Maladaptive Role of IL-6 in Ischemic Acute Renal Failure

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The role of IL-6 was investigated in murine ischemic acute renal failure. The renal pedicles were clamped for 17 min, and the mice were studied at various times after reperfusion. We found that serum IL-6 increased after murine ischemic renal injury. This increase was associated with increased IL-6 mRNA in the ischemic kidney but not in the contralateral kidney or the liver. Maximal IL-6 production occurred at 4 to 8 h and decreased to baseline by 24 h. Reperfusion of the kidney was required for IL-6 production. In situ hybridization and immunohistochemistry showed that macrophages infiltrated areas adjacent to the vascular bundles in the outer medulla within hours of reperfusion and showed that these macrophages produced IL-6 mRNA. For understanding how macrophages were stimulated to produce IL-6, an in vitro model in which S3 proximal tubular cells were injured by reactive oxygen species was set up. These injured cells released molecules that activated macrophages to produce IL-6 in vitro. IL-6 that was produced in response to renal ischemia was maladaptive because transgenic knockout of IL-6 ameliorated renal injury as measured by serum creatinine and histology. IL-6 transgenic knockout mice were lethally irradiated, and their bone marrow was reconstituted with wild-type IL-6 cells. Such bone marrow transfers abolished the protective effects of transgenic IL-6 knockout. It is concluded that macrophages infiltrate the area of the vascular bundles of the outer medulla, these macrophages produce IL-6, and this IL-6 exacerbates ischemic murine acute renal failure.


After ischemia/reperfusion initiates injury to epithelial and vascular cells during ischemic acute renal failure (ARF), maladaptive responses “extend” the injury (see reviews [1,2]). Inflammation is one maladaptive response (2–5), but the regulation of the inflammatory response to ischemic renal injury is not well understood.

This report focuses on IL-6 because, as reviewed in the Discussion section, this cytokine is a major regulator of inflammation. Furthermore, IL-6 production may be a common feature of ischemic injury of any organ. IL-6 not only is found after ischemia of the brain (6), gut (7), and heart (8), but also the amount of IL-6 correlates with the amount of ischemic injury (9). In human renal allografts with ischemia-reperfusion injury, IL-6 is detected in urine, and its level correlates with the severity of that injury (10).

The overall goal of this article is to understand the role of IL-6 on ischemic acute renal failure (ARF). We make the following points: First, IL-6 protein increases in the serum after ischemic renal injury. This increase is associated with increased IL-6 mRNA in the ischemic kidney. Second, in situ hybridization and immunohistochemistry localize IL-6 production to macrophages near the vascular bundles of the outer medulla. Molecules that are released by injured S3 proximal tubular cells activate macrophages to produce IL-6 in vitro. Third, transgenic knockout of IL-6 ameliorates renal injury as measured by serum creatinine and histology. Finally, transfer of IL-6–sufficient macrophages by means of bone marrow transplantation into IL-6 knockout mice restores the susceptibility of the knockout mice to ischemic renal injury.

Materials and Methods

Animals and Surgical Protocols

Male C57Bl/6J [IL-6 (+/+)] and C57Bl/6J-Ile6tm1Kopf [IL-6 (−/−)] 6-wk-old mice were purchased from Jackson Laboratories (Bar Harbor, ME). The genotype was confirmed by genomic PCR of tail snips (http://www.jax.org). The IL-6 (+/+) mice have a 174-bp DNA fragment from the wild-type allele, whereas homozygous IL-6 (−/−) mice have a single 280-bp band as a result of insertion of a neocassette. Mice were handled in accordance with institutional and National Institutes of Health guidelines.

Mice were anesthetized using inhaled isoflurane (Fortec System, Fraser Lake, NY) and maintained at 37°C by a TR100 temperature controlling system with a rectal probe (Fine Science Tools, Foster City, CA). In most mice, first the right kidney was removed and the left renal artery and vein then were clamped for 17 min. In some mice, the left renal artery and vein were clamped, but the right (“contralateral”) kidney was not. Other mice had “sham” surgery: Laparotomy and dissection but not clamping of the left renal pedicle. Peripheral serum
was assayed for creatinine by the Refletron automated system (Roche Diagnostics, Indianapolis, IN) and IL-6 by ELISA (Endogen, Woburn, MA). This ELISA measures an active form of IL-6.

