ischemic ARF and bilateral nephrectomy (Figure 3, C through E). Two cytokines, IL-10 and G-CSF, increased significantly after bilateral nephrectomy but not ischemic ARF (Figure 3, F and G). TNF-α, IL-17, IL-1α, IL-2,

IL-4, IFN-γ, MIP-1α, IL-3, IL-17, IL-5, and IL-12 (p70) did not increase significantly after either ischemic ARF or bilateral nephrectomy (data not shown).

Urine IL-6 after Intravenous IL-6 Administration

Serum and urine IL-6 were measured at baseline and 1 h after vehicle or IL-6 injection. At baseline, IL-6 was undetectable in both serum and urine. IL-6 increased in the serum and urine 1 h after both vehicle and IL-6 injection (Figure 5). These data suggest that the kidney plays a role in the filtration and excretion of IL-6 that is either endogenously produced (vehicle treated) or exogenously administered (IL-6 treated). Serum creatinine was normal in vehicle- and IL-6–treated mice (data not shown).

Pulmonary Histology 24 H after Sham Operation, Unilateral Ischemia, Ischemic ARF, and Bilateral Nephrectomy

Normal alveolar structure was present after sham operation and unilateral ischemia. In contrast, lung histology after ischemic ARF or bilateral nephrectomy was grossly abnormal and characterized by septal edema, hemorrhage, and inflammation. Neutrophils were noted in the interstitium and were found occasionally in the alveolar space. In addition, marked pleural abnormalities, characterized by an inflammatory infiltrate and thickening, were noted (Figure 6).
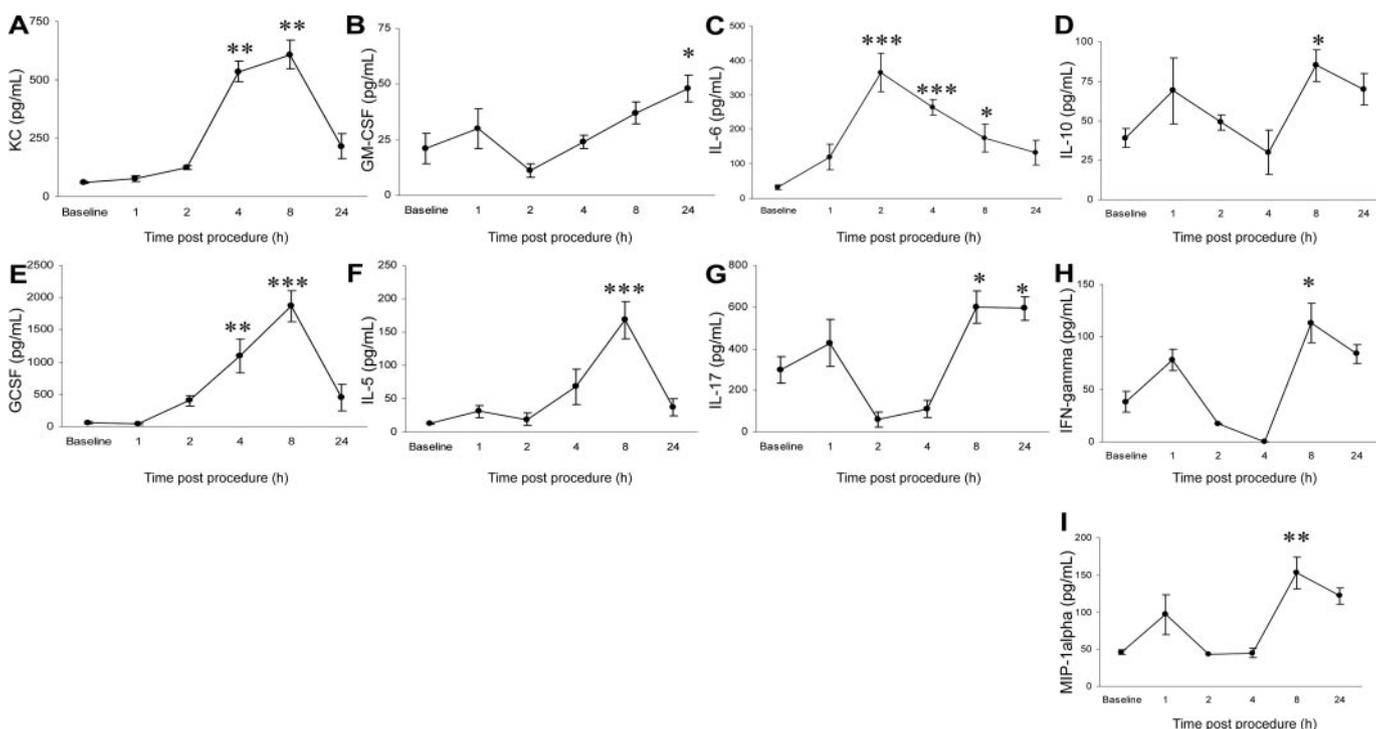

