Splenectomy exacerbates lung injury after ischemic acute kidney injury in mice

Ana Andrés-Hernando,1 Christopher Altmann,1 Nilesh Ahuja,1 Miguel A. Lasnspa,1 Raphael Nemenoff,1 Zhibin He,1 Takuji Ishimoto,1 Pete A. Simpson,1 Mary C. Weiser-Evans,1 Jasna Bacalja,2 and Sarah Faubel1

1Department of Medicine, Division of Renal Diseases and Hypertension, University of Colorado Denver, Aurora, Colorado; and 2Department of Pathology, University Hospital Dubrava, Zagreb, Croatia

Submitted 22 February 2011; accepted in final form 12 June 2011

ACUTE KIDNEY INJURY (AKI) is a common complication in hospitalized patients that is associated with increased morbidity and mortality (3, 27, 28). Increased mortality in AKI may be partly explained by an increase in extrarenal complications such as respiratory failure, acute lung injury, and cardiovascular complications (11, 18). AKI is a proinflammatory state and the proinflammatory cytokines TNF-α, IL-1β, IL-6, and IL-8 are increased in the serum of patients with AKI and increased IL-6 and IL-8 are associated with increased mortality (26). Together, these proinflammatory cytokines mediate the systemic inflammatory response syndrome (SIRS), which is followed by a compensatory anti-inflammatory response syndrome (CARS), during which time anti-inflammatory cytokines, including IL-10, are produced (26). The CARS response is important to limit organ dysfunction and injury from increased proinflammatory cytokines.

Serum proinflammatory cytokines may be increased in AKI due to increased renal and/or extrarenal production. Although evidence suggests that cytokines are produced by the kidney (2, 4, 15), the role of extrarenal organs in cytokine production in AKI is relatively unexplored. In the present study, we sought to examine renal and extrarenal cytokine production in ischemic AKI. In particular, we sought to determine the role of the spleen in cytokine production.

In the present study, we hypothesized that splenectomy would protect against AKI-mediated lung injury due to a reduction in circulating proinflammatory cytokines, particularly IL-6. Surprisingly, we found that serum IL-6 was higher and lung injury was worse after AKI plus splenectomy than with AKI alone. To investigate the cause of the increased proinflammatory response with AKI plus splenectomy, splenic production of the anti-inflammatory cytokine IL-10 was investigated. We found that splenic IL-10 was markedly upregulated. To confirm that IL-10 downregulates the proinflammatory response to noninfections injury. Furthermore, these data suggest that the ability to mount an early compensatory anti-inflammatory response in AKI is necessary to minimize lung injury and other proinflammatory effects of AKI.

METHODS

Animals. Eight- to 10-wk-old C57BL/6J 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 (2,2,2-tribromoethanol; Aldrich, Milwaukee, WI) and surgery for sham operation, ischemic AKI, splenectomy, or AKI and splenectomy was performed. In each group, a midline incision was made. For sham operation, the renal and splenic vasculature was examined and the abdomen was left open for 22 min before closure. For ischemic AKI, 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. This model of ischemic AKI is well described by our laboratory (6, 12, 17). For splenectomy, the two major sources of blood flow (splenic artery and gastric artery) were

http://www.ajprenal.org
1931-857X/11 Copyright © 2011 the American Physiological Society
tied off with suture proximal to the spleen and ligated, and the spleen was removed. For ischemic AKI and splenectomy, splenectomy was performed immediately before 22 min of renal pedicle clamping. For all surgical procedures, the abdomen was closed in one layer and 200 µl of normal saline were administered subcutaneously. None of the mice died as a result of the procedure.

**Blood and tissue collection.** After intraoperative pentobarbital sodium anesthesia, blood was collected by cardiac puncture, and organs were collected.

Blood was allowed to clot for 2 h at room temperature. Samples were centrifugated at 3,000 g for 10 min; serum was collected and centrifugated a second time at 3,000 g for 1 min. An additional centrifugation of 14,000 g for 10 min at 4°C was finally performed to ensure complete removal of red blood cells. Blood samples with notable hemolysis were discarded.

**Assessment of renal function and kidney injury.** Blood urea nitrogen (BUN) and serum creatinine were measured using a QuantiChrom assay kit (BioAssay Systems, Hayward, CA).

