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Cytokine production increases and cytokine clearance decreases in mice with bilateral nephrectomy

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Abstract

Background. Serum cytokines are increased in patients with acute kidney injury (AKI) and predict increased mortality. It is widely assumed that increased renal production of cytokines is the source of increased serum cytokines; the role of extra-renal cytokine production and impaired renal cytokine clearance is less well studied. We hypothesized that cytokine production in AKI was mononuclear phagocyte dependent, independent of production by the kidneys, and that serum cytokine clearance would be impaired in AKI.

Methods. Bilateral nephrectomy was used as a model of AKI to assess cytokine production independent of kidney cytokine production. Mononuclear phagocytes were depleted utilizing intravenous (IV) administration of liposome-encapsulated clodronate (LEC). Twenty-three serum cytokines were determined utilizing a multiplex cytokine kit. Proteins for cytokines were determined in the spleen and liver by enzyme-linked immunosorbent assay. Recombinant cytokines were injected by IV into mice with bilateral nephrectomy to determine the effect of absent kidney function on serum cytokine clearance.

Results. Serum interleukin (IL)-6, chemokine (C-X-C motif) ligand 1 (CXCL1), IL-10, IL-1β, monocyte chemotactic protein 1 (MCP-1), IL-5 and eotaxin were increased in the serum of mice after bilateral nephrectomy and were reduced with LEC. Serum IL-12p40 and regulated upon activation, normal T-cell expressed, and secreted (RANTES) were increased after bilateral nephrectomy and were further increased with LEC. Spleen IL-6, CXCL1, IL-10 and IL-1β and liver IL-6 and IL-10 were increased after bilateral nephrectomy. After IV injection, IL-6, CXCL1, IL-10 and IL-1β had a prolonged serum cytokine appearance in mice with bilateral nephrectomy versus sham operation.

Conclusions. Increased mononuclear phagocyte production and impaired renal clearance contribute to serum cytokine accumulation in AKI, independent of kidney injury. The effect of AKI on cytokine production and clearance may contribute to the increased mortality of patients with AKI.

Keywords: chemokines; CXCL1; cytokines; IL-6; mononuclear phagocytes

Introduction

Serum cytokines are increased in patients with acute kidney injury (AKI), and increased serum interleukin (IL)-6, IL-8 and IL-10 predict mortality in patients with AKI [1]. Cytokines are not just biomarkers of poor outcomes, but mediate deleterious systemic effects. Pro-inflammatory cytokines such as IL-6 and IL-8 mediate distant organ injury and contribute to the systemic inflammatory response syndrome and acute lung injury (ALI). Anti-inflammatory cytokines such as IL-10 may contribute to immune stasis and impair the ability to fight infection. These cytokine-mediated deleterious effects may contribute to the high mortality of patients with AKI. Thus, a better understanding of the production and clearance of cytokines in AKI may shed light onto potential therapeutic treatments for patients with AKI.

The role of the kidneys in cytokine production after AKI is well described in animal models of AKI [2–3]. Less well studied is the contribution that extra-renal cytokine production and impaired renal cytokine clearance may have on serum cytokine accumulation in AKI. We have previously demonstrated that certain cytokines are increased in the serum after bilateral nephrectomy [2], a model of acute renal failure without kidneys. Since kidney production of cytokines cannot explain increased serum cytokines after bilateral nephrectomy, we hypothesized that other mechanisms of serum cytokine accumulation may occur in AKI.

Mononuclear phagocytes, known as monocytes when in the blood and macrophages when in tissues, are a key source of cytokine production after a variety of insults. Mononuclear phagocytes mediate AKI, and the depletion of mononuclear phagocytes with liposome-encapsulated clodronate (LEC) protects against ischemic AKI and reduces renal cytokine production [4–6]. The role of mononuclear phagocytes, independent of the kidney, in cytokine production in AKI is unknown.

In the present study, therefore, we hypothesized that cytokine production in AKI is mononuclear phagocyte dependent, independent of production by the kidneys, and that serum cytokine clearance is impaired in AKI. In order to test this hypothesis, we depleted mononuclear phagocytes by intravenous (IV) injection of LEC, bilateral nephrectomy was performed and serum cytokines were determined. To determine the effect of absent kidney function on serum cytokine clearance, recombinant cytokines were injected by IV into mice with bilateral nephrectomy and serum cytokines were measured.

Materials and methods

Mice

For all the mouse studies, 8- to 10-week-old C57BL/6 mice (Jackson Labs, Bar Harbor, ME) weighing 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 NIH 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.

Sham operation and bilateral nephrectomy protocol

Mice were anesthetized with an intraperitoneal injection of avertin (2,2,2-tribromoethanol: Aldrich, Milwaukee, WI). For bilateral nephrectomy, a midline incision was made and the renal pedicles were tied off with suture and then cut distally. The ureters were pinched off with forceps and the kidneys removed. The bilateral nephrectomy model is well established in our laboratory [2, 7]. Sham surgery consisted of the same procedure except that the renal pedicles were not tied off and the kidneys were not removed.

Assessment of renal function

Blood urea nitrogen (BUN) and serum creatinine were measured using a QuantiChrom assay kit (BioAssay Systems, Hayward, CA).

Lung CXCL1

Lung CXCL1 was performed on whole lung homogenates by enzyme-linked immunosorbent assay (ELISA; R&D Systems, Minneapolis, MN) and corrected for protein. The detection limit is 2.0 pg/mL. Tissue was prepared as described previously [7].

Lung myeloperoxidase activity

One-fourth of the lung was homogenized as described previously [7].