Figure 2. Serum cytokines after sham operation. Serum concentrations (pg/ml) of cytokines were determined at baseline and 1, 2, 4, 8, and 24 h after sham operation as described in Materials and Methods. Cytokines that increased after sham operation relative to baseline levels were keratinocyte-derived chemokine (KC) (A), GM-CSF (B), IL-6 (C), IL-10 (D), G-CSF (E), IL-5 (F), IL-1 (G)7, IFN-γ (H), and macrophage inflammatory protein (MIP)-1α (I). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ *versus* baseline ($n = 3$ to 10).
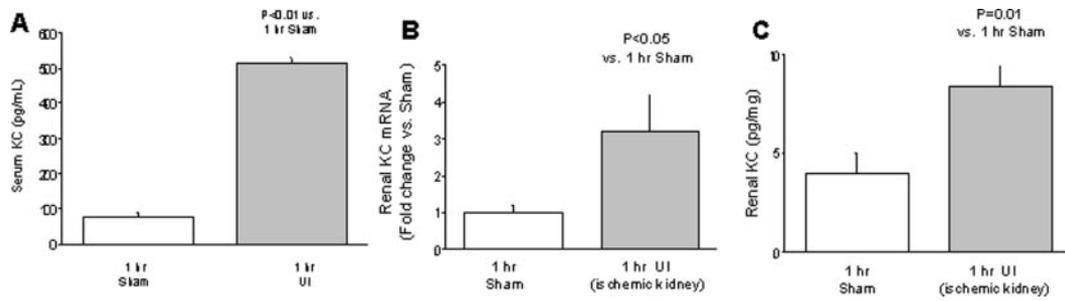
Figure 3. Serum and renal KC after unilateral ischemia. Serum concentrations (pg/ml) of cytokines were determined at baseline and 1, 2, 4, 8, and 24 h after Sham and UI as described in Materials and Methods. (A) Serum KC increased 1 h after UI. $P < 0.01$ *versus* Sham ($n = 6$ to 8). (B) Renal mRNA for KC was determined 1 h after Sham and UI (ischemic kidney). $P < 0.05$ *versus* Sham ($n = 4$). (C) Renal KC protein was determined 1 h after Sham and UI (ischemic kidney). $P = 0.01$ *versus* Sham ($n = 4$ to 5).
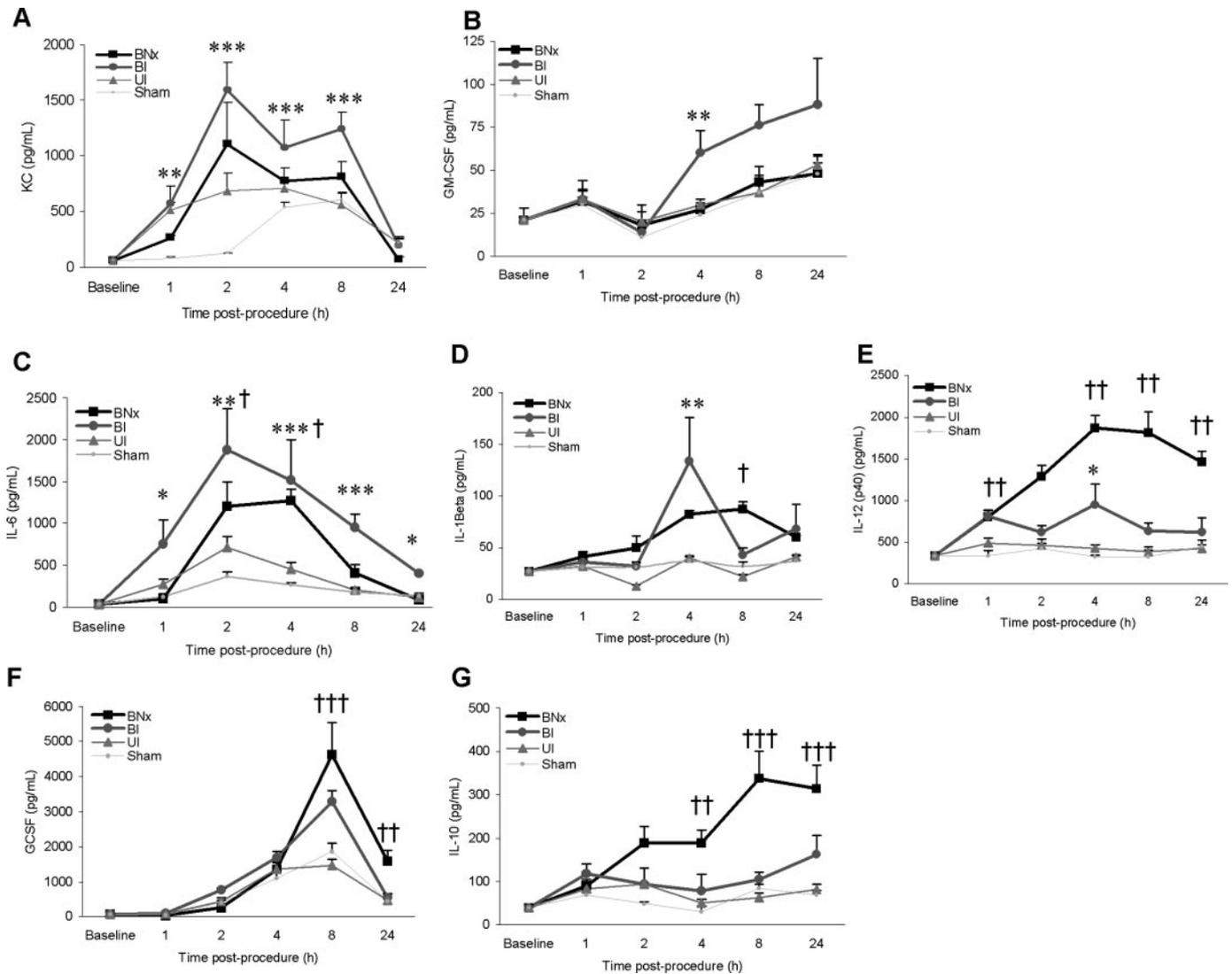