In some experiments, the cortex and outer medulla were isolated by dissection using a ×3 to ×30 operating microscope. These were analyzed by RNase Protection Assay.

**RNase Protection Assay**

Total RNA was extracted from frozen kidneys using RNA-Easy Midi Kits (cat. no. 75144; Qiagen, Santa Clara, CA). Total RNA from S3 proximal tubular cells (see below) was harvested using the RNA-Easy kit (Qiagen). The P32-labeled probes were made using In Vitro Transcription Kits, mCK-2b and mCK-3b templates, and Riboquant RPA kits from Pharmingen (Pasadena, CA). The RNase protection gels were exposed on a phosphor image screen and analyzed with a Molecular Dynamics Storm 820 Phosphorimager (Piscataway, NJ). Densitometry analysis was performed using ImageQuant software (Molecular Dynamics).

**In Situ Hybridization for IL-6 mRNA and Immunohistochemistry for F4/80 Macrophage**

Ischemic and “contralateral” kidneys were harvested 4 h after reperfusion and fixed in 4% buffered paraformaldehyde in PBS, embedded in paraffin, cut into 3-μm sections, and used for in situ hybridization or macrophage immunostaining. The IL-6 fragment for the in situ probe was amplified from an ischemic kidney cDNA library (11). The details of probe preparation and in situ hybridization were published previously by our group (12). To stain with F4/80 antibody, the kidney sections were deparaffinized, blocked with Protein Block Serum (cat. no. 0909; DAKO Labs, Glostrup, Denmark), and incubated overnight at 4°C with 1:200 F4/80 antibody (cat. no. RM2900; Caltag Laboratories, San Francisco, CA). Subsequently, sections were incubated with biotinylated rabbit anti-rat IgG from DAKO (cat. no. DK-2600) at 1:50, then with streptavidin, horseradish peroxidase, and finally with DAB as per the manufacturer’s instruction (cat. no. K0377; DAKO Labs).

**In Vitro Model of Ischemic ARF**

We developed a two-stage assay using the S3 cell line. This cell line was originally dissected from the S3 segment of the proximal tubule of the kidney of an SV40 large T antigen transgenic mouse (13). In stage 1, S3 tubules were injured by reactive oxygen species (ROS) for 12 h; the ROS were generated by the action of reaction of 0.01 U/ml xanthine oxidase on 5 mM hypoxanthine (both from Sigma Chemical Co., St. Louis, MO), as previously reported by our laboratory (12). In stage 2, the supernatant was cultured with a murine macrophage line (J774), and IL-6 mRNA was measured 4 h later by RNase protection assay (Pharmingen).

**In Vivo Injections of Anti–IL-6**

IL-6–sufficient (C57Bl6/J) mice received 1 dose of monoclonal rat anti-IL-6 antibody (MP5-20F3 cat. no. 16-7061; eBioscience, www.ebioscience.com) in the amount of 72 μg per mouse. The control group received an equivalent dose of rat IgG1 (cat 16-4301). The antibodies were suspended in 0.2 ml of 0.1% BSA and injected into the penile vein immediately before the nephrectomy/clamp procedure. Peripheral serum was assayed for creatinine at 24 h after reperfusion. In some mice, peripheral blood was obtained at 4 h after reperfusion and used for measurement of IL-6 levels by the ELISA method (Endogen).

In addition, we used polyclonal goat neutralizing anti-murine IL-6 (AB-406-NA) and control goat IgG (AB-108-C) from R&D systems (www.rndsystems.com). These antibody preparations had <0.01 EU per 1 μg of antibody. According to the manufacturer’s specifications, 3.2 ng of antibody will neutralize 1 ng of IL-6. According to Figure 1, the IL-6 concentration at 4 h after ischemia is 2500 ng/ml. If the mouse weighs 20 g and the volume of distribution of IL-6 is 50%, then the total IL-6 in the mouse is 25,000 ng and the dose of antibody to neutralize is 80 μg. We administered a dose of 125 μg.

