Antecedent acute kidney injury worsens subsequent endotoxin-induced lung inflammation in a two-hit mouse model

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Basu RK, Donaworth E, Wheeler DS, Devarajan P, Wong HR. Antecedent acute kidney injury worsens subsequent endotoxin-induced lung inflammation in a two-hit mouse model. Am J Physiol Renal Physiol 301: F597–F604, 2011. First published June 15, 2011; doi:10.1152/ajprenal.00194.2011.—Acute kidney injury (AKI) contributes greatly to morbidity and mortality in critically ill adults and children. Patients with AKI who subsequently develop lung injury are known to suffer worse outcomes compared with patients with lung injury alone. Isolated experimental kidney ischemia alters distal lung water balance and capillary permeability, but the effects of such an aberration on subsequent lung injury are unknown. We present a clinically relevant two-hit murine model wherein a proximal AKI through bilateral renal ischemia (30 min) is followed by a subsequent acute lung injury (ALI) via intratracheal LPS endotoxin (50 μg at 24 h after surgery). Mice demonstrated AKI by elevation of serum creatinine and renal histopathological damage. Mice with ALI and preexisting AKI had increased lung neutrophilia in bronchoalveolar lavage fluid and by myeloperoxidase activity over Sham-ALI mice. Additionally, lung histopathological damage was greater in ALI mice with preexisting AKI than Sham-ALI mice. There was uniform elevation of monocyte chemoattractant protein-1 in kidney, serum, and lung tissue in animals with both AKI and ALI over those with either injury alone. The additive lung inflammation after ALI with antecedent AKI was abrogated in MCP-1-deficient mice. Taken together, our two-hit model demonstrates that kidney injury may prime the lung for a heightened inflammatory response to subsequent injury and MCP-1 may be involved in this model of kidney-lung cross talk. The model holds clinical relevance for patients at risk of lung injury after ischemic injury to the kidney.

AKI; ALI; cytokines; ischemia-reperfusion

THE INCIDENCE OF ACUTE KIDNEY injury (AKI) in hospitalized patients is significant. Although the incidence has varied based on definition criteria, by the risk, injury, and failure (RIFLE) strata, AKI is present in 36–67% of adults admitted to intensive care units (ICU) and carries 50–80% mortality (21). Data on children admitted to pediatric ICUs (PICU) with AKI are highly variable; however, a large recent study identified 10% of 3,393 hospitalized children with some degree of kidney injury (4, 40). In concert with a vast array of disease processes including sepsis, trauma, burn, and acute respiratory distress syndrome, AKI increases rates of morbidity (5, 13). Once the diagnosis of AKI has been established, the treatment modalities in place are largely supportive and have been unable to improve survival rates. Additionally, AKI is now known to be an independent risk factor for mortality in critical illness (3).

Acute lung injury (ALI) and AKI are bidirectionally deleterious. Mortality rates for patients with combined AKI and ALI are >75% (31, 33). Several recent clinical studies demonstrate that AKI significantly increases the duration of mechanical ventilation, as well as ICU and hospital lengths of stay even in patients with mild ALI (1, 35). Experimental models suggest that hypoxia and hypercapnia seen in ALI induce the expression of proapoptotic signals that trigger cell death in the kidney tissue bed (9, 22). Additionally, animal models of pneumonia display notable rates of kidney injury, a finding believed to be secondary to inflammatory cytokines dispelled by a damaged pulmonary endothelial bed (45).

Experimental kidney injury triggers lung injury. Ischemia in rodent models leads to increased pulmonary vascular permeability, altered sodium-potassium ATPase (Na-K-ATPase) and aquaporin channels, and altered lung fluid balance (28, 37). Models of murine AKI increase systemic levels of IL-6, IL-8 (KC), and macrophage inflammatory protein 2 (MIP-2) (20, 26). Additionally, ischemia-reperfusion injury induces hypercellularity, aberrations in inducible nitric oxide synthase expression, and increased MCP-1 in alveolar fluid (7). Oxidative balance in the kidney governed by heme oxygenase 1 and hypoxia-inducible factor-1 may be altered during AKI and this may contribute to distal lung injury (38). Postischemic kidneys also express increased levels of toll-like receptors, complement activation proteins, and cytokines, indicating a priming effect on the host’s immune response (17). Despite this abundant literature, the effect of AKI-mediated disruption of lung homeostasis on subsequent lung injury is unclear.