Cytokine injection

Mice underwent sham operation or bilateral nephrectomy. Immediately after surgery, 200 ng of recombinant murine IL-6, CXCL1, IL-10 or IL-1β (Peprotech) in 100 µL of phosphate-buffered saline (PBS) with 0.1% bovine serum albumin was administered intravenously. Vehicle-treated mice received 100 µL of PBS with 0.1% bovine serum albumin. Blood was collected by cardiac puncture 60 min after receiving IV cytokine or vehicle.

Mononuclear phagocyte depletion

LEC and empty liposomes were purchased from Encapsula NanoSciences (Nashville, TN). Mice received a tail vein injection of 10 µg of vehicle (empty liposomes) or LEC in 100 µL sterile PBS at 5 and 2 days before sham operation or bilateral nephrectomy.

Serum cytokine measurement

Blood samples were obtained at sacrifice via cardiac puncture. To assure uniformity of serum samples, all samples were processed in the same manner as we have described previously [2, 7]. Twenty-three cytokines [IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-9, IL-10, IL-12 (p40), IL-12 (p70), IL-13, IL-17, eotaxin, granulocyte colony-stimulating factor, granulocyte macrophage colony-stimulating factor, interferon-γ, KC, MCP-1, macrophage inflammatory protein 1 (MIP-1)α, MIP-1β, RANTES and *tumor necrosis factor (*TNF)-α] were determined on serum samples using a bead-based multiplex cytokine kit (Bio-Rad Laboratories Inc., Hercules, CA) in conjunction with flow-based protein detection and the Luminex LabMAP multiplex system (Luminex Corp., Austin, TX) according to the manufacturer's direction. The detection limit for each cytokine was 1.95 pg/mL.

Spleen and liver cytokine measurement by ELISA

The frozen spleen or liver 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 *g* for 15 min. Supernatants were analyzed for protein content using a Bio-Rad DC protein assay kit. Supernatants were analyzed for CXCL1/KC, IL-6, IL-10, IL-1β and TNF-α by ELISA (R&D Systems).

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 the spleen ($1–2 \times 10^6$) were stained for cell surface antigen expression by incubating with antibodies at 4°C for 30 min in the dark, 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% paraformaldehyde for 15–20 min on ice. The following antibodies were used: F4/80-APC and CD11b-PE (eBiosciencies), CD45-V500 and Ly6G-APCCy7 (BD Pharmingen). Cells were gated according to the size and scatter to eliminate dead cells and debris from analysis.

Statistical analysis

All values are expressed as mean ± SE. Non-parametric *t*-tests were performed for the following experimental groups: sham operation versus bilateral nephrectomy and bilateral nephrectomy versus bilateral nephrectomy plus LEC. For cytokine injection experiments, non-parametric *t*-tests were performed for the following: sham plus vehicle versus sham plus cytokine; one-way ANOVA with the Dunnet post-test analysis with bilateral nephrectomy plus cytokine as the control group was performed. For *t*-tests, groups with significant variance were subjected to Welch's correction. A value of $P < 0.05$ was considered statistically significant.

Results

IV administration of LEC reduces splenic mononuclear phagocytes

IV LEC is known to result in the systemic depletion of circulating monocytes and resident mononuclear phagocytes in the spleen and liver. To confirm mononuclear phagocyte depletion with administration in the setting of bilateral nephrectomy, flow cytometry was performed. As shown in Figure 1, IV LEC administration to mice 5 and 2 days before sham operation and bilateral nephrectomy resulted in a significant reduction in splenic mononuclear phagocytes defined as CD45-positive, F4/80-positive and Ly6G-negative cells.