**Histologic Examination**

Three IL-6 (−/−) and three IL-6 (+/+ ) mice were killed for morphologic studies at 24 h after reperfusion. The kidneys were fixed in 10% buffered formalin, cut into 5-μm sections, and stained with hematoxylin and eosin. The morphologic analysis was carried out in a blinded manner as described previously (14). Briefly, the cortex and outer stripe of the outer medulla were evaluated for epithelial cell degeneration and interstitial edema.

**Figure 1.** Ischemic acute renal failure (ARF) increased serum IL-6 protein. Serum IL-6 was measured by ELISA (see Materials and Methods) in three different groups of mice: (1) Sham C57BL/6J mice (■) had their left renal arteries and veins dissected free of surrounding fat, but these vessels were not clamped. (2) Clamp-only mice (□) had their left renal arteries and veins dissected and also clamped for 17 min; this resulted in an ischemic left kidney and a contralateral nonischemic right kidney. The contralateral kidney maintained a normal GFR; both the clamp-only and sham mice had serum creatinines of <0.5 mg/dl. These mice had renal ischemia but no uremia. (3) Clamp + nephrectomy mice (□) had their left renal artery clamped like the above group but, in addition, had their right kidney removed. These mice had serum creatinines of 1.9 mg/dl. They had renal ischemia and uremia. Peripheral blood was taken from the sham group at 4 h after surgery and from the clamp and the clamp + nephrectomy groups at the indicated times of reperfusion. The means and SE are shown. *P < 0.01 versus control by t test. There was no statistically significant difference between the clamp-only and the clamp + nephrectomy groups at 4 h of reperfusion.
lial necrosis, loss of brush border, tubular dilation, and cast formation. The kidney sections were scored on the basis of the percentage of affected tubules as follows: 0, none; 1, <10%; 2, 11 to 25%; 3, 26 to 50%; 4, 51 to 75%; 5, >75%. At least 10 high-power fields (×400) were reviewed for each slide. In addition, leukocyte infiltration in the outer stripe of the outer medulla was counted on hematoxylin and eosin-stained sections. The number of leukocytes were averaged on the basis of at least 5 high-power fields for each slide.

**Bone Marrow Transplant**

Bone marrow cells were isolated from femurs and tibias, filtered through nylon mesh, counted using an electronic particle counter, and washed, and 8 × 10^6 cells in 0.5 ml of PBS were injected intravenously into recipients within 6 h of their receiving two doses of 5 Gy separated by 3 h. We transplanted IL-6+/+ bone marrow into IL-6−/−, or vice versa. The mice were kept in a sterile environment for 8 wk to allow full cellular reconstitution. Full chimerism of each mouse was confirmed by

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**Figure 2.** Reperfusion of the ischemic kidney increased IL-6 mRNA in the injured kidney. Ischemic, contralateral, and sham kidneys are defined in the legend to Figure 1. (A) IL-6 mRNA is increased in the ischemic kidney but not in the liver. RNase protection assays for IL-6 mRNA were performed on the total renal RNA at the indicated reperfusion times. In addition, IL-6 mRNA was assayed from livers that were isolated from a mouse with a sham kidney (a), or from mice with ischemic kidneys at the following renal reperfusion times: 1 h (b), 2 h (c), 8 h (d), and 24 h (e). L32 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) are housekeeping genes. (B) IL-6 mRNA is increased in the ischemic but not the contralateral kidney. Total RNA was taken at the indicated reperfusion times from sham, ischemic, and contralateral kidneys; the indicated cytokines were assayed by RNase protection assays. (C) Reperfusion is required for increased renal IL-6 mRNA. Total RNA was harvested from a sham (control) kidney, from an ischemic kidney without releasing the clamp on the renal artery (no reperfusion), and either 1 or 4 h after release of the clamp (reperfusion). (D) IL-6 mRNA is found in the ischemic outer medulla. Total RNA was isolated from the cortex (C) or outer medulla (M) and was analyzed by RNase protection assay.
genotyping of DNA from peripheral blood and tails using Jackson Lab protocol (see above). Renal ischemia reperfusion was induced as described above.