On the background of clinical and experimental evidence, we hypothesized that ALI induced by direct endotoxin, a surrogate for common nosocomial pathogens, would worsen in hosts with preexisting renal ischemic injury. We created a nonlethal, two-hit model combining primary ischemic AKI and secondary direct ALI. We compared kidney, serum, and lung responses and demonstrate here that AKI primes the lung to a heightened inflammatory response to subsequent endotoxin challenge.

MATERIALS AND METHODS

Animals and Surgical Protocol

The Cincinnati Children’s Hospital Medical Center Institutional Animal Care and Use Committee approved all animal protocols. Male C57BL/6 mice or MCP-1 null mice (C57BL/6 background) (Jackson Laboratories, Bar Harbor, ME), 4–6 wk old, were utilized. All animals were maintained under specific pathogen-free conditions, kept on a 12:12-h light-dark cycle, and given free access to food and water ad libitum.

Mice were anesthetized using 1.5% inhaled isoflurane. Abdominal areas were shaved clean, and mice were placed in a nose-cone inhalation device with continuous 1.5% isoflurane-oxygen mixture (100% oxygen at 0.8l/min flow). Body temperature was maintained...
using a water bath-controlled heating pad underneath the surgical field, and temperature was monitored intermittently using an anal rectal thermometer. A horizontal midline incision was made through the skin and then the fascia. The renal pedicles were exposed using cotton-tipped blunt dissection, isolated using vessel loops, and clamped bilaterally using smooth nontraumatic microvascular clamps (Roboz Surgical Instruments, Gaithersburg, MD) for 30 min. Occlusion was confirmed by visualizing renal cyanosis. A piece of gauze with warm saline was placed over the open abdominal wound during surgery. After clamp removal, reperfusion was visualized with disappearance of cyanosis to each kidney. The fascia and skin were then closed individually with continuous 4.0 vicryl sutures. Animals were given 0.5 ml of subcutaneous normal saline after the procedure and recovered on the heating pad before being returned to their cages. Sham-operated mice underwent identical operations with isolation of renal pedicles but without clamping. The animals were then allowed to recover, with access to food and water ad libitum. At 24 h after ischemia or sham surgery, mice were reanesthetized with 3% isoflurane and suspended using their front incisors. Fifty micrograms of LPS endotoxin [purified *Escherichia coli* LPS (O55:B5, Sigma, St. Louis, MO) stock solution, 1 mg/ml in PBS] was instilled through the mouth into the throat. Pressure was applied to occlude the nares and mice were suspended for at least 60 s while aspiration of liquid was heard. Mice were then returned to their cages. Sham mice received identical treatment but with 50 μl of PBS instead of LPS. Mice were euthanized at 48 h experimental time. Blood was removed by direct intracardiac puncture. Bronchoalveolar lavage (BAL) specimens were taken by instillation of 4 × 0.5 ml PBS after cannulation of the trachea. For histopathology, 30% formalin was directly infused into the right ventricle to perfuse the pulmonary circulation. Formalin was then infused into the lung after cannulation of the trachea. Lobar segments were ligated to preserve inflation. Lungs were excised and snap frozen in liquid nitrogen and kept at −80°C for cytokine and myeloperoxidase (MPO) assays and for separation into cytosolic and nuclear fractions. Kidneys were removed and stored in a similar manner to lungs. Mice are designated as sham surgery-saline (Sham-PBS), sham surgery-LPS (Sham-LPS/ALI), AKI surgery-saline (AKI-PBS), or AKI surgery-LPS (AKI-LPS/ALI). Approximately 8–12 mice/group were used in each experimental condition.

**Sample Preparation**

Blood was fractionated using centrifugation at 10,000 rpm × 10 min into plasma and whole red blood RBC cells. Plasma was stored at −80°C until use. Tissue samples were excised as indicated above and prepared for assays as listed for MPO, cytosol extraction, or nuclear fractionation.

