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Acute Renal Failure in Endotoxemia Is Caused by TNF Acting Directly on TNF Receptor-1 in Kidney

Patrick N. Cunningham,* Hristem M. Dyanov,* Pierce Park,* Jun Wang,† Kenneth A. Newell,† and Richard J. Quigg*

Bacterial endotoxin (LPS) is responsible for much of the widespread inflammatory response seen in sepsis, a condition often accompanied by acute renal failure (ARF). In this work we report that mice deficient in TNFR1 (TNFR1−/−) were resistant to LPS-induced renal failure. Compared with TNFR1+/+ controls, TNFR1−/− mice had less apoptosis in renal cells and fewer neutrophils infiltrating the kidney following LPS administration, supporting these as mediators of ARF. TNFR1+/+ kidneys transplanted into TNFR1−/− mice sustained severe ARF after LPS injection, which was not the case with TNFR1−/− kidneys transplanted into TNFR1+/+ mice. Therefore, TNF is a key mediator of LPS-induced ARF, acting through its receptor TNFR1 in the kidney. The Journal of Immunology, 2002, 168: 5817–5823.

Sepsis is a common and often devastating condition characterized by uncontrolled infection and multisystem organ failure (1). Bacterial endotoxin (LPS) is a cell wall component of Gram-negative bacteria that is responsible for much of the widespread inflammatory response seen in this condition. Administration of LPS to animals causes systemic hypotension and organ dysfunction similar to what occurs in clinical sepsis (2). Several mediators of inflammation have been assigned a key role in sepsis, among them TNF, which is released by macrophages and other cells into the circulation following LPS administration (3, 4). While experiments using TNF-blocking agents have shown protection against LPS-induced mortality in mice (5–7), clinical trials of similar agents in humans have failed to demonstrate any improvement in survival (8). TNF acts through two distinct receptors: TNFR1, which mediates most of the inflammatory actions of TNF, and TNFR2, which has been proposed to have an anti-inflammatory role (9). However, mice deficient in TNFR1 (TNFR1−/−) are not protected from mortality induced by administration of high doses of LPS (9, 10).

Sepsis is a leading cause of acute renal failure (ARF) (11–13), but the mechanisms behind this ARF remain unclear. Although cytokines such as TNF are key mediators of sepsis, little is known about their acute effects on the kidney in the setting of endotoxemia. We previously observed that, despite severe impairment of kidney function following LPS administration, the extent of renal pathologic injury is mild (14). To explain this dissociation between the severe functional impairment, yet the relatively mild pathologic injury seen in LPS-induced ARF, we hypothesized that apoptosis, which is difficult to detect by routine histologic studies, is responsible for renal cellular dysfunction and death. Of note, TNF has been shown to induce apoptosis in a variety of cell types, including renal tubular cells (15, 16). Therefore, we investigated the role and mechanisms of action of TNF in endotoxin-induced ARF.

Materials and Methods

Animals

Mice with a mutation in the Fas gene (B6.MRL-Fas−/−; denoted Fas−/−) or with targeted deletion of TNFR1 (TNFR1−/−) or TNFR2 (TNFR2−/−) were obtained from The Jackson Laboratory (Bar Harbor, ME) and used for experiments at 8 wk of age. Because each of these genotypes is on a C57BL/6 background, age-matched C57BL/6 mice were used as controls. The animals were maintained and experiments were performed in accordance with the guidelines set by the University of Chicago Institutional Animal Care and Use Committee.

ARF model

Under isoflurane inhalational anesthesia, mice were given 0.15 mg Escherichia coli LPS (Sigma-Aldrich, St. Louis, MO) as an i.p. injection. Blood was obtained via retro-orbital bleeding immediately before injection and 24 h later, with sacrifice and harvest of kidney tissue and blood at 48 h after injection. Portions of kidney tissue were frozen in OCT compound (Fisher Scientific, Pittsburg, PA) at −80°C, and also formalin-fixed and stained with periodic acid-Schiff base and H&E. Blood urea nitrogen (BUN) concentrations were determined with a Beckman CX5CE autoanalyzer (Beckman, Palo Alto, CA). At this relatively low dose of LPS, systemic blood pressure falls by <10 mm Hg in C57BL/6 mice (Ref. 17 and our unpublished observations). To evaluate the possibility that TNFR2−/− mice had an enhanced susceptibility to LPS-induced ARF, TNFR2−/− mice and their control group were given LPS at a lower dose of 0.075 mg, the rationale for which is described in greater detail below.