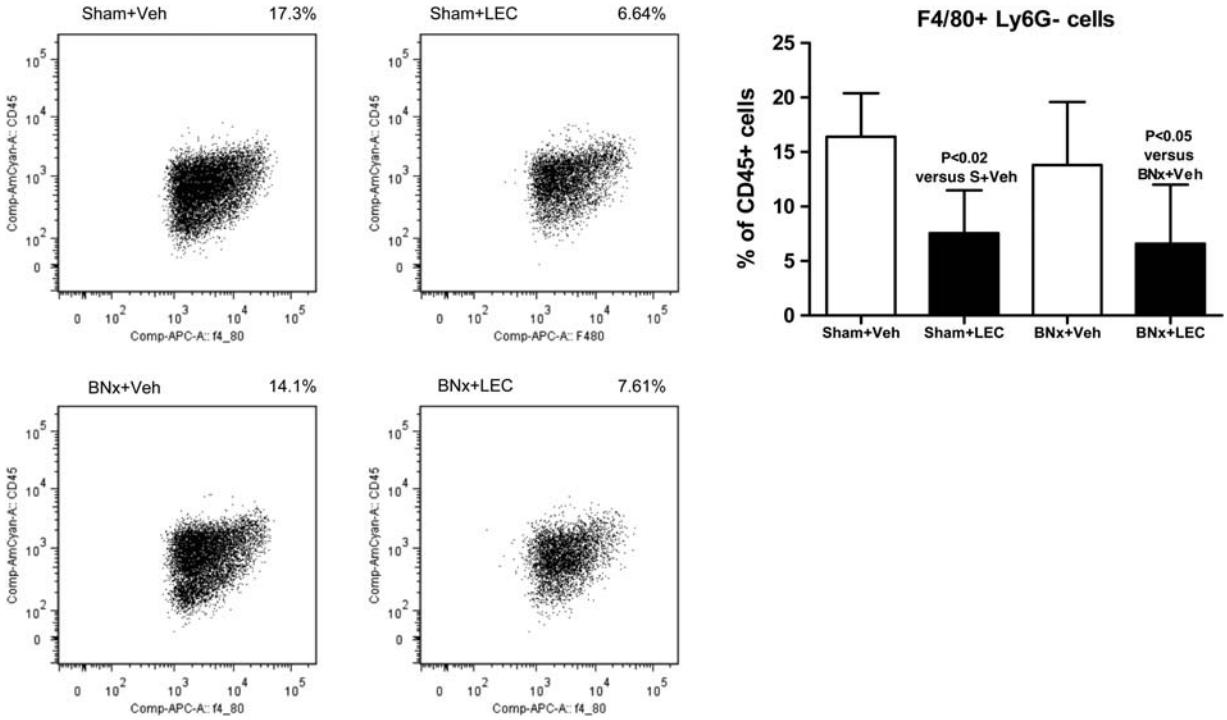

Fig. 1. IV administration of LEC reduces splenic mononuclear phagocytes. Sham operation (Sham) or bilateral nephrectomy (BNx) was performed with or without administration of LEC. Flow cytometry was performed to confirm mononuclear phagocyte depletion. IV LEC administration to before sham operation and bilateral nephrectomy resulted in a significant reduction in splenic mononuclear phagocytes defined as CD45-positive, F4/80-positive and Ly6G-negative cells ($n = 4$–7).

Table 1. Summary of serum cytokine results after bilateral nephrectomy

	Bilateral nephrectomy	Bilateral nephrectomy + LEC
IL-6	↑	↓
CXCL1	↑	↓
IL-1β	↑	↓
IL-10	↑	↓
MCP-1	↑	↓
IL-5	↑	↓
Eotaxin	↑	↓
IL-12 (p40)	↑	↑
RANTES	↑	↑
G-CSF	↑	=

Twenty-three serum cytokines were determined after bilateral nephrectomy and bilateral nephrectomy with LEC treatment.

Serum IL-6, CXCL1, IL-10, IL-1β, MCP-1, IL-5 and eotaxin are increased after bilateral nephrectomy and reduced with LEC administration

To examine the role of mononuclear phagocyte production of cytokines in AKI, 23 serum cytokines were determined in mice 4 h after sham operation or bilateral nephrectomy with or without IV LEC administration (Table 1). Of the 23 serum cytokines measured, 10 were increased after bilateral nephrectomy compared with sham operation: IL-6, CXCL1, IL-10, IL-1β, MCP-1, IL-5, eotaxin, IL-12 (p40), RANTES and G-CSF. Of these, seven were reduced with LEC (IL-6, CXCL1, IL-10, IL-1β, MCP-1, IL-5 and eotaxin) (Figure 2) and two were increased with LEC administration [IL-12 (p40) and RANTES] (Figure 3). LEC did not have a significant effect on serum G-CSF after bilateral nephrectomy (data not shown). None of the other cytokines measured showed significant differences between sham operation versus bilateral nephrectomy or bilateral nephrectomy versus bilateral nephrectomy plus LEC. Confirmatory ELISA for serum IL-6 was performed and similar results were obtained as results for IL-6 determined by the Luminex method (data not shown).

Renal function was assessed after bilateral nephrectomy with administration of IV LEC or vehicle and no significant changes were found in serum creatinine and BUN. Specifically, serum creatinine was 1.28 ± 0.15 mg/dL in bilateral nephrectomy plus vehicle and 1.22 ± 0.17 mg/dL in bilateral nephrectomy plus LEC (P = NS, $n = 5$–8). BUN was 60 ± 5.1 mg/dL in bilateral nephrectomy plus vehicle and 53 ± 6.8 mg/dL in bilateral nephrectomy plus LEC (P = NS, $n = 5$–8).

We have previously shown that lung inflammation occurs by 2 h after bilateral nephrectomy [7]. To determine whether administration of LEC has an effect on lung inflammation, lung myeloperoxidase (MPO) activity, which is a marker of neutrophil infiltration, and lung CXCL1 (a neutrophil chemokine) were assessed 2 h after sham surgery and bilateral nephrectomy with administration of LEC or vehicle. Lung inflammation was not reduced after LEC treatment compared with vehicle after bilateral nephrectomy or sham operation. Specifically, lung MPO activity was 0.475 ± 0.1 Δ/min/mg in sham