Paraformaldehyde (4%)-fixed and paraffin-embedded kidneys were sectioned at 4 µm and stained with periodic acid-Schiff (PAS) by standard methods. All histological examinations were performed by the renal pathologist in a blinded fashion. Histological changes due to acute tubular necrosis (ATN) score were evaluated in the outer stripe of the outer medulla on PAS-stained tissue and were quantified by counting the percent of tubules that displayed cell necrosis, loss of brush border, cast formation, and tubule dilatation as follows: 0, none; 1, <10%; 2, 11 to 25%; 3, 26 to 45%; 4, 46 to 75%; and 5, >76%. At least 10 fields (×200) were reviewed for each slide.

Neutrophil infiltration was quantitatively assessed on PAS-stained tissue by the renal pathologist by counting the number of neutrophils per high-powered field (×400). At least 10 fields were counted in the outer stripe of the outer medulla for each slide.

**Real-time reverse transcriptase-PCR.** Cytosolic RNA was isolated from mice kidney, liver, and spleen using the RNeasy kit (Qiagen, Valencia, CA) and with the TRIzol method (Invitrogen) for the spleen. From mice kidney, liver, and spleen using the RNeasy kit (Qiagen, Valencia, CA) and with the TRIzol method (Invitrogen) for the spleen. Cytosolic RNA was isolated from mice kidney, liver, and spleen using the RNeasy kit (Qiagen, Valencia, CA) and with the TRIzol method (Invitrogen) for the spleen. RT-PCR runs were analyzed by agarose gel electrophoresis and melt curve to verify that the correct amplicon was produced. Real-time reverse transcriptase-PCR. Cytosolic RNA was isolated from mice kidney, liver, and spleen using the RNeasy kit (Qiagen, Valencia, CA) and with the TRIzol method (Invitrogen) for the spleen.

Readymix QPCR kit (Sigma) on a Bio-Rad I-Cycler. RT-PCR runs were performed using 70 nM primers and the SYBR Green JumpStart Taq (Ecoprobes and Jackson Immunoresearch, respectively). Slides were covered with an antifading mounting medium (Vector Laboratories) for confocal microscope analysis. Samples were imaged with a ×40 water-immersion objective by using a laser-scanning confocal microscope (model LSM510; Zeiss). Data were analyzed using the LSM Image Analyzer postacquisition software (Zeiss).

**Protein extraction and Western blotting.** Spleens were homogenized with the M-PER reagent (Thermo Fischer). Protein concentration was determined by the BCA protein assay (Pierce). Thirty micrograms of total protein were loaded per lane for SDS-PAGE (4–12% wt/vol) analysis and then transferred to polyvinylidene di fluoride membranes. Membranes were incubated with primary antibody and visualized by using a horseradish peroxidase secondary antibody and ECL Plus Western Blotting Detection System (Amer sham) as described by the manufacturer. Chemiluminescence was recorded with an Image Station 440CF (Kodak Digital Science), and results were analyzed with one-dimensional Image Software (Kodak Digital Science). Blots were also analyzed for β-actin as a loading control.

**Flow cytometry.** Multicolor multiparameter flow cytometry was performed using a FACSCanto II instrument (BD Biosciences) compensated with single fluorochromes and analyzed using Diva software (BD Biosciences). Freshly isolated cells from spleen (1–2×10⁶) were stained for cell surface antigen expression by incubating with antibody at 4°C for 30 min in the dark and washed twice in 2 ml PBS containing 1% bovine serum albumin and 0.01% sodium azide (FACS Wash). The cells were washed with FACS buffer and stained for cell surface markers before fixation in PBS/1% PFA for 15–20 min on ice. These cells were then permeabilized for 30 min using a saponin-based buffer (1X Perm/Wash, BD Pharmingen in FACS buffer) and stained for inducible nitric oxide synthase (iNOS) or isotype control. The following antibodies were used: F4/80-PE and CD11b-PerCP/Cy5 (eBiosciences), CD45-V500, Ly6G-APC-Cy7 and iNOS (BD Pharmingen), and CD206-APC (Serotec). Cells were gated according to size and scatter to eliminate dead cells and debris from analysis.

**Statistical analysis.** Data were compared with Student's t-test for two experimental groups, or with ANOVA when comparing with more than two experimental groups. Results were expressed as
RESULTS

Proinflammatory cytokines in the kidney, spleen, and liver after AKI. To examine proinflammatory cytokine production after AKI, we determined mRNA expression of IL-6, CXCL1, IL-1β, and TNF-α in the kidney, spleen, and liver at baseline and at 2, 4, and 6 h after sham operation or ischemic AKI. IL-6, CXCL1, and IL-1β mRNA expressions were highly upregulated in the kidney only 2 h after AKI vs. sham-operated mice, while in spleen and liver, increased cytokine expression primarily occurred at 4 and 6 h post-AKI, as shown in Fig. 1.