TUNEL staining

Mice were sacrificed at varying times after i.p. injection of 0.15 mg LPS, and kidney tissue was immediately frozen in OCT compound. TUNEL staining was performed with the TdT-FragEL DNA fragmentation detection kit (Oncogene, Boston, MA) according to the manufacturer’s instructions. In brief, 8-μm cryostat sections were washed in TWEEN 20/TBS and fixed in 4% formaldehyde for 30 min. This was followed by proteinase K treatment for 18 min and incubation in 3% H2O2/methanol for 7 min. Specimens were incubated with TdT at 37°C for 60 min, blocked in dilute BSA for 20 min, and incubated in streptavidin-HRP for 30 min, followed by detection with diaminobenzidine reagent for 15 min. Sections were counterstained with methyl green, and a blinded observer counted the number of positively stained nuclei per high-power field and recorded the average of 10 fields for each sample.

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2 Address correspondence and reprint requests to Dr. Patrick N. Cunningham, Section of Nephrology, MC5100, University of Chicago, 5841 South Maryland Avenue, Chicago, IL 60637. E-mail address: p cunning@medicine.bsd.uchicago.edu

3 Abbreviations used in this paper: ARF, acute renal failure; BUN, blood urea nitrogen; iNOS, inducible NO synthase; LM, ligase-mediated.

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Kidney transplantation

A single kidney was transplanted from 10-wk-old TNFR1−/− to TNFR1+/− mice, and vice versa. Each individual mouse was used only as a recipient or a donor. Mice were anesthetized with 65 mg/kg pentobarbital i.p. The left kidney of donor mice was perfused via the renal artery with 0.3 ml cold saline and resected with artery, vein, and ureter attached. The kidney was stored at 4°C until anastomosis. Next, the recipient underwent midline abdominal incision, followed by suprarenal clamping of aorta and inferior vena cava. The donor kidney was placed in the right flank, and its artery and vein were attached with 10-0 nylon suture via side-to-end anastomoses with the recipient aorta and inferior vena cava. The bladder was punctured with a 21-gauge needle, and the ureter was sewn in place. Native kidneys were then resected. Animals were allowed to recover for 10 days after surgery before LPS injection. Preliminary dosing experiments using syngeneic C57BL/6 kidney transplants showed that mouse kidney transplant recipients have a stable, mild elevation of BUN and creatinine but are somewhat more sensitive to LPS; thus, a dose of 0.1 mg was used in these studies.

Tissue processing

Histologic sections for each animal were assigned a semiquantitative score for tubular injury adopted from Nomura et al. (18). A blinded observer assigned a score ranging from 0 (no injury) to 3 (severe/widespread injury) for tubular injury. A blinded observer calculated separately for the cortex and medulla, and all three variables from both cortex and medulla were summed to arrive at a total injury score for each animal ranging from 0 to 18. For immunohistochemistry, 4-μm kidney cryostat sections were fixed with ether/ethanol, incubated with 0.3% H2O2 for 30 min, and blocked with dilute horse serum. Sections were stained for neutrophils by sequential incubation with rat anti-mouse neutrophil (mAb 7/4; Serotec, Raleigh, NC) at 1/60 dilution for 30 min followed by HRP-conjugated rabbit anti-rat IgG (Sigma-Aldrich) at a 1/60 dilution for 30 min and diaminobenzidine reagent (Vector Laboratories, Burlingame, CA) for 10 min. A blinded observer counted the number of neutrophils per high-power field and recorded the average of 10 fields for each sample. Sections were stained for TNFR1 by incubation with hamster anti-mouse TNFR1 (BD PharMingen, San Diego, CA) at a 1/60 dilution for 30 min, followed by biotin-conjugated mouse anti-hamster IgG (BD PharMingen) at a 1/100 dilution for 30 min, streptavidin-HRP (Vector Laboratories) for 30 min, and diaminobenzidine reagent.