**MPO Activity**

MPO activity was determined as an index of neutrophil accumulation in selected tissues collected 48 h after initial surgery (24 h after “2nd hit”). Excised tissue was homogenized in a solution containing 0.5% hexadecyltrimethylammonium bromide, dissolved in 10 mM potassium phosphate buffer (pH 7), and centrifuged for 30 min at 20,000 g at 4°C. An aliquot of the supernatant was allowed to react

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**Fig. 1. Development of 2-hit acute kidney injury (AKI)-acute lung injury (ALI) model.**

A: increasing ischemic time for kidneys increases weight loss and mortality 48 h after surgery. B: 30 min of ischemia lead to changes in serum creatinine. Analysis of ischemia after 12–48 h reveals a decrease in serum creatinine over time. C: effect of ischemic AKI on lung myeloperoxidase (MPO) activity. Fifteen- to 45-min ischemia against lung MPO at 24 h shows that MPO was highest for 30-min ischemia, demonstrating a 2.9-fold increase over sham. D: effect of harvest time on lung MPO after 30 min of kidney ischemia shows highest MPO levels at 1 day after surgery. E: effect of intratracheal LPS on lung MPO at 24 h shows the highest effect at 100 μg. F: final schematic of model shows that 30 min of ischemia was followed at 24 h by 50 μg of LPS or PBS. Harvest occurred at 48 h. *P < 0.001; n = 8–10 mice/group for all experiments shown.
with a solution of tetramethylbenzidine (1.6 mM) and 0.1 mM hydrogen peroxide. The rate of change in absorbance was measured by spectrophotometry at 650 nm. MPO activity was defined as the quantity of enzyme degrading 1 μmol of hydrogen peroxide/min at 37°C and expressed in units per 100-mg weight of tissue.

**Subcellular Fractionation and Nuclear Protein Extraction**

Tissue samples were homogenized in a buffer containing 0.32 M sucrose, 10 mM Tris·HCl (pH 7.4), 1 mM EGTA, 2 mM EDTA, 5 mM sodium azide, 10 mM β-mercaptoethanol, 20 μM leupeptin, 0.15 μM pepstatin A, 0.2 mM PMSF, 50 mM sodium fluoride, 1 mM sodium orthovanadate, and 0.4 nM microcystin. The homogenates were centrifuged (1,000 g, 10 min), and the supernatant (cytosol plus membrane extract) was collected. The pellets were solubilized in Triton buffer [1% Triton X-100, 150 mM NaCl, 10 mM Tris·HCl (pH 7.4), 1 mM EGTA, 1 mM EDTA, 0.2 mM sodium orthovanadate, 20 μM leupeptin A, and 0.2 mM PMSF]. The lysates were centrifuged (15,000 g, 30 min, 4°C), and the supernatant (nuclear extract) was collected.

**Histological Examination of Lung Tissue**

A scoring system for histopathology of lung tissue was used as described previously (29). Briefly, lung sections were inflated ex vivo with 30% formalin, set for 24 h in formalin, embedded in paraffin, and processed for light microscopy. Sections were stained with hematoxylin-eosin. A scoring grid for lung damage was used: degree of alveolar congestion + hemorrhage + neutrophil infiltration in air space/vessel wall + thickness of alveolar wall/hyaline membrane.

**Histological Examination of BAL Fluid**

After removal of BAL fluid (BALF) by the method described above, isolates were spun at 1,800 rpm for 10 min at 4°C. Supernatant fluid was stored at −80°C for future analysis. Cell pellets were resuspended in 1 ml 10% PBS/FBS (Sigma) suspension. Two hundred microliters of each suspension was centrifuge-plated onto slides for microscopic analysis and counting via a hemocytometer. Cell counts were manually performed using randomly selected areas of each slide, counting 500 cells total.

**Serum Creatinine Measurements**

A colorimetric-based assay (Genzyme Diagnostics, Framingham, MA) was used to determine serum creatinine concentration. Values were plotted against a standard curve after calibration and measurement at 545 nm.