Fig. 1. mRNA expression of proinflammatory cytokines in the kidney, spleen, and liver after acute kidney injury (AKI). The mRNA expression of kidney, spleen, and liver IL-6, CXCL1, IL-1β, and TNF-α was measured by qPCR at baseline and at 2, 4, and 6 h after AKI or sham operation in C57/B6 mice. A: IL-6, CXCL1, and IL-1β mRNA expressions were highly upregulated in the kidney only 2 h after AKI vs. sham-operated mice. IL-6, CXCL1, and IL-1β mRNA expressions were elevated after AKI vs. sham operation in the spleen (B) and liver (C), 2 and 6 h post-AKI. Changes in TNF-α mRNA expression were found at 2 h post-AKI in the liver and 6 h post-AKI in the kidney and spleen. All the graphs are expressed as arbitrary units corrected by the loading control β-actin. NS, not significant.

*P < 0.05, **P < 0.01, ***P < 0.001 (n = 3–5).

Fig. 2. Lung inflammation and lung capillary leak 4 h after AKI plus splenectomy. Lung myeloperoxidase (MPO) activity, lung CXCL1, and lung capillary leak were assessed 4 h after sham operation (Sham), splenectomy (Splnx), ischemic AKI (AKI), and ischemic AKI plus Splnx (AKI+Splnx). A: MPO activity significantly increased in AKI compared with sham or Splnx and was further increased in AKI plus Splnx compared with AKI alone. B: lung CXCL1 increased in AKI compared with sham or Splnx and was further increased in AKI plus Splnx compared with AKI alone. C: capillary leak, as assessed by extravasation of Evans blue dye (EBD) into the lungs, was increased in AKI plus Splnx, indicating increased capillary leak (n = 6–10).
Lung MPO activity and lung CXCL1 after AKI plus splenectomy. To determine the role of the spleen in lung injury after AKI, MPO activity and CXCL1 expression were measured in lung tissue 4 and 24 h after sham operation, splenectomy, AKI, and AKI plus splenectomy. MPO activity is widely used as a biochemical marker for tissue neutrophil content (5, 9) and correlates with lung neutrophil accumulation (17); CXCL1, also known as KC, is a neutrophil chemokine.

As depicted in Fig. 2, A and B, respectively, lung MPO activity and CXCL1 significantly increased 4 h after ischemic AKI compared with sham operation or splenectomy alone. There was a further increase in both MPO activity and lung CXCL1 after AKI plus splenectomy compared with AKI alone. As shown in Fig. 3, A and B, respectively, lung MPO activity and lung CXCL1 were not increased 24 h after AKI compared with sham or splenectomy alone, but they were still significantly increased 24 h after AKI plus splenectomy.

Lung capillary leak after AKI plus splenectomy. To further examine lung injury after AKI plus splenectomy, lung capillary leak as assessed by lung infiltration of EBD was determined at 4 and 24 h after AKI and AKI plus splenectomy. Lung EBD significantly increased in AKI plus splenectomy compared with AKI at 4 and 24 h as shown in Figs. 2C and 3C, respectively.

Serum IL-6 and other cytokines after AKI plus splenectomy. We previously demonstrated that IL-6 mediates lung injury after AKI (17). Therefore, to determine whether increased IL-6 might explain the exacerbation in lung injury with splenectomy, serum IL-6 was determined 4 and 24 h after sham operation, splenectomy, AKI, or AKI plus splenectomy. As depicted in Fig. 4A, serum IL-6 significantly increased 4 h after ischemic AKI compared with sham operation or splenectomy alone. Serum IL-6 was further increased after AKI plus splenectomy compared with AKI alone. As shown in Fig. 4B, 24 h postprocedure, no significant difference was found in serum IL-6 after AKI compared with sham or splenectomy, but a significant increase in serum IL-6 was observed after AKI plus splenectomy compared with sham, splenectomy, and AKI.