Figure 4. Serum cytokines after ischemic ARF (bilateral ischemia) and bilateral nephrectomy. Eighteen serum cytokines were measured at baseline and 1, 2, 4, 8, and 24 h after Sham, UI, BI, and BNx. Serum cytokines with significant differences after ischemic ARF or bilateral nephrectomy are shown; data points for Sham and UI are shown for the purpose of comparison. KC (A) and GM-CSF (B) increased after ischemic ARF but not bilateral nephrectomy; IL-6 (C), IL-1β (D), and IL-12 (p40) (E) increased after both ischemic ARF and bilateral nephrectomy; G-CSF (F) and IL-10 (G) increased after bilateral nephrectomy but not ischemic ARF. $*P < 0.05$ for BI *versus* Sham; $**P < 0.01$ for BI *versus* Sham; $***P < 0.001$ BI *versus* Sham; $^{†}P < 0.05$ for BNx *versus* Sham; $^{††}P < 0.01$ for BNx *versus* Sham; $^{†††}P < 0.001$ for BNx *versus* Sham ($n = 3$ to 11).
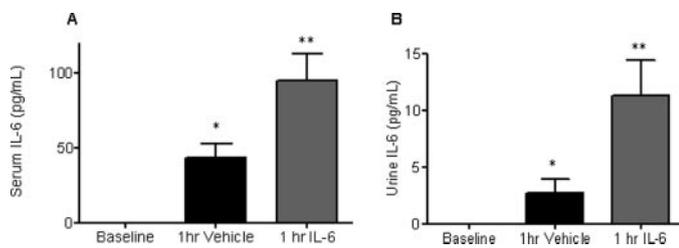
Figure 5. Serum and urine IL-6 were determined at baseline and 1 h after intravenous administration of vehicle (1 hr Vehicle) or IL-6 (1 hr IL-6). (A) Serum IL-6 (pg/ml) was 0 ± 0 at baseline, 80 ± 8 1 h after vehicle administration (*$P < 0.05$ *versus* baseline; $n = 6$ to 12), and 127 ± 31 at 1 h after IL-6 administration (**$P < 0.01$ *versus* baseline and 1 hr Vehicle; $n = 6$ to 12). (B) Urine IL-6 (pg/ml) was 0 ± 0 at baseline, 3 ± 1 at 1 h after vehicle administration (*$P < 0.05$ *versus* baseline; $n = 6$ to 12), and 11 ± 3 at 1 h after IL-6 administration (**$P < 0.01$ *versus* baseline and 1 hr Vehicle; $n = 6$ to 12).

Effect of IL-10 on Pulmonary Injury after Bilateral Nephrectomy

We hypothesized that the excess proinflammatory cytokines that were identified after bilateral nephrectomy may mediate pulmonary injury through the upregulation of chemokine production and subsequent neutrophil infiltration. To test this, we treated mice with the anti-inflammatory cytokine IL-10, which inhibits production and action of numerous proinflammatory cytokines (26). Pulmonary architecture in the IL-10–treated mice was improved with reduced septal edema, congestion, and neutrophil infiltration (Figure 7, A and B). For further characterization of lung injury after ARF from bilateral nephrectomy and evaluation of the protective effects of IL-10, total BAL fluid protein was determined and was increased after bilateral nephrectomy and reduced with IL-10 treatment (Figure 7C). We next examined the effect of IL-10 on neutrophil accumulation and found that pulmonary myeloperoxidase, a biochemical parameter of neutrophil infiltration, was reduced in the IL-10–treated mice (Figure 7D). Finally, we examined pulmonary MIP-2, a neutrophil chemoattractant, which was increased after bilateral nephrectomy and reduced by IL-10 treatment (Figure 7E).

Discussion

In this study, we sought to evaluate the systemic consequences of ARF regarding serum cytokines and pulmonary injury. We found that ARF after ischemia and bilateral nephrectomy had unique cytokine profiles, and both were associated with pulmonary injury. Furthermore, we demonstrate that pulmonary injury after ARF from bilateral nephrectomy can be ameliorated with anticytokine treatment. Our data have significant implications regarding the deleterious systemic effects that are associated with ARF as well as the complex role of the kidney in affecting systemic cytokines and lung tissue homeostasis.

Before analyzing the effect of ischemia and ARF on systemic cytokines, we first examined the effect of sham operation. Notably, of the 17 cytokines examined, KC, GM-CSF, IL-6, IL-10, G-CSF, IL-5, IL-17, IFN-γ, and MIP-1α increased after sham

operation compared with baseline. Cytokine production after surgical procedures is a well-described phenomenon in patients that has been linked to postsurgical multisystem organ failure and mortality (27,28). Abdominal surgery, in particular, is associated with increased serum IL-6, G-CSF (27), and IL-10 (28). Cytokine production in this setting is a transient phenomenon that is thought to occur by activation of neutrophils, monocytes, and fibroblasts in the operative field (27). To our knowledge, IL-5, IL-17, and MIP-α have not been shown previously to increase after surgical procedures. Because of the increase in serum cytokines after sham operation, cytokine values after unilateral ischemia, ischemic ARF, and bilateral nephrectomy were analyzed relative to sham operation at the corresponding time point.