Measurement of TNF release

In a parallel set of experiments, blood was collected from wild-type C57BL/6 mice (n = 8) at baseline and again 2 h after LPS injection. Serum was analyzed for TNF levels using a commercially available TNF ELISA kit (R&D Systems, Minneapolis, MN) according to the manufacturer’s instructions.

mRNA transcript profiling in LPS-injected mice

For microarrays, normal C57BL/6 mice were given 0.15 mg LPS, and 4 or 8 h later tissue was harvested and kidney RNA was isolated using TRIzol reagent (Life Technologies, Grand Island, NY). RNA from the kidney of a mouse injected with saline served as the control. RNA was processed and hybridized with murine genome U74A arrays (Affymetrix, Santa Clara, CA) according to the manufacturer’s instructions.

Real-time RT-PCR for inducible NO synthase (iNOS) was performed as follows. A portion of whole kidney from each TNFR1−/− and TNFR1+/− mouse was frozen at −80°C, from which total RNA was purified using the RNeasy Mini RNA purification kit (Qiagen). To remove all traces of genomic DNA, samples were then treated with RNase-free RQ1 DNase (1 U per 4 μg RNA; Promega, Madison, WI) in 10 μl reaction buffer (final concentration, 40 nM Tris-HCl, 10 mM MgSO4, and 1 mM CaCl2; pH 8) at 37°C for 30 min. This was followed by addition of 1 μl 20 mM EGTA (pH 8) to stop the reaction and incubation at 65°C for 10 min to inactivate the DNase. cDNA was generated from RNA using random hexamers as primers with the SuperScript first-strand synthesis kit (Life Technologies), according to the manufacturer’s instructions. CDNA was prepared in an analogous fashion from kidneys of mice sacrificed 6 h after LPS injection and compared with sham-injected kidney cDNA for analysis of early TNF and TNFR1 mRNA (n = 6 per group).

Real-time PCR was performed using a Smart Cycler (Cepheid, Sunnyvale, CA). For iNOS and GAPDH measurements, probes were labeled at the 5′ end with the reporter dye molecule FAM (6-carboxyfluorescein; emission λmax = 518 nm) and at the 3′ end with the quencher dye TAMRA (6-carboxytetramethyl-rhodamine; emission λmax = 582 nm). Reaction was conducted in a total volume of 25 μl with 1X TaqMan Master Mix (PE Applied Biosystems, Foster City, CA), 3 μl sample or standard cDNA, primers at 200 nM each, and probe at 100 nM. PCR was conducted with a hot start at 95°C (5 min), followed by 45 cycles of 95°C for 15 s and 60°C for 1 min. For each sample, the number of cycles required to generate a given threshold signal (Ct) was recorded. Using a standard curve generated from serial dilutions of splenic cDNA, the ratio of iNOS to GAPDH expression was calculated for each experimental animal and normalized relative to an average of ratios from TNFR1−/− kidney unexposed to LPS (n = 5). Measurements of TNF and TNFR1 mRNA expression relative to GAPDH were performed in an analogous fashion, but using the SybrGreen intercalating dye method with HotStar DNA polymerase (PE Applied Biosystems) instead of labeled probes. Reaction conditions were according to the manufacturer’s instructions. The reaction temperature profiles for TNF and TNFR1 were as described above, except that the annealing temperature was 58°C. Primers were synthesized by Integrated DNA Technologies (Corvalis, VA) and probes by Syntegen (Houston, TX). The sequences of primers/probes were as follows: iNOS forward primer, 5′-CAGCTGGGCTGTACAAACCTT-3′; iNOS reverse primer, 5′-TGAATGTGATTTGCTTGCG-3′; iNOS probe, 5′-CGG GCAAGCTTGTAGACCTTTTG-3′; GAPDH forward primer, 5′-GGCA AATTCACCGGACAGT-3′; GAPDH reverse primer, 5′-AGATGGTG ATGGGTCCTCC-3′; GAPDH probe, 5′-AAGGGCAGAATGGGAAAC TTGGTAC-3′; TNF forward primer, 5′-CGATGGTGTAGCTTTTTCT GC-3′; TNF reverse primer, 5′-GATGGTGGAGGACCATG-3′; TNFR1 forward primer, 5′-GGACCGGAGAAGGATAG-3′; and TNFR1 reverse primer, 5′-GTTCCTTGTGACCTGATG-3′.