Results

Ischemic ARF Increased Serum IL-6 Protein and IL-6 mRNA in the Ischemic Kidney

Figure 1 shows that mice with ischemic kidneys (“clamp only” and “clamp + nephrectomy”) had increased serum IL-6 protein at 4 h after reperfusion compared with mice with non-ischemic sham-operated kidneys. The ischemic left kidney had similarly increased serum IL-6, in the presence or absence of a functioning right kidney. This finding showed that the increased IL-6 was not due to decreased renal elimination by the ischemic left kidney and suggested hepatic, rather than renal, elimination of IL-6 (15).

Figure 2A shows that the increased serum IL-6 originated in the ischemic kidney, not the liver. In these experiments, liver and a kidney were taken from the same mouse. Minimal amounts of IL-6 mRNA were present in the sham-operated kidney and the associated liver. In the ischemic kidney, IL-6 mRNA abundance increased until 8 h of reperfusion and decreased at 24 h of reperfusion; little IL-6 mRNA was present in the livers of these mice.

Figure 2B compares IL-6 mRNA abundance in sham-operated kidneys and ischemic left versus contralateral right non-ischemic kidneys at various times after reperfusion. IL-6 mRNA increased in the ischemic kidney at 1 h, peaked at 4 to 8 h, and decreased by 24 h. The IL-6 mRNA did not increase in the contralateral or in the sham kidneys. These data confirm previous observations (16). Figure 3 summarizes RNase protection assays, quantified by densitometry, on four ischemic versus four sham kidneys. The IL-6 mRNA was significantly increased at 1 and 4 h and then decreased to baseline at 72 h.

Figure 2C compares IL-6 mRNA abundance in ischemic kidneys before release of the renal arterial clamp (“ischemia, no reperfusion”) with ischemic kidneys after 1 or 4 h of reperfusion. IL-6 mRNA abundance did not increase unless the kidney was reperfused.

In addition to IL-6, the Pharmingen RNase protection assays provided information about other cytokines. These were not the focus of our studies, but we comment on them briefly. Figure 2B shows that mRNA for IL-1β, IL-1Ra, and IL-18 increased in the ischemic kidney and to a lesser extent in the contralateral kidney. These molecules are expressed by the ischemic kidney (16,17). However, expression by the contralateral kidney to our knowledge has not been previously reported. Such expression is consistent with the relatively minor inflammation there and may result from the systemic release of cytokines from the ischemic kidney (18). In addition, Figure 2D shows increased leukemia inhibitory factor expression in the ischemic kidney and confirms a previous report (16).

Macrophages in the Ischemic Outer Medulla Express IL-6 mRNA

To determine which part of the ischemic kidney expressed IL-6 mRNA, we dissected the cortex and the outer medulla. Figure 2D shows that the greatest increase in IL-6 mRNA at 4 and 8 h was in the ischemic outer medulla.

To identify the cell population expressing IL-6 mRNA in the ischemic kidney, we performed in situ hybridization. We selected the 4-h reperfusion time point on the basis of the time course for IL-6 mRNA expression shown in Figure 2. Low-power darkfield photomicrographs localize IL-6 expression to the ischemic outer medulla: Figure 4A shows that the S35-labeled antisense IL-6 mRNA bound to IL-6 mRNA in the outer medulla of the ischemic kidney, Figure 4B shows absent staining of the ischemic kidney by control S35-labeled sense IL-6 mRNA, and Figure 4C shows absent staining of the contralateral nonischemic kidney by S35-labeled antisense IL-6 mRNA.

Figure 3. Increased IL-6 mRNA abundance in the ischemic kidney after reperfusion: Composite of four experiments. L32 is a housekeeping gene. The means and SE of four kidneys per group are shown. By t test: *P < 0.04 sham versus ischemic 1-h reperfusion; **P < 0.01 sham versus ischemic 4-h reperfusion.
Higher power views show that the IL-6–expressing cells are mononuclear cells and that the mononuclear cells are located adjacent to the vascular bundles. Figure 4D is a medium-power brightfield photomicrograph of the ischemic outer medulla. Four mononuclear cells that express IL-6 are designated by arrows and outlined in boxes. The neighboring vascular bun-
Control experiments established that resting renal tubular cells released molecules into the outer medulla (Figure 5, bottom). After 4 h of reperfusion, F4/80 macrophages were found adjacent to the vascular bundles of the outer medulla (Figure 5, bottom).