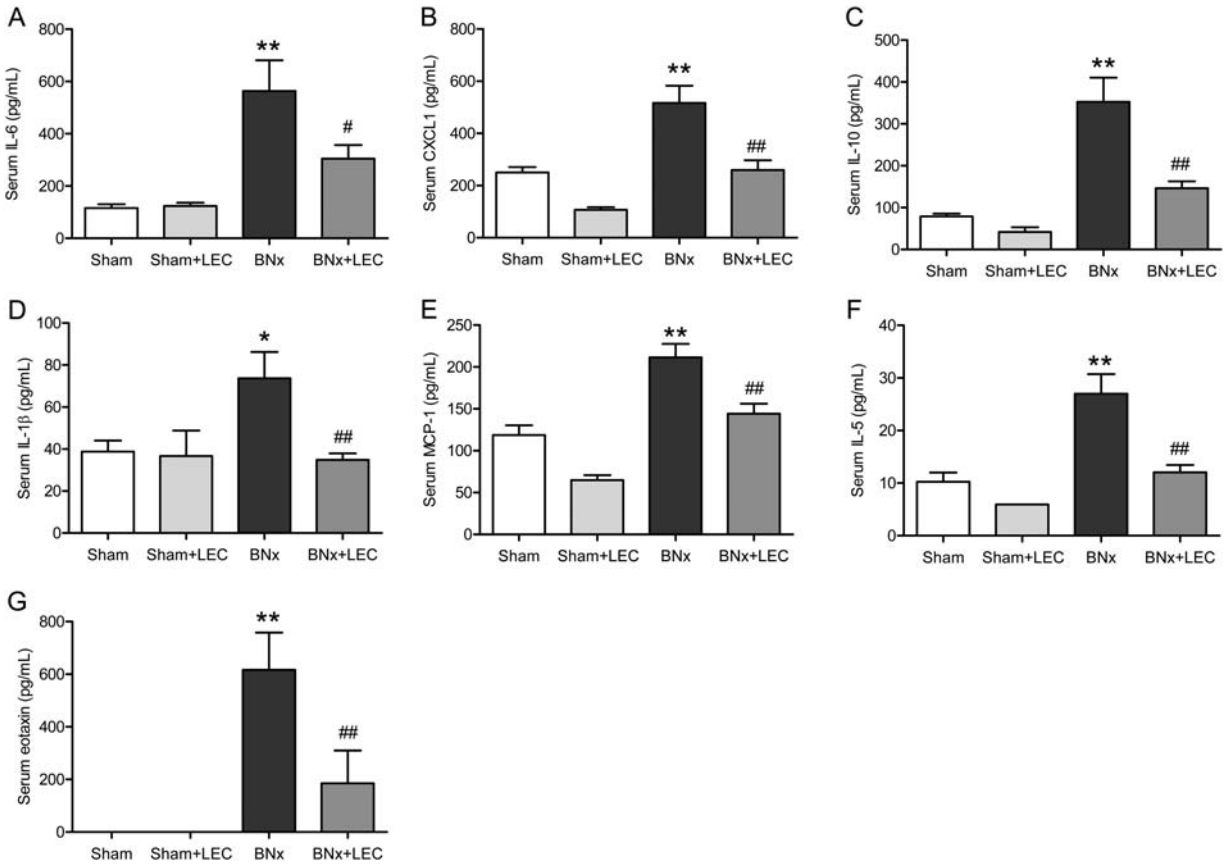

Fig. 2. Serum IL-6, CXCL1, IL-10, IL-1β, IL-5, MCP-1 and eotaxin were increased after bilateral nephrectomy and reduced with LEC treatment. Sham operation (Sham) or bilateral nephrectomy (BNx) was performed with or without administration of LEC to deplete mononuclear phagocytes. Serum (**A**) IL-6, (**B**) CXCL1, (**C**) IL-10, (**D**) IL-1β, (**E**) IL-5, (**F**) MCP-1 and (**G**) eotaxin were all increased in mice with bilateral nephrectomy versus sham operation and reduced with LEC treatment. *$P < 0.05$ versus Sham,**$P < 0.01$ versus Sham, $^{\#}P < 0.05$ versus BNx, $^{\#\#}P < 0.01$ versus BNx ($n = 4$–7).

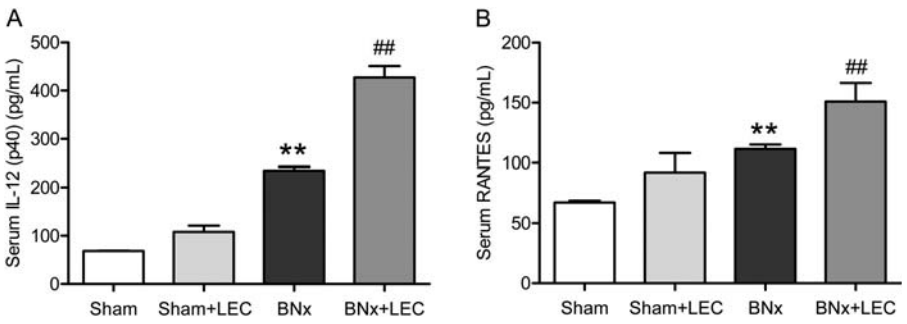

Fig. 3. Serum IL-12 (p40) and RANTES were increased after bilateral nephrectomy and further increased with LEC treatment. Sham operation (Sham) or bilateral nephrectomy (BNx) was performed with or without administration of LEC to deplete mononuclear phagocytes. Serum (**A**) IL-12 (p40) and (**B**) RANTES were both increased in mice with bilateral nephrectomy versus sham operation and were further increased with LEC treatment. **$P < 0.01$ versus Sham, $^{\#\#}P < 0.01$ versus BNx ($n = 4$–7).

plus vehicle and 0.65 ± 0.103 (Δ/min/mg) in sham plus LEC (P = NS, $n = 5$–8) and was 1.44 ± 0.22 (Δ/min/mg) in bilateral nephrectomy plus vehicle and $1.52 \pm .16$ (Δ/min/mg) in bilateral nephrectomy plus LEC (P = NS, $n = 5$–8). Lung CXCL1 was $2723. \pm 54.7$ pg/mg in sham plus vehicle and 219 ± 75.2 pg/mg in sham plus LEC (P = NS, $n = 5$–8) and was 438 ± 58.7 pg/mg in bilateral nephrectomy plus vehicle and 409 ± 43.6 pg/mg in bilateral nephrectomy plus LEC (P = NS, $n = 5$–8). Since we have previously demonstrated that IL-6 is an important mediator of lung inflammation after bilateral nephrectomy [7], we speculate that the reduction in serum IL-6 with LEC, while significant, was insufficient to prevent the development of lung inflammation after bilateral

nephrectomy. Furthermore, we have demonstrated that alveolar macrophages are important in CXCL1 production and neutrophil accumulation after ischemic AKI; alveolar macrophages are not depleted with IV LEC and may thus also contribute to lung inflammation after bilateral nephrectomy [8]. Indeed, mononuclear phagocytes are likely to have a complex role in the development of lung inflammation after AKI.