Statistics

Data were analyzed with Minitab software (State College, PA). Unless noted otherwise, data are given as the mean ± SEM. Groups were compared by two-sample t test. When comparing the severity of ARF between two groups, the slope of BUN vs time was determined for each individual animal by least squares regression, and the individual slopes in the two groups were compared with the Mann-Whitney rank-sum test. A value of p ≤ 0.05 was considered significant.

Results

Apoptosis occurs in the kidney after endotoxin administration

Several factors present in sepsis can serve as triggers of apoptosis, including hypoxia and reactive oxygen species (19, 20). Additionally, apoptosis can be induced in a variety of cell types, including renal tubular cells, in a receptor-mediated fashion, via Fas, TNF-Fas, and TNFR1-TRAIL. Apoptosis can be induced in a variety of cell types, including renal tubular cells, in a receptor-mediated fashion, via Fas, TNF-Fas, and TNFR1-TRAIL. Apoptosis occurs in the kidney after endotoxin administration.
Apoptosis occurs in the kidney after LPS administration. A, TUNEL-stained apoptotic nuclei are rare in normal mouse kidney. B, At time points ranging from 3 to 48 h after LPS administration, darkly staining apoptotic nuclei were visible at an increased frequency (arrows). Apoptotic nuclei were predominantly observed in the tubules. Kidney harvested 8 h after LPS injection is shown. Methyl green counterstain; magnification, ×400.

FIGURE 1. Apoptosis occurs in the kidney after LPS administration. A, TUNEL-stained apoptotic nuclei are rare in normal mouse kidney. B, At time points ranging from 3 to 48 h after LPS administration, darkly staining TUNEL-stained apoptotic nuclei are rare in normal mouse kidney.

Further quantify the extent of apoptosis, LM-PCR was performed to amplify fragmented DNA from kidney specimens. This demonstrated that the limited extent of apoptosis present in normal kidney was increased in a time-dependent fashion following LPS administration (Fig. 2).

Mice deficient in TNFR1 are resistant to endotoxin-induced ARF

In addition to the release of TNF into the circulation that occurs after LPS administration, Fas and Fas ligand have been shown to be up-regulated on the surface of renal tubular cells following LPS administration in vivo (24). Thus, we hypothesized that one or both of these pathways could account for renal cell apoptosis and ARF following LPS administration. Fas−/− mice developed ARF to the same extent as Fas+/− controls following LPS injection (Fig. 3A). In contrast, TNFR1−/− mice were resistant to LPS-induced ARF (Fig. 3B; n = 8 per group; p = 0.005 compared with TNFR1+/− mice). In addition to protection from renal functional impairment, TNFR1−/− mice had less renal tubular injury (tubular injury scores of 3.9 ± 0.6 vs 7.1 ± 1.1 in TNFR1−/− and TNFR1+/− mice, respectively; p = 0.027; Fig. 4). Therefore, TNF, acting through TNFR1, is a key mediator of LPS-induced ARF. Renal apoptosis, as assessed by LM-PCR, was greater in the TNFR1−/− controls than in the TNFR1+/− group. Furthermore, the amount of apoptosis in individual animals correlated with the extent of renal functional impairment (Fig. 5), suggesting that apoptosis is intimately involved in LPS-induced ARF.