To determine whether other serum cytokines were affected by AKI plus splenectomy, 23 serum cytokines, including CXCL1, IL-1β, TNF-α, and IL-10, were examined by multiplex analysis in mice 4 h after AKI or AKI plus splenectomy. Only serum CXCL1 and IL-10 were different in mice with AKI plus splenectomy vs. AKI alone and were both higher in AKI plus splenectomy vs. AKI alone. Serum CXCL1 was 1,321 ± 130 in AKI and was 2,508 ± 202 in splenectomy plus AKI (P = 0.0011, n = 5); serum IL-10 was 132 ± 12 in AKI and was 291 ± 64 in AKI plus splenectomy (P = 0.0411, n = 9). (Results for serum CXCL1, IL-1β, TNF-α, and IL-10 were confirmed by ELISA and similar values were obtained). To determine whether the increase in serum IL-10 after AKI plus splenectomy was coming from the liver, IL-10 mRNA levels were measured in the liver 4 h after sham, splenectomy, AKI, and AKI plus splenectomy. No significant difference was observed between AKI and AKI plus splenectomy. This result indicates that splenectomy does not lead to liver IL-10 increase and thus liver IL-10 is not responsible for the increase in serum IL-10 after AKI and splenectomy.

Serum creatinine and BUN after AKI plus splenectomy. To determine whether splenectomy affected renal function, serum creatinine and BUN were measured 4 h after sham operation, splenectomy, AKI, and AKI plus splenectomy. No significant difference was found in serum creatinine and BUN after AKI compared with sham, splenectomy, and AKI (P < 0.05).
Creatinine and BUN were measured 4 and 24 h after sham, splenectomy, ischemic AKI, and ischemic AKI plus splenectomy. No significant change was found in serum creatinine or BUN after ischemic AKI plus splenectomy vs. ischemic AKI alone (Fig. 5, A and B).

To confirm that kidney injury, not detected by changes in serum creatinine and BUN, was similar, ATN scores were determined 24 h after AKI and AKI plus splenectomy. No significant change was found in ATN scores after AKI (4.4/1.3) compared with AKI plus splenectomy (4.7/0.6). ATN scores were as follows: 0, no ATN; 1, less or equal 10%; 2, 11–25%; 3, 26–45%; 4, 46–75%; 5, >75% of parenchyma affected with ATN.

**IL-6 production in the kidney and the liver after ischemic AKI plus splenectomy.** To determine whether splenectomy increased IL-6 production, IL-6 mRNA in the kidney and in the liver 4 h after sham operation, splenectomy, ischemic AKI, and ischemic AKI plus splenectomy was determined. No increase in IL-6 mRNA was found in the kidney (Fig. 5C) or in the liver (Fig. 5D) in mice with ischemic AKI plus splenectomy vs. AKI alone.

**Upregulation of anti-inflammatory cytokine IL-10 mRNA in the spleen after AKI.** To determine whether worse lung injury after ischemic AKI plus splenectomy was due to the loss of a beneficial effect of the spleen, splenic IL-10 mRNA was measured at 2, 4, and 6 h after sham operation or ischemic AKI. As depicted in Fig. 6A, IL-10 mRNA significantly increased by 2 h after ischemic AKI, indicating that the spleen acts as an anti-inflammatory organ early after ischemic AKI. Figure 6B shows a statistically significant increase in liver IL-10 at 2, 4, and 6 h after AKI but to a lesser degree than splenic IL-10. Figure 6C shows no upregulation of IL-10 mRNA in the kidney after AKI vs. sham at any time points.

**Fig. 5.** Renal function and IL-6 mRNA production in kidney and liver after AKI plus Splnx. Serum creatinine (A) and blood urea nitrogen (BUN; B) were measured at 4 and 24 h postprocedure and were elevated after AKI and AKI plus Splnx compared with sham or Splnx. No change was found in serum creatinine and BUN in AKI plus Splnx compared with AKI alone (n = 8–10). mRNA expression of kidney and liver IL-6 was measured by QPCR at 4 h after sham operation, Splnx, AKI, and AKI plus Splnx. No increase in IL-6 mRNA was found in the kidney (C) or in the liver (D) in mice with AKI plus Splnx compared with AKI alone. (n = 3–4).