**Cytokine Analysis**

Serum and tissue cytosolic cytokine levels of TNF-α, IL-1β, IL-4, IL-10, IL-12β, IL-6, MCP-1, MIP-2, and KC were analyzed using Luminex xMAP multiplex array system technology (Luminex, Austin, TX).

**Statistical Analysis**

Due to the nonparametric nature of some data, results of analyses are expressed as means ± SE, with unpaired t-tests as tests of significance, or as medians with interquartile ranges, using Mann-Whitney tests as tests of significance. P < 0.05 was inferred as statistically significant. All statistical analyses were performed using SigmaStat 3.1 (Systat Software, San Jose, CA).

**RESULTS**

**Development of Two-Hit Model**

Published models of AKI vary from unilateral or bilateral renal pedicle clamping (20–60 min) to degrees of nephrec-
tomy. Initial nephrectomy data (not shown) and ischemic times >30 min had high morbidity and mortality, which limited the ability to discern the effects of secondary ALI. Bilateral ischemia was chosen to mimic clinical renal hypoperfusion seen in sepsis, hypovolemia, or surgical procedures which limit renal blood flow [suprarenal aortic cross-clamping for cardiopulmonary bypass or aneurysm repair]. Varied ischemic times were attempted before the decision for 30 min, a time which allowed for sufficient AKI (measured by serum creatinine) without mortality (Fig. 1, A and B). The interval of time between AKI and ALI was chosen based on the rise in MPO levels (after 24 h, ischemic AKI induced a lung MPO change of ~2.6-fold) (Fig. 1D) and to allow mice sufficient time to recover from abdominal surgery. ALI was detected after direct intratracheal instillation of LPS, an acceptable model of ALI and was based on increases in lung MPO at 24 h in naive mice receiving escalating concentrations of the endotoxin (Fig. 1E). Determination of the appropriate time after the endotoxic-ALI to perform analysis was based on preliminary experiments demonstrating optimal lung inflammation 16–24 h after LPS challenge (Fig. 2, A–D). The final two-hit model was thus established (Fig. 3).

Ischemia Results in Kidney Inflammation and Injury

There was no mortality at time of harvest (48 h) after 30 min of bilateral ischemia followed 24 h later by intratracheal injection of 50 μg of LPS. AKI-PBS and AKI-LPS mice had significantly higher serum creatinine levels than sham controls, but the second injury did not further increase creatinine (Fig. 4A). Representative histopathological changes in the surgical model are shown in Fig. 4B. Compared with the sham surgeries, the AKI surgeries demonstrate tubular dilation, cast formation, necrosis, and a leukocytic infiltrate at 48 h, a time when serum creatinine remains significantly elevated over sham-operated animals.

AKI and ALI Lead to Increased Lung Neutrophil Chemotaxis

MPO activity was measured in lung tissue after a two-hit injury among the four experimental groups to determine the level of neutrophil content. MPO, a lysosomal peroxidase enzyme, is released by activated neutrophils after entry into sites of inflammation and is both a marker of inflammation in the lungs and a direct mediator of tissue injury. MPO activity significantly increased in mice who received LPS in both sham and AKI surgeries (Fig. 5A). Notably, MPO activity was significantly higher in mice with ALI and preexisting AKI vs. mice without AKI. BALF was sampled from each experimental group to determine the effect of the model on intra-alveolar cytokines and protein content. On gross examination, fluid removed from mice with ALI and preexisting AKI appeared more hemorrhagic compared with fluid from mice without AKI. Microscopic analysis of BALF demonstrates that mice with ALI and preexisting AKI had increased levels of neutrophilia, hemorrhage, and macrophage infiltration (Fig. 5B). Quantification of the cell populations for Sham-LPS vs. AKI-LPS revealed a statistically significant increase in neutrophils and a cellular shift from monocytic predominance to neutrophilic predominance (Fig. 5C). Protein quantification was performed on BALF and demonstrates that total protein concentration trended higher in mice with ALI and preexisting AKI vs. mice without AKI (Fig. 5D).

AKI and ALI Lead to Increased Damage to Lung Parenchyma

As demonstrated on BALF analysis, mice with ALI and preexisting AKI had increased pulmonary hemorrhage based on gross and microscopic analysis (Fig. 5B). Representative histological cross sections from inflated and fixed lungs demonstrate that AKI-LPS mice had increased areas of hemorrhage and alveolar congestion vs. Sham-LPS mice (Fig. 6).