We next examined the effect of unilateral ischemia on serum cytokines. Only KC increased after unilateral ischemia, and it increased at only 1 h after ischemic reperfusion. The mouse chemokine KC shares 70% homology with the human CXC chemokine IL-8. For determination of whether the increase in KC was due to increased renal production, renal mRNA and protein for KC were measured in the ischemic kidney 1 h after unilateral ischemia and were increased. These results complement previous observations regarding KC in the serum and kidney after ischemic ARF (29,30) as well as ischemia-reperfusion injury of other organs.

We next examined the effect of ischemic ARF on serum cytokines and found that serum KC also increased after ischemic ARF. The sustained increase, at 1, 2, 4, and 8 h, suggests that the increase may be due to renal KC production coupled with impaired renal elimination from bilateral renal dysfunction. In this regard, the role of the kidney in the metabolism of a CXC chemokine was determined recently (31). Other cytokines that increased after ischemic ARF were GM-CSF, IL-6, IL-1β, and IL-12 (p40). Renal production may explain their increase, because increased renal production of these cytokines (mRNA or protein) in ischemic ARF has been reported (32–35). The lack of an increase in these cytokines after unilateral ischemia may be because the extent of ischemia was insufficient to affect serum levels appreciably; alternatively, elimination by the normally functioning contralateral kidney may have occurred.

Finally, we examined the effect of ARF after bilateral nephrectomy and found that serum IL-6, IL-1β, IL-12 (p40), G-CSF, and IL-10 were increased. IL-6, IL-1β, IL-12, and G-CSF are proinflammatory, and IL-10 is anti-inflammatory. Increased extrarenal production coupled with absent renal elimination is a likely explanation for the increased levels of cytokines that are seen after bilateral nephrectomy. Extrarenal cytokine production after bilateral nephrectomy likely occurred as a result of the surgical procedure itself, as demonstrated by the increase in multiple cytokines after sham operation. Extrarenal cytokine production is known to be stimulated by a variety of insults, such as trauma and sepsis. Even mild insults, such as restraint, may cause an increase in cytokine production (36); this stress response explains the increase in IL-6 that we observed after tail-vein injection of vehicle (Figure 5). Uremia is another known stimulus of cytokine production; for example, stimu-
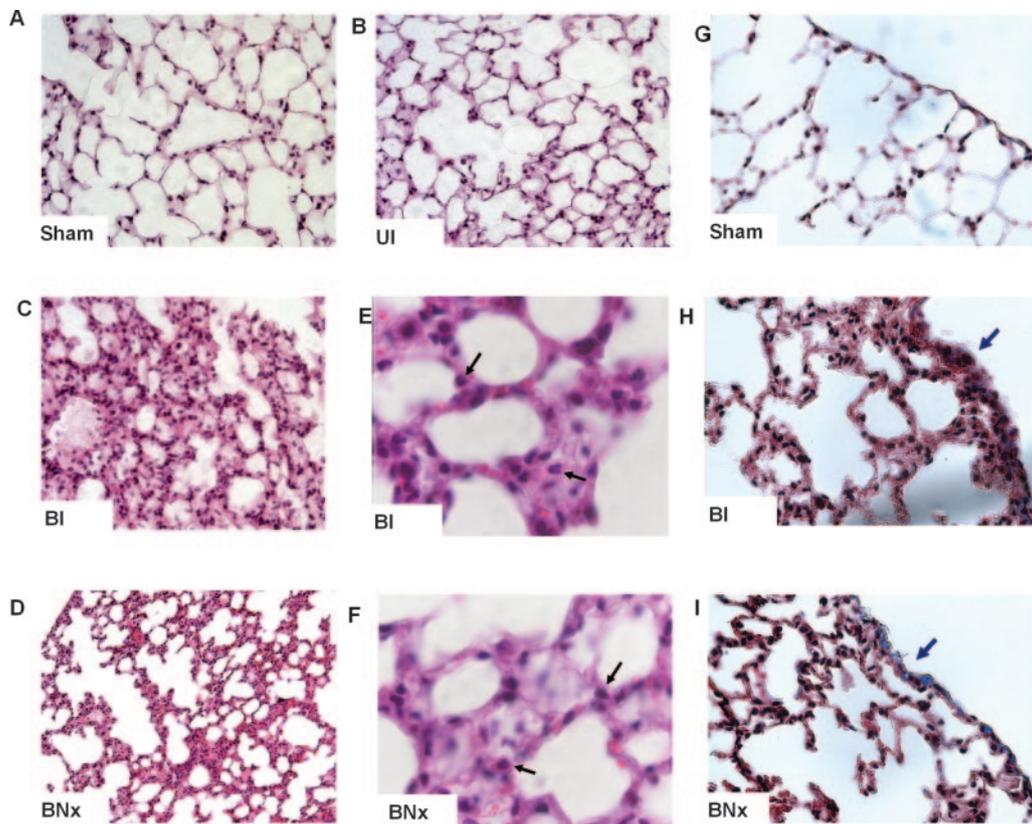
Figure 6. Pulmonary characteristics after Sham, UI, BI, and BNx. (A through D) Lung histopathology 24 h after Sham (A), UI (B), BI (C), and BNx (D) is shown. Appearance after Sham and UI is normal; appearance after BI and BNx is characterized by engorged septae, hemorrhage, and pockets of inflammation in the interstitium. (E and F) Lung histopathology for neutrophils 24 h after BI (E) and BNx (F) demonstrates neutrophils in the interstitium (black arrows). Pleural thickness is normal after Sham (G); however, marked pleural thickening (blue arrows) is demonstrated after BI (H) and BNx (I). All figures are representative images of at least three separate experiments. Magnifications: ×40 in A through D (hematoxylin and eosin) and G through I (Trichrome staining); ×100 in E and F.