The role of TNFR2 in apoptosis and inflammation is less clear. While apoptotic signaling has been clearly demonstrated to occur through TNFR2 (25), an antiapoptotic role has also been ascribed to TNFR2 (26, 27). Also, soluble TNFR2 may serve to down-regulate TNF action (9). Thus, we thought it conceivable that TNFR2−/− mice would be more sensitive than TNFR2+/− mice to TNF in vivo (24). Thus, we hypothesized that one or both of these pathways could account for renal cell apoptosis and ARF following LPS administration. Fas−/− mice developed ARF to the same extent as Fas+/− controls following LPS injection (Fig. 3A). In contrast, TNFR1−/− mice were resistant to LPS-induced ARF (Fig. 3B; n = 8 per group; p = 0.005 compared with TNFR1+/− mice). In addition to protection from renal functional impairment, TNFR1−/− mice had less renal tubular injury (tubular injury scores of 3.9 ± 0.6 vs 7.1 ± 1.1 in TNFR1−/− and TNFR1+/− mice, respectively; p = 0.027; Fig. 4). Therefore, TNF, acting through TNFR1, is a key mediator of LPS-induced ARF. Renal apoptosis, as assessed by LM-PCR, was greater in the

FIGURE 2. Time course of apoptosis in the kidney after LPS administration. DNA was isolated from C57BL/6 mice at various time points after injection of 0.15 mg LPS and subjected to LM-PCR. The small amount of apoptosis occurring in kidney at baseline (BL) is markedly increased following LPS administration, peaks at 8–12 h, and is still apparent 48 h after injection.

FIGURE 3. TNFR1−/− mice are resistant to LPS-induced ARF. Renal function, as measured by BUN, is shown at 24 and 48 h following LPS injection. A, Both Fas−/− and Fas+/− mice developed ARF following injection of 0.15 mg LPS (n = 10 in each group). B, TNFR1−/− mice did not develop ARF following LPS administration, in marked contrast to TNFR1+/− control mice (n = 8 in each group; p = 0.005). C, Both TNFR2−/− and TNFR2+/− mice developed ARF after injection of 0.075 mg LPS (n = 8 in each group).

The role of TNFR2 in apoptosis and inflammation is less clear. While apoptotic signaling has been clearly demonstrated to occur through TNFR2 (25), an antiapoptotic role has also been ascribed to TNFR2 (26, 27). Also, soluble TNFR2 may serve to down-regulate TNF action (9). Thus, we thought it conceivable that TNFR2−/− mice would be more sensitive than TNFR2+/− mice to LPS-induced ARF. However, using a lower dose of 0.075 mg LPS, TNFR2−/− mice developed ARF to a similar extent as TNFR2+/− mice (Fig. 3C) and tubular injury scores (5.6 ± 0.3 vs 5.3 ± 0.3 in TNFR2−/− and TNFR2+/− mice, respectively; p = 0.42). By LM-PCR, TNFR2−/− and TNFR2+/− mice had a similar amount of apoptosis in the kidney after LPS administration (data not shown). Therefore, TNFR2 does not appear to have a role in mediating the ARF or renal apoptosis induced by LPS.
TNFR1 mediates LPS-induced neutrophil infiltration in the kidney

In addition to apoptosis, there are other mechanisms by which TNFR1 could mediate LPS-induced renal injury. Although relatively mild, the pathologic changes seen in TNFR1−/− mice after LPS administration cannot be explained by the noninflammatory process of apoptosis. TNF has been shown to stimulate the oxidative burst of neutrophils (28–30) and to up-regulate the expression of chemokines (31) and adhesion molecules (32–34) that mediate neutrophil recruitment to tissues following an inflammatory stimulus. Neutrophils have been implicated in the pathogenesis of LPS-induced injury of the liver and lung (35–37) and in ischemia-reperfusion injury of the kidney (38, 39). We confirmed that neutrophils infiltrated the kidney following LPS administration, but to a significantly lesser extent in TNFR1−/− mice compared with TNFR1+/− controls (5.4 ± 0.8 vs 9.0 ± 1.3 neutrophils per high-power field in TNFR1−/− and TNFR1+/− mice, respectively; p = 0.039; Fig. 6). Additionally, the extent of renal neutrophil accumulation correlated with the degree of renal functional impairment in individual animals, as measured by BUN levels (r = 0.89; p < 0.001), further supporting a role for TNF-mediated neutrophil recruitment in the pathogenesis of LPS-induced ARF.