Expression of MCP-1 Increases in AKI and ALI

In the preliminary experiments, a subjective mouse wellness score (25) was worse in the first 2 days for the LPS groups, but was essentially the same for all groups from day 4 to day 7 (data not shown). Additionally, although the mice lost weight over the first 48–72 h, by the end of the observation period...
average group weights were not statistically different (data not shown). To determine whether systemic inflammation was manifest after injury, expression profiles of inflammatory cytokines and chemokines was performed on tissue samples from kidney, lung, blood, and BALF (Table 1). Proinflammatory mediators IL-6 and KC were elevated in both groups receiving LPS in serum, lung, and BALF. In the kidney, expression of IL-6, KC, and MCP-1 was elevated in both AKI groups. Notably, MCP-1 was uniformly elevated in all tissues tested in AKI-ALI mice vs. Sham-ALI, with significant elevations in serum and BALF.

MCP-1 Elimination Reduces AKI Lung Priming Effect on ALI

To begin analyzing the role that MCP-1 plays in the AKI-dependent priming of the lung, commercially available MCP-1 null mice were subjected to the two-hit model along with a cohort of wild-type mice. Results demonstrate an abrogation of
Table 1. Expression profiles of proinflammatory chemokines

<table>
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<tr>
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<th>Kidney</th>
<th>Serum</th>
<th>Lung</th>
<th>BALF</th>
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<tbody>
<tr>
<td></td>
<td>Sham-PBS (pg/mg tissue)</td>
<td>Sham-LPS (pg/mg tissue)</td>
<td>AKI-PBS (pg/ml)</td>
<td>AKI-LPS (pg/ml)</td>
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<tr>
<td>IL-6</td>
<td>1.2 (0.8–1.7)</td>
<td>0.9 (0.7–1.1)</td>
<td>1.5 (1.4–1.7)</td>
<td>1.3 (0.8–2.1)</td>
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<tr>
<td>KC</td>
<td>3.5 (2.7–4.3)</td>
<td>3.1 (2.3–3.3)</td>
<td>16.9 (10–23.8)</td>
<td>10.5 (8.1–24.6)</td>
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<tr>
<td>MCP-1</td>
<td>3.2 (2.5–3.9)</td>
<td>2.6 (2.2–2.9)</td>
<td>9.5 (5.2–13.2)</td>
<td>4.0 (2.6–8.2)</td>
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<tr>
<td>Serum</td>
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<tr>
<td>IL-6</td>
<td>54 (27–81)</td>
<td>93 (55–130)</td>
<td>56 (37–75)</td>
<td>97 (60–133)</td>
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<tr>
<td>KC</td>
<td>512 (276–748)</td>
<td>549 (378–819)</td>
<td>728 (474–980)</td>
<td>735 (273–1,160)</td>
</tr>
<tr>
<td>MCP-1</td>
<td>59 (43–75)</td>
<td>69 (56–73)</td>
<td>108 (53–161)</td>
<td>139 (87–230)</td>
</tr>
<tr>
<td>Lung</td>
<td></td>
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<tr>
<td>IL-6</td>
<td>0.7 (0.5–0.9)</td>
<td>16 (8–24)</td>
<td>0.4 (0.3–0.5)</td>
<td>7 (2–11)</td>
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<tr>
<td>KC</td>
<td>5.1 (2.8–7.4)</td>
<td>45 (22–67)</td>
<td>6.8 (2.9–10.7)</td>
<td>36 (22–50)</td>
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<tr>
<td>MCP-1</td>
<td>1.1 (0.7–1.5)</td>
<td>9 (6–10)</td>
<td>1.4 (0.9–1.8)</td>
<td>11 (7–14)</td>
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<tr>
<td>BALF</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-6</td>
<td>85 (31–120)</td>
<td>3,696 (2,410–4,975)</td>
<td>59 (21–147)</td>
<td>2,705 (414–4,996)</td>
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<tr>
<td>KC</td>
<td>5 (3.1–10.4)</td>
<td>610 (355–865)</td>
<td>4 (2.5–14.3)</td>
<td>827 (332–1,322)</td>
</tr>
<tr>
<td>MCP-1</td>
<td>13.8 (7.2–25.8)</td>
<td>767 (707–1,107)</td>
<td>12 (5.2–13)</td>
<td>5,388 (4,024–6,662)</td>
</tr>
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Proinflammatory chemokines IL-6, KC, and MCP-1 were analyzed in kidney, serum, lung, and bronchoalveolar lavage fluid (BALF) after 2-hit injury. AKI, acute kidney injury. Data are shown as median with interquartile range expressed in pg/mg tissue for kidney and lung and pg/ml for serum and BALF. Results show increased expression of all 3 chemokines in the kidney in AKI groups and increased expression of IL-6 and KC in LPS groups for serum, lung, and BALF. Expression of MCP-1 was increased in all tissue compartments but was significantly increased in both serum and in BALF. *P values shown compare Sham-LPS with AKI-LPS groups; n = 11–12 for all cytokines.