lated monocytes from patients with ESRD produce excess IL-10 (37). The role of the kidney in the elimination of numerous cytokines (*e.g.*, IL-6 [38], IL-1β [39], G-CSF [40], IL-10 [41]) has been suggested. Available evidence suggests that the primary mechanism by which cytokines are eliminated by the kidney is *via* proximal tubule metabolism (31). To examine the role of filtration and excretion, we examined the urinary fate of IL-6 that was injected into normal mice. We found that IL-6 increased significantly in the urine after injection, suggesting that filtration and excretion also may play a role in renal cytokine elimination. The return of some cytokines to baseline levels 24 h after bilateral nephrectomy suggests that nonrenal mechanisms of cytokine clearance also were active.

The increased cytokines after ischemic ARF (IL-6, KC, GM-CSF, IL-1β, and IL-12[p40]) and bilateral nephrectomy (IL-6, IL-1β, IL-12, G-CSF, and IL-10) illustrate the previously unappreciated role of normal kidney function in the acute regulation of these cytokines. It is interesting to note that the cytokine profiles after ischemic ARF and bilateral nephrectomy are distinct, even though the development of uremia, as judged by creatinine and BUN, occurred at the same rate. In patients, it may be postulated that ischemic ARF has a different cytokine profile compared with ARF from other causes; this may have

important clinical implications in regard to the development of biomarkers of ARF as well as the development of interventions for the systemic consequences of ARF. The clinical relevance of our findings is highlighted by the fact that serum IL-8 (KC), IL-10, and IL-6 are increased in patients with ARF and predict mortality (9). We observed that KC increased after ischemic ARF, IL-10 increased after bilateral nephrectomy, and IL-6 increased after both ischemic ARF and bilateral nephrectomy. The systemic effects of an altered cytokine milieu has been proposed as a mechanism to explain the increased mortality in patients with ARF (9,11): Proinflammatory cytokines such as IL-6 mediate organ dysfunction, whereas anti-inflammatory cytokines, such as IL-10, predispose to infections.