Protein for IL-6, CXCL1, IL-10, IL-1β and TNF-α in the spleen and liver after bilateral nephrectomy

Proteins for IL-6, CXCL1, IL-10, IL-1β and TNF-α were determined by ELISA in the spleen and liver 2 h after sham operation or bilateral nephrectomy. As shown in Figure 4, IL-6, CXCL1, IL-10 and IL-1β were increased in the spleen 2 h after bilateral nephrectomy versus sham; IL-6 and IL-10 were also significantly increased in the liver 2 h after bilateral nephrectomy versus sham. TNF- α did not increased in the liver or spleen after bilateral nephrectomy.

To determine whether administration of LEC would influence cytokine production, proteins for IL-6, CXCL1, IL-10, IL-1β and TNF-α were determined by ELISA in the spleen and liver 2 h after bilateral nephrectomy with LEC or vehicle administration. As shown in Figure 5, we found a reduction in IL-6 and CXCL1 production 2 h after bilateral nephrectomy and LEC administration compared with vehicle in the spleen, but no change in these cytokines was found in the liver. IL-1β was reduced in the liver after bilateral nephrectomy and LEC administration compared with vehicle with no significant change in the spleen. No change in IL-10 or TNF-α production was found in the spleen or liver after bilateral nephrectomy with or without LEC administration.

Injection of cytokines to mice with bilateral nephrectomy

To determine whether lack of kidney function resulted in delayed serum cytokine clearance in mice with bilateral nephrectomy, 200 ng of IL-6, CXCL1, IL-10, IL-1β, TNF-α or vehicle was administered by tail vein injection

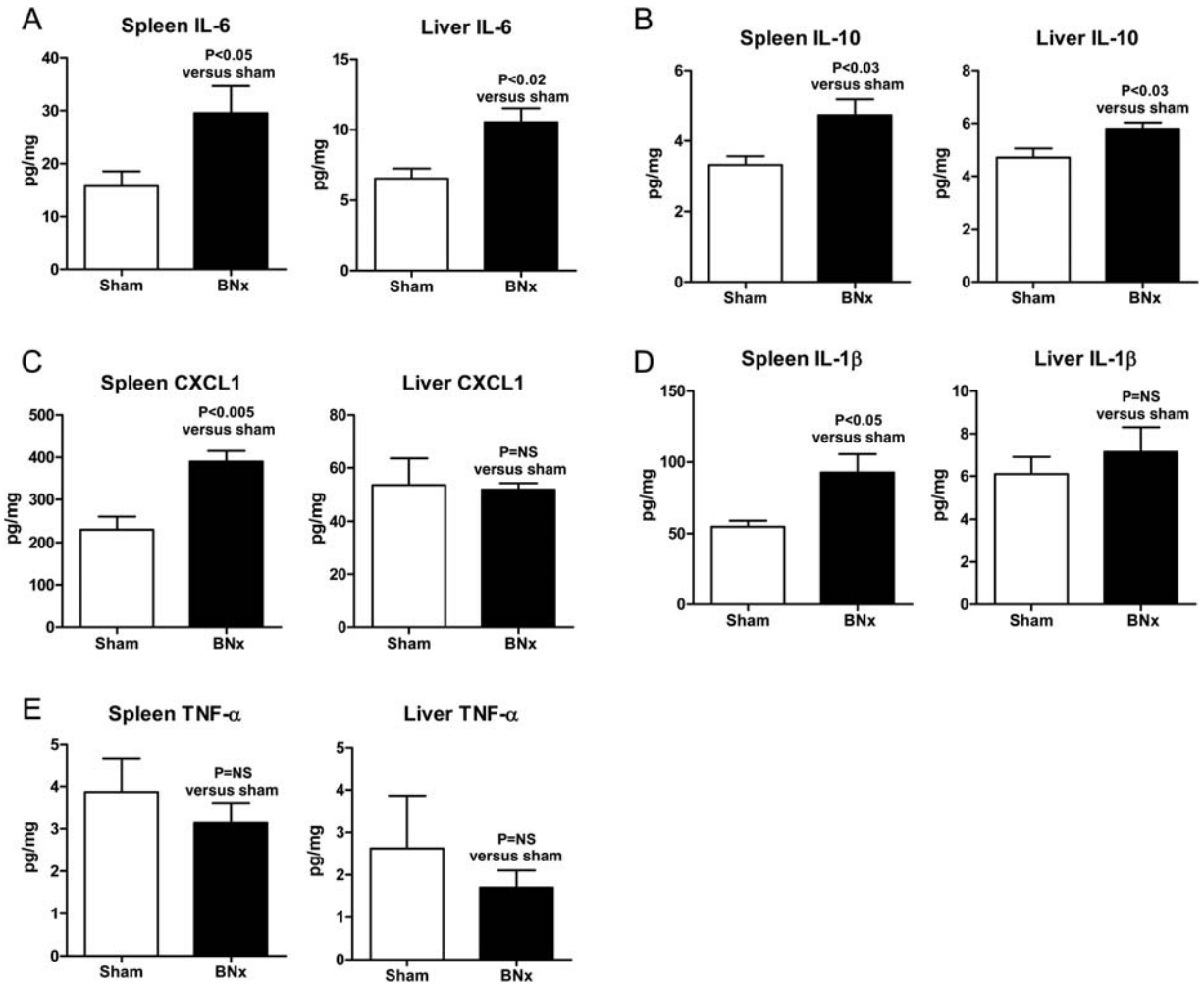

Fig. 4. IL-6, CXCL1 and IL-10 protein are increased in the spleen 2 h after bilateral nephrectomy. Sham operation (Sham) or bilateral nephrectomy (BNx) was performed, and (**A**) IL-6, (**B**) IL-10, (**C**) CXCL1, (**D**) IL-1β and (**E**) TNF-α were measured in the spleen and liver by ELISA 2 h post-procedure. IL-6 and IL-10 were significantly increased in both organs. CXCL1 and IL-1β was significantly increased in the spleen but not the liver. TNF-α did not increase in either organ ($n = 5–8$).