**Fig. 6.** Splenic, hepatic, and renal IL-10 mRNA production after AKI. Splenic, hepatic, and renal IL-10 mRNA was measured at baseline and at 2, 4, and 6 h after sham operation or ischemic AKI. A: splenic IL-10 mRNA was highly upregulated by 2 h after AKI compared with sham-operated mice. B: hepatic IL-10 was upregulated by 2 h but to a lesser extent than splenic IL-10. C: only basal levels were found in the kidney of mice after AKI or sham operation. *P < 0.05, ***P < 0.001 (n = 3–5).
Cellular source of IL-10 in the spleen after AKI. To determine the cellular source of IL-10 in the spleen after AKI, we performed immunofluorescence on spleen from wild-type mice 4 h after sham or AKI. As shown in Fig. 7A, no IL-10 expression was found in the spleen after sham operation. In contrast, Fig. 7B shows that IL-10 was markedly expressed in the spleen after AKI. IL-10 (in green) colocalized (merge in yellow) with CD11b, CD4, and B220 but not with Ly6G (all in red) in the spleens of wild-type mice 4 h after AKI (Fig. 7B). IL-10-positive cells (in green) were found to be positive for F4/80 (in red), but they did not colocalize. F4/80 is the most specific marker for macrophages, CD11b is a marker for both monocytes/macrophages and neutrophils, and Ly6G is a specific marker for neutrophils. Our data show that IL-10 was expressed by both F4/80 and CD11b cells but not Ly6G, indicating that macrophages but not neutrophils are a source of IL-10 in the spleen 4 h after AKI. IL-10-positive cells were found to be positive for the T cell marker CD4 and for the B cell marker B220. These data demonstrate that the splenic sources of IL-10 4 h after AKI are macrophages, T and B cells.

Since macrophages produce IL-10 in the spleen after AKI, we hypothesized that macrophage programming to an M1 or M2 phenotype may occur in the spleen. Therefore, we analyzed splenic macrophages 4 h after sham operation or AKI by flow cytometry and Western blot. To examine macrophage programming, expressions of iNOS (M1 marker) and CD206 (M2 marker) were analyzed in F4/80-positive, CD11b-positive, and Ly6G-negative cells by flow cytometry. In addition, iNOS (M1) and arginase (M2) expression were analyzed by Western blot. As shown in Fig. 8A, no difference in splenic iNOS (M1) or CD206 (M2) was found 4 h after AKI compared with sham-operated mice by flow cytometry. No difference in splenic iNOS protein expression was found by Western blot 4 h after AKI compared with sham operation (Fig. 8B). Arginase was not detected in the spleen by Western blot. Thus, macrophage programming to M1 or M2 phenotype does not occur in the spleen 4 h after AKI.

Effect of IL-10 administration on lung injury after AKI plus splenectomy. To test whether restoration of IL-10 would improve lung injury in splenectomized mice with AKI, mice underwent ischemic AKI alone or ischemic AKI plus splenectomy with IL-10 or vehicle treatment. At 4 h, lung MPO

---

**Fig. 7.** Splenic IL-10 production is increased 4 h after AKI. Immunofluorescence in OCT-embedded spleens from wild-type mice was analyzed by confocal microscopy 4 h after sham operation or AKI. A: IL-10 (in green) colocalized (merge in yellow) with CD11b, CD4, and B220 but not with Ly6G (all in red) in the spleens of wild-type mice 4 h after AKI. IL-10-positive cells (in green) were found to be positive for F4/80, but they did not colocalize. CD11b is a monocyte/macrophage and neutrophil marker, F4/80 is a macrophage marker, Ly6G is a neutrophil marker, CD4 is a T cell marker, and B220 is a B cell marker. B: no IL-10 was found in the spleen of wild-type mice after sham operation \((n = 5–6)\).
activity (Fig. 9A) and lung CXCL1 (Fig. 9B) were improved in the IL-10-treated mice with ischemic AKI plus splenectomy compared with the vehicle-treated mice.

**Effect of IL-10 administration on serum IL-6 after AKI plus splenectomy.** To test whether restoration of IL-10 in splenectomized mice with AKI would reduce serum IL-6, mice underwent AKI alone or AKI plus vehicle treatment. At 4 h, the increase in serum IL-6 after AKI plus splenectomy was significantly reduced in IL-10-treated mice vs. vehicle-treated mice, as depicted in Fig. 9C.

**Effect of IL-10 administration on kidney and liver IL-6 mRNA expression after AKI plus splenectomy.** To test whether the reduction in serum IL-6 after IL-10 administration to mice undergoing AKI plus splenectomy was due to a reduction of IL-6 production by other organs, mRNA was extracted from kidney and liver 4 h post-AKI plus splenectomy IL-10 or vehicle-treated animals. As shown in Fig. 9D, IL-10 administration reduced the increase in kidney and liver IL-6 mRNA upregulation after AKI plus splenectomy.