Because excess cytokine production and impaired cytokine elimination may have deleterious systemic effects in ARF, we examined end points of lung injury. Pulmonary histology 24 h after sham operation and unilateral ischemia was normal. In contrast, lung histology after both ischemic ARF and bilateral nephrectomy was characterized by septal edema, leukocyte infiltration, hemorrhage, and marked pleural abnormalities. The histologic appearance of the lungs after ischemic ARF is similar to that reported by others (16,17). Assessment of fluid status by pre- and postsurgical total body weight revealed that
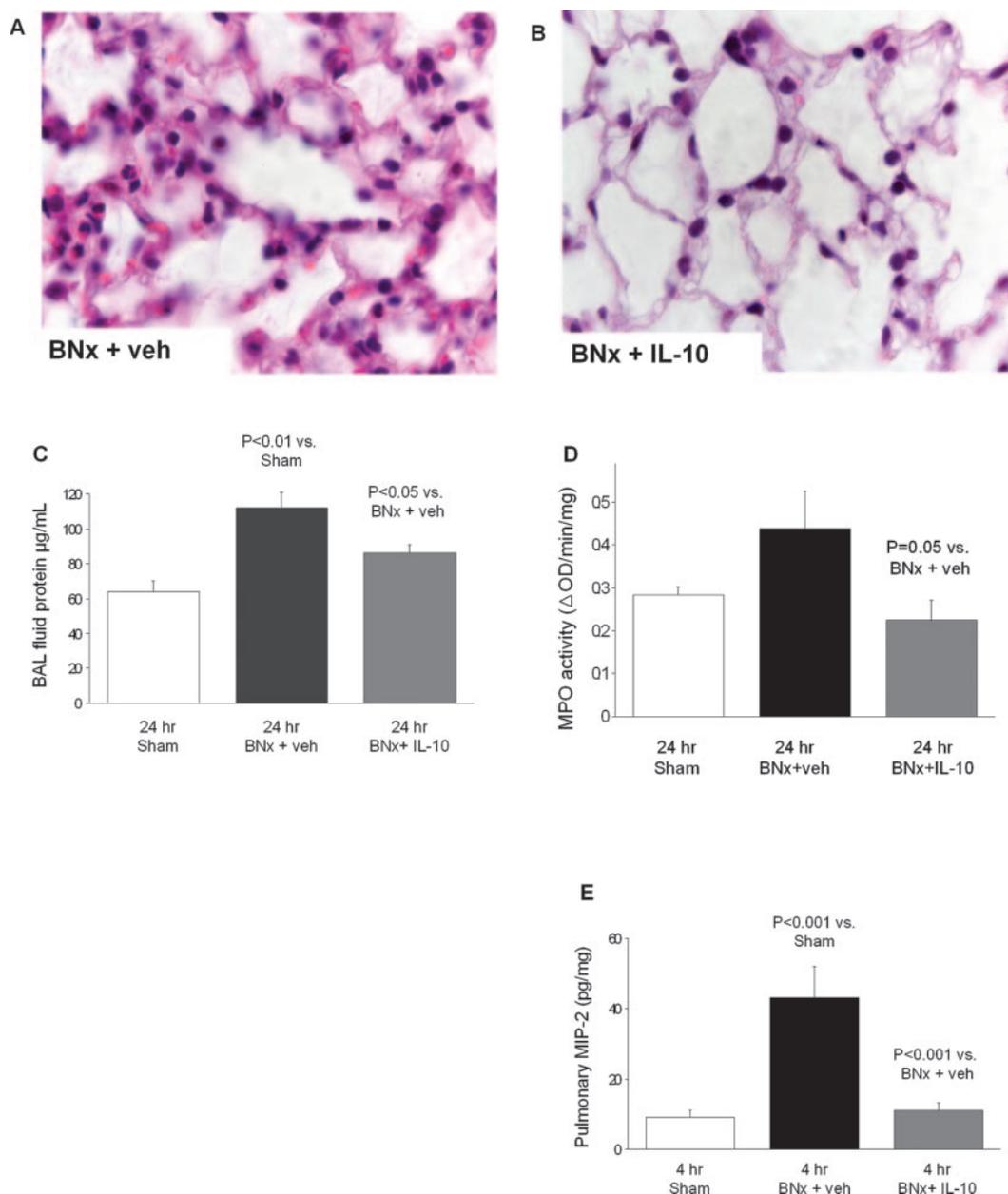
Figure 7. Administration of IL-10 ameliorates lung injury after BNx. A total of 1 μg of IL-10 or vehicle (veh) was administered before BNx. Pulmonary histology from vehicle-treated (A) and IL-10–treated (B) mice 24 h after BNx is shown. Pulmonary histology in the IL-10–treated mice was improved with reduced septal edema, hemorrhagic congestion, and neutrophil infiltration. Images are representative of at least three separate experiments. (C) Total bronchoalveolar lavage (BAL) fluid, which increases in inflammatory conditions, was increased after bilateral nephrectomy and reduced with IL-10 treatment ($n = 6$ to 10). (D) Pulmonary myeloperoxidase (MPO) activity, a biochemical marker of neutrophil infiltration, was reduced 24 h after BNx in IL-10–treated compared to vehicle-treated mice ($n = 6$ to 10). (E) Pulmonary MIP-2 increased 4 h after BNx and was reduced in the IL-10–treated mice ($n = 6$ to 10). Magnification, ×100 (hematoxylin and eosin).

all experimental groups lost weight, suggesting that volume overload that causes increased hydrostatic pressure and pulmonary edema is unlikely to be the primary mechanism of lung injury. To characterize further lung injury after bilateral nephrectomy, we examined total BAL fluid protein. Total BAL fluid protein typically increases in inflammatory conditions as a result of the loss of capillary membrane integrity and was increased after bilateral nephrectomy. Together, these data demonstrate that a component of lung injury after ARF is due to noncardiogenic pulmonary edema.

Studies of numerous models of systemic injury, such as sepsis and organ ischemia-reperfusion injury, suggest that proinflammatory cytokines mediate remote pulmonary injury *via* the upregulation of pulmonary adhesion molecules and chemokines, causing neutrophil infiltration and subsequent tissue damage (20,22). In fact, injection of TNF-α directly mediates

lung injury that is characterized by increased permeability, edema, and trapping of neutrophils in the microvasculature (14). We hypothesized that excess proinflammatory cytokines that are identified after ARF from bilateral nephrectomy may mediate pulmonary injury by a similar mechanism. Therefore, we examined the effect of the anti-inflammatory cytokine IL-10 on lung injury after ARF from bilateral nephrectomy. IL-10 inhibits both production and action of numerous proinflammatory cytokines, such as IL-1β and IL-6, both of which were elevated after bilateral nephrectomy (26). IL-10 improved histologic appearance and reduced BAL fluid protein, pulmonary MIP-2, and pulmonary myeloperoxidase activity. MIP-2 is a neutrophil chemoattractant and known mediator of lung injury (22). Together, these data suggest that ARF, even in the absence of renal ischemia, leads to pulmonary inflammation and subsequent tissue injury *via* chemokine upregulation and neutrophil infiltration.

Conclusion

Our data suggest that lung injury after ARF from either ischemia or bilateral nephrectomy is characterized by noncardiogenic pulmonary edema and inflammation. Furthermore, our data demonstrate that the kidney plays an important role in the production and elimination of mediators of pulmonary injury and that prolonged exposure to these mediators contributes to pulmonary injury. Our data highlight the critical role of the kidney in the regulation of systemic cytokines and pulmonary homeostasis. Further exploration of the mediators and mechanisms of the deleterious pulmonary effects of ARF may lead to interventions to improve the mortality that is seen in patients with this common, life-threatening complication.