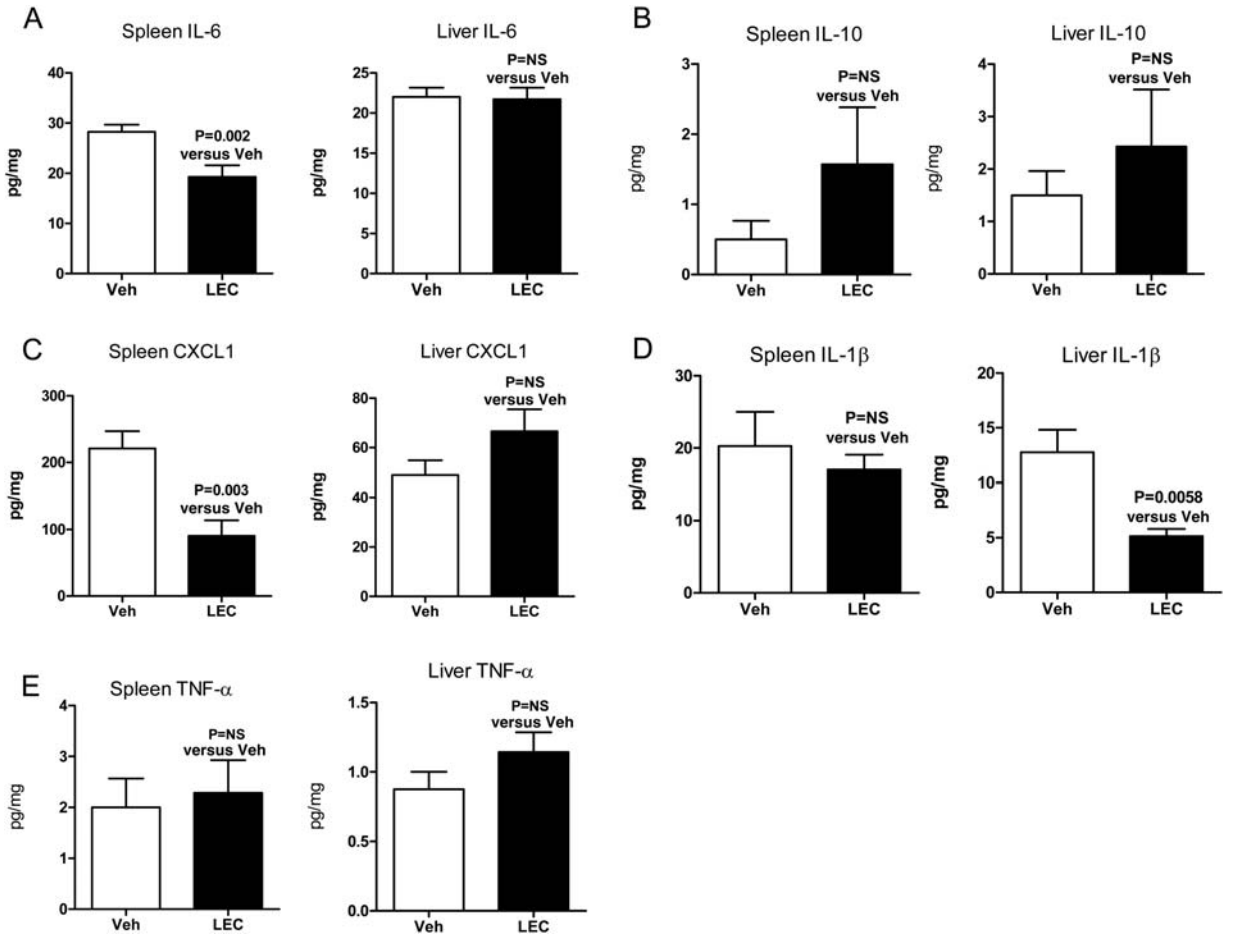

Fig. 5. IL-6 and CXCL1 are reduced in the spleen 2 h after bilateral nephrectomy and LEC. Bilateral nephrectomy (BNx) with LEC or Vehicle administration was performed, and (**A**) IL-6, (**B**) IL-10, (**C**) CXCL1, (**D**) IL-1β and (**E**) TNF-α were measured in the spleen and liver by ELISA 2 h post-procedure. IL-6 and CXCL1 were significantly decreased in the spleen after LEC treatment. IL-1β was significantly decreased in the liver after LEC treatment. IL-10 and TNF-α did not change in either organ ($n = 5–8$).

immediately after surgery to sham-operated mice or mice with bilateral nephrectomy, and serum cytokine levels were determined 1 h post-injection.

As shown in Figure 6, the injection of a cytokine to sham-operated mice resulted in a detectable and significant increase in the cytokine versus vehicle injection. These data demonstrate that even with normal kidney function, the dose of cytokine injection was not able to be completely cleared from the serum within 1 h. For IL-6, CXCL1, IL-10 and IL-1β, serum cytokine levels greater than 2-fold higher were detected in the setting of bilateral nephrectomy versus sham operation. As shown in Figure 7, bilateral nephrectomy did not affect the serum clearance of TNF-α.

Discussion

AKI complicates ∼20% of hospital admissions [9] and 30–50% of intensive care unit admissions [10]. AKI confers an independent, excess risk of mortality [11–15], regardless of whether the AKI is mild [16–17] or requires renal replacement therapy [18–19]. The high mortality associated with AKI suggests that there are deleterious systemic effects of AKI which are potentially fatal.