**Effect of IL-10 administration on renal function after AKI plus splenectomy.** To determine whether IL-10 treatment affected renal function, serum creatinine was determined 4 h after AKI or AKI plus splenectomy with IL-10 treatment or vehicle treatment. Serum creatinine was lower with IL-10 treatment vs. vehicle in AKI alone; serum creatinine (mg/dl) was 0.88 ± 0.06 in vehicle-treated AKI and was 0.57 ± 0.08 in IL-10-treated AKI ($P = 0.067; n = 5–14$); serum creatinine was similar in vehicle-treated and IL-10-treated AKI plus splenectomy; serum creatinine (mg/dl) was 0.76 ± 0.06 in vehicle-treated AKI plus splenectomy and was 0.64 ± 0.12 in IL-10-treated AKI plus splenectomy ($P = 0.33; n = 5–14$).

**DISCUSSION**

Increased proinflammatory cytokine production occurs after a wide variety of illnesses and insults and can cause the SIRS, acute lung injury (ALI), and multiple organ dysfunction syndrome (MODS). AKI is considered to be a proinflammatory state (26), but it is currently not considered to be a cause of SIRS, ALI, or MODS. Clinically, the effect of AKI on remote organ injury is difficult to ascertain because AKI is typically diagnosed very late, thus, AKI often appears to be a consequence of ALI or MODS. Recently, however, it has been appreciated that AKI can initiate a proinflammatory response and that the proinflammatory effects of AKI occur early. For example, we recently demonstrated that serum IL-6 and IL-8 are increased by patients with AKI and that the increase in these cytokines is associated with prolonged mechanical ventilation (20). In the present study, we examine the early proinflammatory response of AKI in an animal model of ischemic AKI. Herein, we demonstrate that renal and extrarenal cytokine production increase within 6 h after AKI. Furthermore, we demonstrate that if the early proinflammatory burst associated with AKI goes unchecked, the inflammatory response after AKI is exacerbated and prolonged. Our data have important implications regarding the pro- and anti-inflammatory responses that occur in AKI and provide further evidence...
supporting the notion that, in patients, AKI may be a potential trigger for ALI and MODS via proinflammatory cytokine production.

To investigate potential sources of proinflammatory cytokine production after AKI, we first examined the time course of renal cytokine production. In the kidney, IL-6, CXCL1, and IL-1β were increased by 2 h, and TNF-α production was increased by 6 h. Because we previously demonstrated that serum cytokines are increased after bilateral nephrectomy (12), we reasoned that extrarenal cytokine production might also occur after AKI. Indeed, hepatic and splenic production of the proinflammatory cytokines IL-6, CXCL1, IL-1β, and TNF-α were all increased. Although proinflammatory cytokine production after ischemic AKI has been extensively examined in the kidney and lung (8, 10, 12, 17), splenic and hepatic cytokine production after ischemic AKI has not been systematically analyzed. Taken together, data demonstrate that serum cytokines are increased after bilateral nephrectomy (12), we reasoned that extrarenal cytokine production might also occur after AKI. Indeed, hepatic and splenic production of the proinflammatory cytokines IL-6, CXCL1, IL-1β, and TNF-α were all increased. Although proinflammatory cytokine production after ischemic AKI has been extensively examined in the kidney and lung (8, 10, 12, 17), splenic and hepatic cytokine production after ischemic AKI has not been systematically analyzed. Taken together, data demonstrate that AKI is an early proinflammatory state and that the kidney, lung, spleen, and liver all participate in cytokine production after ischemic AKI. In general, proinflammatory cytokine production appears to increase in the kidney first (within 2 h) and in the spleen and liver later (4 to 6 h).

Since proinflammatory cytokines were increased in the spleen, we hypothesized that splenectomy might reduce serum proinflammatory cytokines and thus protect against AKI-mediated lung injury. In fact, splenectomy improved lung injury after liver (14) or intestinal ischemic reperfusion injury (25) in mice; the effect of splenectomy on remote organ injury after organ ischemia may be dependent on the site of ischemia. Splenectomy itself did not appear to be deleterious as serum IL-6 and lung injury were similar after splenectomy and sham operation; furthermore, extrarenal IL-6 production, renal function, and renal injury were similar after AKI plus splenectomy vs. AKI alone.