Disclosures

None.

References

1. Star RA: Treatment of acute renal failure. *Kidney Int* 54: 1817–1831, 1998
2. Levy EM, Viscoli CM, Horwitz RI: The effect of acute renal failure on mortality. A cohort analysis. *JAMA* 275: 1489–1494, 1996
3. Bates DW, Su L, Yu DT, Chertow GM, Seger DL, Gomes DR, Dasbach EJ, Platt R: Mortality and costs of acute renal failure associated with amphotericin B therapy. *Clin Infect Dis* 32: 686–693, 2001
4. Chertow GM, Levy EM, Hammermeister KE, Grover F, Daley J: Independent association between acute renal failure and mortality following cardiac surgery. *Am J Med* 104: 343–348, 1998
5. Lassnigg A, Schmidlin D, Mouhieddine M, Bachmann LM, Druml W, Bauer P, Hiesmayr M: Minimal changes of serum creatinine predict prognosis in patients after cardiothoracic surgery: A prospective cohort study. *J Am Soc Nephrol* 15: 1597–1605, 2004
6. Parikh C, McSweeney P, Korular D, Merouani A, Slat-Vasquez V, Shpall E, Jones R, Bearman S, Schrier RW: Acute renal failure in allogeneic hematopoietic cell transplantation. *Kidney Int* 62: 566–573, 2002
7. Metnitz PG, Krenn CG, Steltzer H, Lang T, Ploder J, Lenz K, Le Gall JR, Druml W: Effect of acute renal failure requiring renal replacement therapy on outcome in critically ill patients. *Crit Care Med* 30: 2051–2058, 2002
8. du Cheyron D, Bouchet B, Parienti JJ, Ramakers M, Charbonneau MP: The attributable mortality of acute renal failure in critically ill patients with liver cirrhosis. *Intensive Care Med* 31: 1693–1699, 2006
9. Simmons EM, Himmelfarb J, Sezer MT, Chertow GM, Mehta RL, Paganini EP, Soroko S, Freedman S, Becker K, Spratt D, Shyr Y, Ikizler TA; for the PICARD Study Group: Plasma cytokine levels predict mortality in patients with acute renal failure. *Kidney Int* 65: 1357–1365, 2004
10. Thornberry NA: The caspase family of cysteine proteases. *Br Med Bull* 53: 478–490, 1998
11. Uchino S, Kellum JA, Bellomo R, Doig GS, Morimatsu H, Morgera S, Schetz M, Tan I, Bouman C, Macedo E, Gibney N, Tolwani A, Ronco C; Beginning and Ending Supportive Therapy for the Kidney (BEST Kidney) Investigators: Acute renal failure in critically ill patients: A multinational, multicenter study. *JAMA* 294: 813–818, 2005
12. Prezant DJ: Effect of uremia and its treatment on pulmonary function. *Lung* 168: 1–14, 1990
13. Schrier RW, Wang W, Poole B, Mitra A: Acute renal failure: Definitions, diagnosis, pathogenesis, and therapy. *J Clin Invest* 114: 5–14, 2004 [published erratum appears in *J Clin Invest* 114: 598, 2004]
14. Okusawa S, Gelfand JA, Ikejima T, Connolly RJ, Dinarello CA: Interleukin 1 induces a shock-like state in rabbits. Synergism with tumor necrosis factor and the effect of cyclooxygenase inhibition. *J Clin Invest* 81: 1162–1172, 1988
15. Thickett DR, Armstrong L, Christie SJ, Millar AB: Vascular endothelial growth factor may contribute to increased vascular permeability in acute respiratory distress syndrome. *Am J Respir Crit Care Med* 164: 1601–1605, 2001
16. Kramer AA, Postler G, Salhab KF, Mendez C, Carey LC, Rabb H: Renal ischemia/reperfusion leads to macrophage-mediated increase in pulmonary vascular permeability. *Kidney Int* 55: 2362–2367, 1999
17. Deng J, Hu X, Yuen PS, Star RA: Alpha-melanocyte-stimulating hormone inhibits lung injury after renal ischemia/reperfusion. *Am J Respir Crit Care Med* 169: 749–756, 2004
18. Welbourn R, Goldman G, O'Riordain M, Lindsay TF, Paterson IS, Kobzik L, Valeri CR, Shepro D, Hechtman HB: Role for tumor necrosis factor as mediator of lung injury following lower torso ischemia. *J Appl Physiol* 70: 2645–2649, 1991
19. Colletti LM, Cortis A, Lukacs N, Kunkel SL, Green M, Strieter RM: Tumor necrosis factor up-regulates intercellular adhesion molecule 1, which is important in the neutrophil-dependent lung and liver injury associated with hepatic ischemia and reperfusion in the rat. *Shock* 10: 182–191, 1998
20. Colletti LM, Kunkel SL, Walz A, Burdick MD, Kunkel RG,

Wilke CA, Strieter RM: Chemokine expression during hepatic ischemia/reperfusion-induced lung injury in the rat. The role of epithelial neutrophil activating protein. *J Clin Invest* 95: 134–141, 1995

21. Seekamp A, Mulligan MS, Till GO, Smith CW, Miyasaka M, Tamatani T, Todd RF 3rd, Ward PA: Role of beta 2 integrins and ICAM-1 in lung injury following ischemia-reperfusion of rat hind limbs. *Am J Pathol* 143: 464–472, 1993