LPS causes up-regulation of iNOS expression independently of TNFR1

NO is a key mediator of a wide range of inflammatory as well as anti-inflammatory processes and has been shown to have direct cytotoxic effects on renal tubular cells (40) as well as complex actions on the renal vasculature (41). Inducible NOS has been shown to be strongly up-regulated in a wide variety of tissues, including the kidney, after administration of LPS (42). By real-time RT-PCR, expression of iNOS mRNA in the kidney was markedly up-regulated in both TNFR1−/− and TNFR1+/− mice given LPS compared with the baseline value, but with no statistical differences between the two groups (4.7 ± 1.3- and 8.7 ± 3.0-fold increases in TNFR1−/− and TNFR1+/− mice, respectively; p = 0.25; Fig. 7). Furthermore, iNOS expression did not correlate with BUN levels (r = 0.326; p = 0.24). Thus, despite induction of iNOS mRNA as in wild-type controls, TNFR1−/− mice are protected from LPS-induced ARF.

TNF mediates ARF by acting on its receptor in kidney

As has been documented by others, the injection of LPS led to profound release of TNF into the circulation within 2 h (0.02 ± 0.02 ng/ml at baseline vs 2.70 ± 0.65 ng/ml 2 h after LPS; p < 0.001). The release of TNF soon after LPS administration is responsible for the later appearance of other circulating cytokines (43, 44). Also, injection of TNF in vivo duplicates the severe hypotension seen in septic shock (45). Thus, TNF could mediate LPS-induced ARF indirectly, by inducing systemic hypotension and/or by leading to the release of other inflammatory mediators into the circulation that could act on the kidney. Alternatively, LPS-induced ARF could be mediated by TNF acting directly on renal TNFR1. In support of the latter possibility, kidney sections from normal C57BL/6 mouse kidney stained positively for TNFR1, predominantly in cortical tubules (data not shown). In addition, using a microarray approach, we found TNFR1 mRNA to be up-regulated in the kidney by factors of 2.3- and 5.9-fold at 4 and 8 h after LPS administration, respectively. These preliminary data were confirmed in subsequent experiments using real-time RT-PCR, showing that TNFR1 mRNA was up-regulated 3.0 ± 0.2-fold in kidney at 6 h after LPS administration in comparison with sham-injected controls (n = 6 per group; p < 0.001).

To address the question of the level of action of TNF, we transplanted kidneys between TNFR1+/+ and TNFR1−/− mice and examined their responses to LPS. TNFR1−/− kidneys in TNFR1−/− mice developed severe ARF after LPS administration, which was not the case for TNFR1−/− kidneys in TNFR1+/+ recipients (n = 4 per group; p = 0.029; Fig. 8). As with native kidneys in TNFR1−/− mice, pathologic specimens of transplanted TNFR1−/− kidneys had less injury than transplanted TNFR1+/+ kidneys (tubular injury scores of 3.6 ± 0.6 vs 5.8 ± 0.4; p = 0.062). These data prove that TNF causes LPS-induced ARF.
The pathogenesis of sepsis and the associated end-organ failure is highly complex, involving multiple inflammatory mediators (2). Although an important role for TNF in sepsis has been established, the role of TNF in LPS-induced ARF has only recently been examined. Knotek et al. (17) found that the administration of a TNF-binding protein protected mice against LPS-induced ARF, but they did not find evidence of renal apoptosis or neutrophil infiltration described herein. In this work we show that LPS-induced ARF can be attributed to TNF acting directly on its receptor, TNFR1, in kidney.

The multisystem organ failure that occurs in sepsis is often ascribed to widespread hypotension and impairment of oxygen delivery. This explanation is especially intuitive in the kidney, where hemodynamic forces drive glomerular filtration, and a high metabolic activity exists in the transporting tubular epithelium (47, 48). TNF is well known to lead to the release of multiple other cytokines into the circulation (43, 44). Thus, we posed the question of whether TNF mediates LPS-induced ARF indirectly, by leading to hypotension and/or the release of other inflammatory mediators into the circulation. However, our data showing severe LPS-induced ARF in TNFR1−/− kidneys transplanted into TNFR1−/− recipients, with relative protection of TNFR1−/− kidneys in TNFR1+/+ recipients, prove that TNF mediates LPS-induced ARF directly in the kidney. In addition, the fact that TNFR1−/− mice are fully sensitive to LPS-induced mortality (9, 10) yet resistant to LPS-induced ARF further supports a renal-specific role for TNF.