Proper model conditions required both injuries to be sublethal, that ischemic AKI reproduced aberrations in lung homeostasis reported by other investigators, and that sufficient recovery time existed for the mice between insults. Although several patient populations carry high risk for renal ischemia [patients undergoing abdominal aneurysm repair requiring suprarenal aortic cross clamping (32) or patients undergoing cardiopulmonary bypass (8, 36)], it can be argued that systemic illness from isolated renal ischemia is likely a clinical non sequitur. However, the proposed model seeks to underscore the potential contribution of the kidney to deleterious lung priming during common ischemic states such as hypovolemia. Bilateral renal pedicle clamping for 30 min induced AKI based on marked increases in serum creatinine and distortion in the renal architecture vs. sham mice. Although serum creatinine decreased after 12 h posts ischemia, and may be indicative of restoration of glomerular filtration after ischemic injury, kidney histological injury remained notable at 48 h. This indicates that persistent damage exists in the AKI mice (when creatinine is still markedly elevated over sham levels). Interestingly, the addition of LPS did not significantly increase the serum creatinine in mice subjected to AKI, nor did it make renal histological damage worse (Fig. 4B). This may be secondary either to a lack of systemic inflammation induced by intratracheal LPS or possibly to a further decrease in creatinine production in an acutely ill host as postulated by prior authors (16).

We believe that the interval change in mouse wellness score and weight loss (Fig. 1A) are indicative of systemic illness, which was greater in the mice exposed to two insults than either alone or the sham cohorts. An obvious limitation to our model development, however, was of the lack of data involving fluid balance. Future studies will address this issue by use of metabolic cages to directly measure intake and output of mice in different experimental conditions.
Previously published data demonstrate that increased lung MPO activity, BALF protein and cellularity, and alveolar leukocytosis all occur after isolated experimental AKI (7, 12, 20). Faulk and colleagues (26) highlighted neutrophil chemokinesis in the systemic circulation and in lung 2–6 h after AKI (17, 18). In our model, lung MPO activity increases at 24 h after ischemic AKI vs. sham surgery but drops precipitously absent a secondary stimulus (Fig. 1D). This may be a direct manifestation of a priming phenomenon, wherein AKI is sufficient for a mild ALI but also triggers the recruitment of participants which contribute to augmented inflammation during the subsequent lung insult. This notion is supported by our findings. MPO activity was significantly increased in endotoxic-ALI after antecedent AKI compared with ALI alone (Fig. 5A). BALF specimens were grossly more hemorrhagic and microscopically demonstrated more neutrophilia. Furthermore, histopathology of lungs after ALI with antecedent AKI demonstrates increased alveolar septal thickening, hemorrhage, and neutrophilia over sham-ALI.