Macrophages Express IL-6 in Response to Molecules Released by S3 Proximal Tubular Cells In Vitro

Molecules that are released by injured cells activate macrophages in vitro (see reviews [20]). To determine whether this occurs in the ischemic kidney, we injured S3 renal tubular cells with ROS in vitro (Figure 6). Such ROS are produced during ischemic ARF (21), and ROS injury of renal tubular cells in vitro has previously been used to model ischemic ARF in vitro (e.g., 22). The injured renal tubular cells released molecules into the supernatant that activated macrophages to express IL-6 mRNA. Control experiments established that resting renal tubular cells did not release activating molecules (Figure 6, Grp C). Grp D was ROS and medium incubated overnight in the absence of S3 tubules. The ROS of Grp D were so unstable that they degraded during the overnight incubation and were not a factor in stage 2.

Altogether, these in vitro experiments support the in vivo experiments of Figures 4 and 5 that show that renal macrophages express IL-6 in response to ischemic ARF. Further support for macrophages as the source of IL-6 in ischemic kidneys will be provided when we discuss our bone marrow chimera experiments at the end of the Results section.

Removal of IL-6 Ameliorates Ischemic ARF

To determine whether IL-6 exacerbates ischemic ARF, we injected two different anti–IL-6 antibodies intravenously at the time of renal ischemia and measured the effect on renal injury. First, we injected monoclonal rat anti–IL-6 (MP5–20F; eBio-science) intravenously at a dose seven times larger than that previously used successfully to neutralize IL-6 and ameliorate murine septic shock (23). Control mice received an equivalent amount of rat IgG. The anti–IL-6 decreased the peripheral blood IL-6 at 4 h of reperfusion from 7000 ± 1000 ng/ml in the rat IgG–injected group to 1600 ± 600 ng/ml in the anti–IL-6–injected group (mean ± SE; n = 6; P < 0.01 by t test). Despite this 77% decrease in peripheral blood IL-6, there was no significant effect on ischemic ARF; the serum creatinine in the anti–IL-6–treated group (mean ± SE; n = 6; P = 0.38 by t test) in a second experiment, we injected 125 μg of neutralizing polyclonal goat anti-murine IL-6 (AB-108-C; R&D Systems). This antibody preparation contained <0.01 EU of endotoxin per 1 μg of antibody. We chose this dose because it exceeds that necessary to neutralize the 2500 ng/ml of IL-6 found in blood at 4 h after ischemia (see Figure 1 and Materials and Methods). We increased the clamp time to induce greater injury in these experiments. The serum creatinine was 1.6 ± 0.3 mg/dl in the goat control IgG-injected mice and 1.5 ± 0.4 mg/dl in the anti–IL-6–injected mice. Again, the anti–IL-6 did not ameliorate ischemic ARF. The inability of anti–IL-6 to ameliorate ischemic ARF most likely represents an inability of sufficient quantities of the large antibody molecule (molecular weight = 160 k) to access the outer medulla, where the IL-6 is located.

To determine further the role of IL-6 in ischemic ARF, we examined the effect of transgenic knockout of IL-6 as assessed by both function (Figure 7) and morphology (Figure 8). We used C57BL/6/J-Il6tm1Kopf [IL-6 (−/−)] and C57BL/6J wild-type [IL-6 (+/+)] mice. These mice are the same except for the nonfunctional IL-6 gene in the former. Figure 7 compares renal function during ischemic ARF. The serum creatinine (Scr) was determined at 24 h after reperfusion. The mean serum creatinine level in the IL-6 (−/−) group was 0.89 ± 0.136 mg/dl versus 1.83 ± 0.1 mg/dl in the wild-type group (mean and SE of at least eight mice per group; P < 0.05).

Figure 8, A and B, are low-power photomicrographs that compare the pathology of the IL-6 (−/−) versus IL-6 (+/+) kidneys at 24 h of reperfusion. The IL-6 (+/+) kidneys had severe injury (Figure 8B). There were many necrotic tubules in the outer medulla, indicated by *. These tubules were filled...
with casts. There was less but still significant injury in the cortex. There were many neutrophils in the interstitial spaces, indicated by arrows. In comparison, the IL-6 (H11002/H11002/H11002) kidneys (Figure 8A) had much less injury by all of the above criteria.