22. Yoshidome H, Lentsch AB, Cheadle WG, Miller FN, Edwards MJ: Enhanced pulmonary expression of CXC chemokines during hepatic ischemia/reperfusion-induced lung injury in mice. *J Surg Res* 81: 33–37, 1999

23. Melnikov VY, Faubel SG, Siegmund B, Lucia MS, Ljubanovic D, Edelstein CL: Neutrophil-independent mechanisms of caspase-1- and IL-18-mediated ischemic acute tubular necrosis in mice. *J Clin Invest* 110: 1083–1091, 2002

24. Faubel SG, Ljubanovic D, Poole B, Dursun B, Cushing S, He Z, Gill RG, Edelstein CL: Peripheral CD4 T cell depletion is not sufficient to prevent ischemic acute renal failure. *Transplantation* 80: 643–649, 2005

25. Kasahara Y, Tuder RM, Taraseviciene-Stewart L, Le Cras TD, Abman S, Hirth PK, Waltenberger J, Voelkel NF: Inhibition of VEGF receptors causes lung cell apoptosis and emphysema. *J Clin Invest* 106: 1311–1319, 2000

26. Bocci V: Interleukins. Clinical pharmacokinetics and practical implications. *Clin Pharmacokinet* 21: 274–284, 1991

27. Kato M, Suzuki H, Murakami M, Akama M, Matsukawa S, Hashimoto Y: Elevated plasma levels of interleukin-6, interleukin-8, and granulocyte colony-stimulating factor during and after major abdominal surgery. *J Clin Anesth* 9: 293–298, 1997

28. Kato M, Honda I, Suzuki H, Murakami M, Matsukawa S, Hashimoto Y: Interleukin-10 production during and after upper abdominal surgery. *J Clin Anesth* 10: 184–188, 1998

29. Daemen MA, de Vries B, Van't Veer C, Wolfs TG, Buurman WA: Apoptosis and chemokine induction after renal ischemia-reperfusion. *Transplantation* 71: 1007–1011, 2001

30. Molls RR, Savransky V, Liu M, Bevans S, Mehta T, Tuder RM, King LS, Rabb H: Keratinocyte-derived chemokine is an early biomarker of ischemic acute kidney injury. *Am J Physiol Renal Physiol* 290: F1187–F1193, 2006

31. Hepburn TW, Hart TK, Horton VL, Sellers TS, Tobia LP, Urbanski JJ, Shi W, Davis CB: Pharmacokinetics and tissue distribution of SB-251353, a novel human CXC chemokine, after intravenous administration to mice. *J Pharmacol Exp Ther* 298: 886–893, 2001

32. Kelly KJ, Williams WW Jr, Colvin RB, Bonventre JV: Antibody to intracellular adhesion molecule-1 protects the kidney against ischemic injury. *Proc Natl Acad Sci U S A* 91: 812–816, 1994

33. Kelly KJ, Williams WW Jr, Colvin RB, Bonventre JV: Intracellular adhesion molecule-1 deficient mice are protected against ischemic renal injury. *J Clin Invest* 97: 1056–1063, 1996

34. Kielar ML, John R, Bennet M, Richardson JA, Shelton JM, Chen L, Jeyarajah DR, Zhou XJ, Zhou H, Chiquett B, Nagami GT, Lu CY: Maladaptive role of IL-6 in ischemic acute renal failure. *J Am Soc Nephrol* 16: 3315–3325, 2006

35. Daemen MA, Van't Veer C, Wolfs TG, Buurman WA: Ischemia-reperfusion-induced IFN-gamma up-regulation: Involvement of IL-12 and IL-18. *J Immunol* 162: 5506–5510, 1999

36. Huang M, Pang X, Karalis K, Theoharides TC: Stress-induced interleukin-6 release in mice is mast cell-dependent and more pronounced in apolipoprotein E knockout mice. *Cardiovasc Res* 59: 241–249, 2003

37. Brunet P, Capo C, Dellacasagrande J, Thirion X, Mege JL, Berland Y: IL-10 synthesis and secretion by peripheral blood mononuclear cells in haemodialysis patients. *Nephrol Dial Transplant* 13: 1745–1751, 1998

38. Bocci V, Paulesu L, Pessina GP: The renal catabolic pathways of cytokines. *Contrib Nephrol* 101: 55–60, 1993

39. Poole S, Bird TA, Selkirk S, Gaines-Das RE, Choudry Y, Stephenson SL, Kenny AJ, Saklatvaa J: Fate of injected interleukin 1 in rats: Sequestration and degradation in the kidney. *Cytokine* 2: 416–422, 1990

40. Tanaka H, Tokiwa T: Influence of renal and hepatic failure on the pharmacokinetics of recombinant human granulocyte colony-stimulating factor (KRN8601) in the rat. *Cancer Res* 50: 6615–6619, 1990

41. Rachmawati H, Beljaars L, Reker-Smit C, Loenen-Weemaes AM, Hagens WI, Meijer DK, Poelstra K: Pharmacokinetic and biodistribution profile of recombinant human interleukin-10 following intravenous administration in rats with extensive liver fibrosis. *Pharm Res* 21: 2072–2078, 2004