Clinical and experimental data are mounting that AKI causes widespread distant organ injury [20–21] and pro-inflammatory cytokines have been shown in animal models to mediate lung, gastrointestnal, cardiac and brain injury after AKI [2, 22–24]. In patients, the role of pro-inflammatory cytokines in mediating distant organ injury, ALI and multiple organ dysfunction syndrome is well described [25–26]. The pro-inflammatory response is typically followed by an anti-inflammatory response known as the compensatory anti-inflammatory response syndrome [27]. Clinical and experimental data indicate that an anti-inflammatory response also occurs in AKI characterized by increased splenic and hepatic IL-10 production [28–29] and increased serum IL-10 [1–2, 28]. Although IL-10 may be necessary to counter the pro-inflammatory response after AKI, excess IL-10 may impair infection fighting ability and it is well known that infection is a key cause of death in patients with AKI [30–31]. Thus, both pro- and anti-inflammatory cytokines may contribute to the increased mortality observed in patients with AKI. Supporting this notion are clinical data demonstrating that

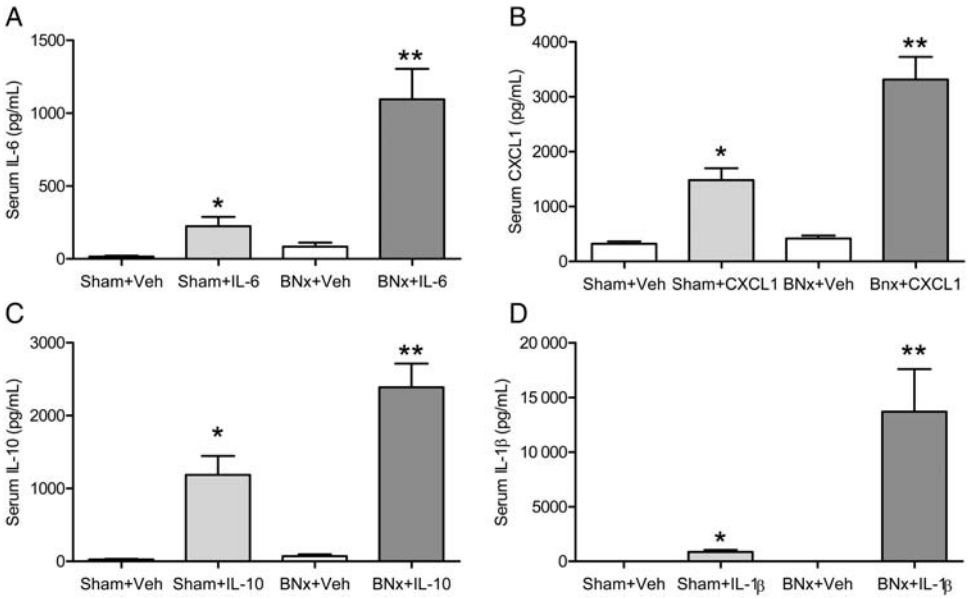

Fig. 6. Serum clearance of IL-6, CXCL1, IL-10 and IL-1β is impaired in mice with bilateral nephrectomy. Vehicle (Veh) or 200 ng of (**A**) IL-6, (**B**) CXCL1, (**C**) IL-10 or (**D**) IL-1β were administered intravenously by tail vein injection to mice immediately after sham operation (Sham) or bilateral nephrectomy (BNx). Serum cytokine levels were determined 60 min post-injection. *$P < 0.05$ versus Sham + Veh; **$P < 0.05$ versus all other groups ($n = 3$–7).

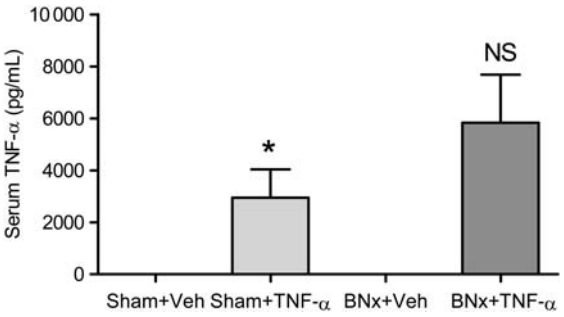

Fig. 7. Serum clearance of TNF-α is not affected by bilateral nephrectomy. Vehicle (Veh) or 200 ng of TNF-α was administered intravenously by tail vein injection to mice immediately after sham operation (Sham) or bilateral nephrectomy (BNx). Serum cytokine levels were determined 60 min post-injection. *$P < 0.05$ versus Sham + Veh; NS, not significant versus Sham + TNF-α ($n = 4$–10).

serum IL-6, IL-8 and IL-10 are increased and predict increased morality in patients with AKI [1].

Serum cytokines may accumulate in AKI through many mechanisms including increased renal production, increased extra-renal production and impaired renal clearance. Since AKI is characterized by injury to kidney tissue, it has been presumed that renal cytokine production from injury—whether ischemic or nephrotoxic—is the inciting event which leads to serum cytokine accumulation. Indeed, mRNA or protein of multiple cytokines has been demonstrated in the kidney after ischemic or cisplatin-induced AKI [2–3, 32–33].

In the present study, we sought to determine whether serum cytokine accumulation in AKI was 'independent' of injured kidney tissue and we therefore studied cytokine production and clearance after bilateral nephrectomy, a model of acute renal failure without kidney injury. Our novel data demonstrate that mononuclear phagocytes mediate serum cytokine accumulation, independent of injured kidney.