Figure 8, C and D, are high-power photomicrographs of the outer medulla of the ischemic IL-6 (H11002/H11002/H11002) versus IL-6 (H11001/H11001/H11001) kidneys. Necrotic tubules and inflammation are present in the IL-6 (H11001/H11001/H11001) kidneys. The morphometric analysis (Figure 9) confirmed the decreased medullary and cortical damage and the decreased inflammation in the IL-6 (H11002/H11002/H11002) kidneys.

Production of IL-6 by Bone Marrow–Derived Cells Increases Ischemic ARF

Figure 10 shows that chimeric mice with IL-6 (H11002/H11002/H11002) renal parenchymal cells and IL-6 (H11001/H11001/H11001) macrophages have greater ischemic ARF than chimeric mice with IL-6 (H11001/H11001/H11001) renal parenchymal cells and IL-6 (H11002/H11002/H11002) bone marrow–derived cells. In these experiments, C57BL/6J-Ile61tm1Kopf [IL-6 (H11001/H11001/H11001)] mice were lethally irradiated and then received bone marrow transplants from C57BL/6J [IL-6 (H11002/H11002/H11002)] mice. Similarly, lethally irradiated IL-6 (H11002/H11002/H11002) mice received IL-6 (H11001/H11001/H11001) bone marrow. Because these mice differed only in the IL-6 gene, there was no graft-versus-host or host-versus-graft disease. Genomic PCR (see Materials and Methods) of the radioresistant tail and radiosensitive peripheral blood confirmed the chimerism.

Discussion

This article makes several points. IL-6 is produced by the ischemic kidney. Immunohistology and in situ hybridization show that macrophages adjacent to the ischemic vascular bundles of the outer medulla produce IL-6. An in vitro model of ischemic ARF suggests that this production is stimulated by molecules that are produced by ischemic renal tubular cells. Transgenic knockout of IL-6 ameliorates renal injury. In chimeric mice whose renal parenchymal cells and macrophages were or were not capable of producing IL-6, maximal ischemic injury required IL-6–producing macrophages.
Our transgenic mice carry the caveat of all such experiments, i.e., mice with transgenic knockout of IL-6 may produce molecules that compensate for the absence of IL-6 from birth. It is possible that the lesser ischemic ARF of the knockout mice is due to these compensatory molecules and not the deficiency of IL-6. One way to overcome this caveat is by inhibiting IL-6 in wild-type mice. Although others ameliorated ischemic ARF with anti–IL-6 injections (24), we found that exogenous anti–IL-6 did not ameliorate ischemic ARF. Our antibody experiments are inconclusive because the antibodies may not access the interstitial spaces of the injured kidney. We have overcome this caveat using bone marrow chimeras. These experiments demonstrate a specific effect of wild-type IL-6–producing macrophages. Thus, wild-type IL-6–producing macrophages in the IL-6 knockout kidneys produce more ischemic ARF than IL-6 knockout macrophages in wild-type kidneys (Figure 10). In other words, bone marrow transfer of IL-6–producing wild-

Figure 7. Transgenic knockout of IL-6 ameliorated ischemic ARF: Renal function. We removed the right kidney and clamped the left renal pedicle for 17 min. The serum creatinine was measured after 24 h of reperfusion. ■, homozygous IL-6 knockout [IL-6 (-/-)] mice; □, wild-type mice [IL-6 (+/+)]. The means and SE are shown.

Panel A: IL6 (-/-) 200x

Cortex Outer Medulla

Panel B: IL6 (+/+) 200x

Cortex Outer Medulla

Panel C: IL6 (-/-) 400x

Cortex Outer Medulla

Panel D: IL6 (+/+) 400x

Cortex Outer Medulla

Figure 8. Transgenic knockout of IL-6 ameliorated ischemic ARF: Pathology. Kidneys were removed after 24 h of reperfusion, fixed in formalin, sectioned, and stained with hematoxylin and eosin. (A) IL-6 (-/-). (B)IL-6 (+/+). (C) IL-6 (-/-) view of the outer medulla. (D) IL-6 (+/+) view of the outer medulla. G, glomeruli; *, necrotic tubules. Arrows show neutrophils. Magnification, ×200 in A and B; ×400 in C and D.
type macrophages overcame the protective effect of IL-6 knock-
out to ischemic renal injury.