Proinflammatory chemokine expression after AKI is time dependent. Grigoryev et al. (17) demonstrate that IL-6, KC, and MCP-1 all increase until 6 h and then decrease. Hoke et al. (20) show that KC increases and then decreases after 8 h (20), and Supavekin et al. (43) illustrate that IL-6 increases and then decreases after 8 h (43). In these studies, all mentioned chemokines continue downward at 24 h. While our finding of decreased IL-6 expression may be secondary to the late time point of our measurement, ischemic preconditioning may have also played a role (6). We observed increased expression of IL-6 and KC in the serum, kidney, lung, and BALF of all mice receiving LPS, but no significant differences in mice receiving AKI before ALI (Table 1). No demonstrable changes were seen with IL-10, IL-12, IL-1β, IL-2, IL-4, or TNF-α (data not shown). Interestingly, we found uniform elevation in the levels of MCP-1 in AKI-ALI mice vs. Sham-ALI mice across all tissue compartments analyzed, with significant elevations in serum and BALF.

Our findings parallel previous authors investigating extrarenal effects of AKI and secondary injury. Increased morbidity was demonstrated in mice with chemical AKI with secondary sepsis by cecal ligation and puncture (15). In a subtotal nephrectomy model of AKI, a secondary ischemic insult resulted in increased lethality (42). In prior two-hit models involving AKI and lung injury, Dodd et al. (14) showed that mice subjected to ischemic AKI had less pulmonary capillary leak after high tidal volume ventilation (30 ml/kg) than their sham cohorts (14). Similarly, Zarbock et al. (47) highlighted a potential protective effect of uremic neutrophils in mice challenged with intratracheal hydrochloric acid. Interestingly, in Dodd et al. (14) the lung inflammation after moderate secondary injury (low tidal volume ventilation) was made more severe by antecedent AKI. Additionally, both of these experimental models analyzed result at time points significantly earlier than our model (Dodd: 4 h after 2nd hit and Zarbock: 2 h after 2nd hit) (14, 47). Demonstration of impaired ventilatory mechanics and gas exchange would support a hypothesis that AKI leads to a functionally impaired lung after a direct secondary insult; however, with sublethality (no mortality up to 7 days), differences in murine lung mechanics can be subtle and difficult to elucidate.

The correlation between the ubiquitous elevation in MCP-1 expression and lung inflammation/neutrophilia is unclear. MCP-1 is expressed by multiple cell lineages during injury and exerts chemotactic effects by binding to its dedicated receptor (CCR-2) on circulating monocytes and macrophages. MCP-1 is known to be involved in the progression of numerous nephropathies and may even have utility as a biomarker for ischemic renal tubular injury (10, 34, 44, 46). MCP-1 has been underscored as a contributor to the heightened systemic inflammation seen in autoimmune diseases (19, 39). Also, MCP-1 levels are associated with increased inflammation and macrophage infiltration in numerous models of acute lung injury (27, 41, 48), including those with intratracheal LPS (23, 24). Interestingly, pulmonary vascular permeability after rat renal ischemia was significantly abrogated by a macrophage pacificant (28). In preliminary experiments, antecedent AKI-mediated, augmented lung inflammation after secondary insult, as measured by lung neutrophilia, is abrogated in MCP-1 null mice. The explanation for this is uncertain, as MCP-1 is typically a monocyte chemoattractant and has minimal effects on circulating neutrophils. Additionally, the source of MCP-1 in this model remains speculative, although persistently distorted renal architecture after ischemia indicates that injured tubular epithelia or glomerular endothelia may be a contributing factor. Finally, the kinetics of chemokine expression and chemotaxis are not addressed by our study and is a limitation to the analysis. While the influx of MCP-1 along with its monocyte-macrophage partners may contribute to inflammation, it may also herald the beginning of the reparative phase seen after ALI (11). There is certainly evidence that the presence of alveolar macrophages and MCP-1 helps attenuate lung injury in the longer term evaluation after bacterial lung injury models (2). Further analysis of our model, through biochemical and transgenic manipulation of both MCP-1 and its receptor CCR-2, is needed.

Conclusions

Clinical and laboratory evidence implies early and lasting dysregulation in pulmonary function as a result of AKI. We report lung inflammation induced by direct lung injury is worsened by preexisting kidney injury, a clinically relevant model. AKI may actually prime the lung. Further investigation into this model is required to elucidate the role of potential key mediators, especially MCP-1, and to highlight potential strategies to reduce the deleterious impact of these disease processes with significant clinical comorbidity.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

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