To examine the role of mononuclear phagocytes in cytokine production after AKI, independent of renal cytokine production, we administered IV LEC to mice with bilateral nephrectomy. The use of LEC is a well-described strategy to deplete mononuclear phagocytes [34–37], although dendritic cells are also depleted with this strategy [38–40]. Mononuclear phagocytes and dendritic cells ingest the liposomes causing death via apoptosis; cell death occurs without cytokine release. The population of cells depleted depends on the route of administration; IV LEC, as used in this study, reduces mononuclear phagocytes from the bone marrow, blood, liver and spleen [34, 41].

Our data in the present study suggest that mononuclear phagocytes and dendritic cells not derived from the kidney contribute extensively to the systemic inflammatory state of AKI. Of the 10 cytokines that increased after bilateral nephrectomy, 7 (IL-6, CXCL1, IL-10, IL-1β, MCP-1, IL-5 and eotaxin) were reduced with LEC treatment. These data are consistent with what is known in patients with end-stage kidney failure in whom extra-renal cytokine production occurs in part due to the uremic environment [42]. For example, stimulated monocytes from end-stage renal disease patients produce excess IL-10 [43]. In contrast, the expression of RANTES and IL-12(p40) was further increased with LEC treatment. One can speculate that mononuclear phagocytes and/or dendritic cells are responsible for the secretion of soluble factors that may inhibit the production of RANTES and IL-12p40 after bilateral nephrectomy.

In light of our data, it seems possible that the uremic environment of 'acute' renal failure is also a stimulus for mononuclear phagocyte cytokine production. Indeed, products normally cleared by the kidney accumulate rapidly as judged by the 3-fold increase in BUN just 4 h after bilateral nephrectomy; thus, some factor(s) normally cleared by the kidney may be the stimulus for mononuclear phagocyte/dendritic cell cytokine production after AKI.

To further assess cytokine production after bilateral nephrectomy, proteins for IL-6, CXCL1, IL-10, IL-1β and TNF-α were determined in the liver and spleen. All except TNF-α were found to be increased in the spleen 2 h after bilateral nephrectomy, and IL-6, CXCL1 and IL-10 were also increased in the liver 2 h after bilateral nephrectomy. LEC treatment decreased splenic IL-6 and CXCL1 production and hepatic IL-1β. We chose to examine these five cytokines as they are the most clinically relevant in patients with AKI and critical illness in general. All of these cytokines have been shown to be increased in the serum of patients with AKI [1].

Pharmacokinetic studies have demonstrated that the kidney plays a role in cytokine clearance via filtration and proximal tubule metabolism [44–45]. Other key mechanisms of cytokine clearance are liver metabolism and cytokine binding to a specific cytokine receptor. Several other organs such as the gut, spleen and lungs have an additional but small catabolic role [46]. Generally, it has been observed that the kidney accounts for ∼15–20% cytokine metabolism versus other mechanisms of clearance. For example, in a study in which normal rats were injected with radiolabelled IL-10, 23% accumulated in the kidney, 11% in the liver and less than 5% in other organs and urine [47]. In another study, also performed in normal rats, radiolabelled IL-6 was injected and 80% accumulated in the liver and 15% in the kidney [48]. Whether impaired renal metabolism has any meaningful effect on serum cytokine accumulation, however, has not been well studied. We [49] and others [50] have reported serum cytokine accumulation after cytokine injection to mice with bilateral nephrectomy; however, these studies were done utilizing recombinant 'human' forms of IL-6 and IL-10 to mice. Human, rat and mouse cytokines do not have a 100% homology; in fact, human and rat IL-6 and IL-10 only share 39 and 74% homology, respectively, at the protein sequence level. Therefore, impaired clearance in these studies could represent non-specific impaired clearance of a foreign protein.

Thus, in the present study, we tested whether absent kidney function had a direct effect on serum cytokine accumulation by injecting recombinant murine forms of IL-6, CXCL1, IL-10, IL-1β or TNF-α to mice with bilateral nephrectomy. We demonstrate that absent kidney function has a significant effect on IL-6, CXCL1, IL-10 and IL-1β accumulation in the serum, with values 2-fold higher in bilateral nephrectomy compared with sham-operated mice; these data are especially clinically relevant as these cytokines are increased in the serum of patients with AKI and suggest that absent kidney function does indeed affect serum cytokine accumulation. IL-6, CXCL1 (analog of human IL-8) and IL-10 are particularly relevant to patients with AKI as their increase is associated with increased mortality [1]. Interestingly, we were not able to demonstrate that absent kidney function affected serum cytokine accumulation for TNF-α, suggesting that intact kidney function is not as necessary for TNF-α elimination as it is for the other cytokines examined in our study.

Together with what is already known regarding cytokine metabolism and production, our data suggest that AKI has the following effects on cytokines: (i) increased renal cytokine production, (ii) increased extra-renal cytokine production, (iii) increased mononuclear phagocyte cytokine production and (iv) impaired renal cytokine clearance. Furthermore, these factors work together to cause serum cytokine accumulation in AKI. Higher levels of serum cytokines are associated with adverse outcomes in AKI such as prolonged mechanical ventilation [51] and increased mortality [1]. Serum cytokine accumulation may simply reflect worse AKI and thus be the explanation for worse outcomes; alternatively, serum cytokines may have adverse distant organ effects and may thus directly contribute to organ injury and increased mortality in AKI.

In conclusion, we demonstrate that serum cytokines increase during AKI due to increased mononuclear phagocyte production and impaired renal clearance, independent of kidney injury. Since higher levels of serum cytokines are associated with adverse outcomes in patients with AKI, further study of the deleterious effects of circulating cytokines in AKI may hold the key to reducing the increased mortality of patients with AKI.

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Conflict of interest statement. None declared.

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