A leading experimental model of ARF is that of ischemia-reperfusion, in which the renal artery is completely occluded for a period of time and then released. However, this is not an accurate simulation of what occurs in most cases of human ARF. Instead, sepsis with attendant multisystem organ failure is a leading cause of human ARF in most series (11, 12). Most pathologic specimens from human ARF show the same relatively mild degree of histologic damage that we note in our model (49, 50). Therefore, the conclusions we present here are especially relevant to ARF in humans.

Given the complexity of sepsis, the pathogenesis of LPS-induced ARF is probably multifactorial. Our studies point to two possible mechanisms by which TNF causes ARF: renal cell apoptosis and neutrophil infiltration. Apoptosis is increasingly recognized to play a key role in ischemia-reperfusion injury in the kidney and other organs (21, 51), and inhibition of apoptosis with caspase inhibitors has been shown to protect against ischemia-reperfusion renal injury (52). Overall, given our findings and the fact that TNF acting on TNFR1 is a well-described trigger of apoptosis (15, 16), it seems likely that in the setting of LPS-induced TNF release (3, 4), renal TNFR1 is activated and results in apoptosis. However, other known triggers of apoptosis, such as hypoxia or reactive oxygen intermediates, the latter perhaps derived from infiltrating neutrophils, which are present in the setting of sepsis could be relevant as well. Further delineation of the precise apoptotic pathways involved in LPS-induced ARF is the subject of ongoing work. A role for neutrophils in LPS- and ischemia-reperfusion-induced ARF has also been suggested by others (29, 38, 39). Our findings that TNFR1−/− mice given LPS were protected from ARF and had fewer neutrophils infiltrating the kidney are consistent with these previous observations. Possible mechanisms...
for the recruitment of neutrophils to the kidney include TNF acting through TNFR1 to up-regulate adhesion molecules such as E- and P-selectin and ICAM-1 (32–34) or chemotactic molecules such as IFN-inducible protein-10 and macrophage inflammatory protein-2 (31). The fact that apoptosis and neutrophil accumulation each correlated with the degree of LPS-induced renal failure in individual animals supports a causative role, but additional experiments must be conducted to determine the extent to which these mechanisms individually contribute to LPS-induced ARF and the extent to which these processes are interrelated.

NO has received great attention as an important mediator in ARF. Its role is complex, with both noxious and protective effects (53). Specific inhibition of iNOS, which is markedly up-regulated in the kidney after LPS administration, but not broad-spectrum NO inhibition, has been shown to be protective against LPS-induced ARF, implying that it is the inducible isoform of NO synthase that mediates injury in this disease model (41). Because TNF has been shown to up-regulate iNOS in a variety of cell types, including rat proximal tubule cells (54), we hypothesized that the TNFR1−/− mice were resistant to LPS-induced ARF in part because of a lesser induction of iNOS. However, our finding that iNOS was up-regulated to a similar extent in both TNFR1−/− and TNFR1−/+ kidneys shows that signaling through TNFR1 is not required for iNOS transcription and that the resistance to LPS-induced ARF which we observed in TNFR1−/− mice must be due to other mechanisms. This finding that iNOS has little role in LPS-induced ARF in mice is in agreement with the observations of Knotek et al. (17) that iNOS−/− mice are still susceptible to LPS-induced ARF.

Clinical sepsis is a complex phenomenon, due to a variety of bacterial products released intermittently or continuously, often in the setting of considerable comorbidities, and is therefore imperfectly simulated by animal models. Nevertheless, the key role of bacterial endotoxin in severe sepsis is undisputed. Although human trials of TNF-blocking agents in septic shock have been disappointing because they do not appear to affect mortality (8), preclinical models of sepsis occurring during the course of sepsis.

References