Therefore, we questioned whether the spleen might produce a beneficial factor after AKI and found that production of the anti-inflammatory cytokine IL-10 was massively increased in the spleen 2 h after AKI. Colocalization experiments revealed that the cellular sources of IL-10 production were macrophages, T cells, and B cells, but not neutrophils. Since macrophages were producing IL-10 after AKI, we questioned whether programming to an M1 or M2 phenotype may have occurred; however, no difference in the M1 marker iNOS or the M2 marker CD206 was observed. The lack of macrophage programming is likely due to the early time point (4 h) studied (1, 19, 21).

The anti-inflammatory effect of IL-10 has broad clinical relevance. In patients, the presence of an IL-10 polymorphism that increases IL-10 production is associated with reduced mortality in critically ill patients with ALI (7) as well as...
patients with AKI requiring renal replacement therapy (13). Conversely, IL-10 polymorphisms associated with reduced IL-10 production are associated with increased mortality. Thus, our novel data that endogenous IL-10 production is necessary to limit inflammatory lung injury after AKI are consistent with these clinical observations. Clinically, it may be feasible to screen patients undergoing procedures at high risk for AKI (e.g., cardiac surgery) for low-producing IL-10 polymorphisms and prophylaxis against AKI and ALI with exogenous IL-10 administration.

Even though IL-10 administered before splenectomy dampened the proinflammatory response, it is important to recognize that splenectomy with AKI was associated with increased serum IL-10 levels. In fact, serum IL-6, CXCL1 (a murine analog to human IL-8), and IL-10 were all higher in splenectomy with AKI vs. AKI alone. These findings are especially clinically relevant as serum IL-6, IL-8, and IL-10 are also increased in patients with AKI and predict increased mortality (26). The presence of the increase in both pro- and anti-inflammatory cytokines in the serum is the hallmark of a dysregulated inflammatory response that has been shown to contribute to ALI and MODS (22). Notably, increased serum IL-10 is also associated with increased mortality in patients with ALI (23). Furthermore, in a cohort of patients, individuals with high circulating levels of both IL-6 and IL-10 cytokines had a markedly increased risk of severe sepsis and death (16). In the present study, when serum IL-6, CXCL1, and IL-10 were the highest, lung injury was the worst. One can speculate that there is an attempt to downregulate the proinflammatory response via IL-10 resulting in increased serum levels; however, if the increase in IL-10 production occurs late, then a tipping point has already been reached where the proinflammatory response has accelerated and lung injury and other organ dysfunctions have already occurred.

The clinical relevance of our findings may also extend to patients who require splenectomy. The most feared consequence of acute splenectomy is death from overwhelming sepsis and the development of MODS. The normal role of the spleen in battling infections has been attributed to a lack of B cell IgM production and reduced bacterial opsonization. Our data suggest that splenic IL-10 production is necessary to downregulate the proinflammatory response to injury. Thus, one may speculate that death from overwhelming sepsis after splenectomy may also be related to an inability to downregulate the septic proinflammatory response, thus leading to dysregulated cytokine production and MODS. Currently, it is thought that control of inflammation by IL-10 is a nonredundant process (24), thus compensation for a loss of a source of IL-10 production during splenectomy may not occur. Conclusion. In the present study, we demonstrate that splenectomy notably exacerbates the proinflammatory response that occurs in ischemic AKI by removal of the anti-inflammatory cytokine IL-10. These data do have broad implications in understanding the deleterious systemic effects that occur in patients with acute splenectomy as well as AKI. Currently, AKI is not generally regarded as a cause of ALI or MODS; our data, however, demonstrate that AKI in the setting of an inadequate counter-inflammatory response results in exuberant systemic inflammation. Further study of the conditions that affect the counterinflammatory response in AKI may be the key to understanding and reducing the increased mortality that is observed in patients with AKI.

GRANTS
This study was supported by the following research grants: American Heart Association Beginning Grant in Aid, American Society of Nephrology Career Development Grant (Gottschalk Award), National Institutes of Health funding from National Heart, Lung, and Blood Institute: R01 HL005363 to S. Faubel, MD and American Heart Association Postdoctoral Fellowship to A. Andrés-Hernando.

Portions of this work were presented in abstract form at the 42nd and 43rd annual meetings of the American Society of Nephrology, October 2009, San Diego, CA and November 2010, Denver, CO.

DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

REFERENCES

AJP-Renal Physiol • VOL 301 • OCTOBER 2011 • www.ajprenal.org