In addition to our group, many other laboratories (25–27) found macrophages in the outer medulla early during ischemic ARF. Preventing the macrophage infiltration ameliorates renal injury (26,28,29). However, how macrophages injure the kidney was not known. We now suggest that one mechanism is the production of IL-6.

The maladaptive renal effects of IL-6 remain to be elucidated in detail. We favor the following hypothesis. Macrophages are a major component of the initial inflammatory response to renal injury, as shown in Figure 5 and previously reported by others (26,27). These macrophages release IL-6, which further increases renal inflammation by recruiting more neutrophils into the injured kidney. This hypothesis is consistent with our observations (Figures 8 and 9), which show decreased inflammation in the kidney at 24 h in the IL-6 (−/−) ischemic kidney. This hypothesis is also consistent with previous reports that the peak neutrophil infiltrate after renal ischemia does not occur until 12 h (30,31), well after we and others (26) found IL-6–expressing renal macrophages (Figures 4 and 5).

Also consistent with this hypothesis are the known proinflammatory effects of IL-6. These include stimulation of neutrophil release from the bone marrow (32), prevention of neutrophil apoptosis (33), activation of neutrophils to produce toxic enzymes (34), and activation of endothelial cells to express intercellular adhesion molecule 1 and chemokines (35,36). These effects of IL-6 are confirmed by the decreased inflammatory response in transgenic knockout mice after ischemia in the lung (37), gut (7,38), or brain (6); after injections of sterile turpentine (39) or carrageen (35); and after infections. (40,41). Further support of the proinflammatory effects of IL-6 are the increased isletitis in mice overexpressing IL-6 driven by the insulin promoter (42) and the anti-inflammatory effects of removing IL-6 in experimental (43) and clinical arthritis (44). Furthermore, IL-6 contributes to neutrophilic influx and increased damage in the models of hemorrhagic shock (45) and spinal cord injury (46).

Another issue raised by our experiments is what activates the macrophages after they have entered the kidney. Figure 6 shows that injured renal tubules release molecules that activate macrophages in vitro. Ongoing experiments in our laboratory aim at identifying these molecules. One possibility is that macrophages are stimulated by molecules that ordinarily reside inside renal tubular cells but are released into the extracellular space after these cells are injured. Heat-shock proteins are examples of such molecules. Extracellular heat-shock proteins do activate macrophages via their Toll-like receptor 4 (TLR4) receptor (20,47–49). Consistent with the idea that TLR4 participates in ischemic ARF are our data that TLR4-deficient mice
suffer less injury after renal ischemia (R.J. and C.Y.L., unpublished observations, 2005).

Although our data and most reports in the literature, reviewed in the beginning of this article, indicate that IL-6 exacerbates ischemic injury, no fair discussion would be complete without acknowledging that, in a few models, IL-6 ameliorates rather than exacerbates injury (37, 50). Why IL-6 exacerbates injury in most models but ameliorates injury in a few models remains to be explained.

In summary, this article makes the following major points: First, IL-6 protein increases in the serum after ischemic renal injury. This increase is associated with increased IL-6 mRNA in the ischemic kidney. Second, in situ hybridization and immunohistochemistry localize IL-6 production to macrophages near the vascular bundles of the outer medulla. Molecules that are released by injured S3 proximal tubular cells activate macrophages to produce IL-6 in vitro. Third, transgenic knockout of IL-6 ameliorates renal injury as measured by serum creatinine and histology. Finally, transfer of IL-6–sufficient macrophages by means of bone marrow transplantation into IL-6 knockout mice increases ischemic renal injury.

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References

24. Patel NS, Chatterjee PK, Di Paola R, Mazzon E, Britti D, De Sarro A, Cuzzocrea S, Thiemer mann C: Endogenous interleukin-6 enhances the renal injury, dysfunction, and in-
flammation caused by ischemia/reperfusion. J Pharmacol Exp Ther 312: 1170–1178, 2005