TNF-α neutralization ameliorates obstruction-induced renal fibrosis and dysfunction


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Meldrum KK, Misseri R, Metcalfe P, Dinarello CA, Hile KL, Meldrum DR. TNF-α neutralization ameliorates obstruction-induced renal fibrosis and dysfunction. Am J Physiol Regul Integr Comp Physiol 292: R1456–R1464, 2007. First published December 14, 2006; doi:10.1152/ajpregu.00620.2005.—Upper urinary tract obstruction results in tubulointerstitial fibrosis and a progressive decline in renal function. Although several inflammatory mediators have been implicated in the pathophysiology of renal obstruction, the contribution of TNF-α to obstruction-induced fibrosis and renal dysfunction has not been thoroughly evaluated. To study this, male Sprague-Dawley rats were subjected to left unilateral ureteral obstruction vs. sham operation. Rats received either vehicle or a pegylated form of soluble TNF receptor type 1 (PEG-sTNFR1) every 84 h. The kidneys were harvested 1, 3, or 7 days postoperatively, and tissue samples were analyzed for TNF-α expression (ELISA), macrophage infiltration (ED-1 staining), transforming growth factor-β1 expression (ELISA, RT-PCR), collagen I and IV activity (Western Blot, immunohistochemistry), α-smooth muscle actin accumulation (immunohistochemistry, Western blot analysis), and angiotsinogen expression (Western blot). In a separate arm, the glomerular filtration rate (inulin clearance) of rats subjected to unilateral ureteral obstruction vs. sham operation indicates that TGF-β1 (TGF-β1), Renal cortical TGF-β1 levels increase in response to obstruction (8, 17, 31, 44), and evidence indicates that TGF-β1 is a major regulator of fibrosis via stimulation of fibroblast proliferation (23, 35, 37), ECM synthesis (i.e., collagen types I, III, and IV, proteoglycans, laminin, and fibronectin) (1, 5, 11, 15, 31, 37), and the simultaneous inhibition of collagenase and degradative matrix metalloproteinases (5, 6, 11, 37).

The profibrotic effect of TGF-β1 is regulated by a number of different inflammatory mediators. ANG II is widely considered to be the major initiating factor in the cascade of events culminating in tubulointerstitial fibrosis by stimulating increased expression of TGF-β1 (14, 16, 17, 33), NF-κB (21), and TNF-α (14, 18, 21). Inhibition of ANG II production and/or activity only partially ameliorates the development of obstruction-induced tubulointerstitial fibrosis (16, 17, 33), suggesting that other mediators contribute to this process.

TNF-α production is increased during renal obstruction (18, 28), and preliminary evidence suggests that TNF-α has a role in the development of tubulointerstitial fibrosis independent of the renin-angiotensin system (13, 14). Although ANG II stimulates obstruction-induced renal TNF-α production (14, 18, 21), angiotensin-converting enzyme inhibition only reduces TNF-α production early in the course of obstruction (i.e., 4 h) (18). Furthermore, Guo et al. (13, 14) have shown that TNF receptor I (TNFR1) and receptor 2 (TNFR2) knockout mice demonstrate a reduction in obstruction-induced interstitial volume in the presence of an intact renin-angiotensin signaling pathway (13, 14). Although these studies suggest a role for TNF-α in obstruction-induced renal fibrosis, the impact of TNF-α production on TGF-β1 expression, ECM synthesis, and renal dysfunction has not previously been determined. The purposes of this investigation were therefore to ascertain 1) the kinetics of TNF-α production, 2) the kinetics of TGF-β1 mRNA expression and protein production, 3) the expression of collagen I and IV, 4) the expression and accumulation of α-smooth muscle actin (α-SMA), 5) the expression of angiotensinogen, 6) changes in renal function, and 7) the impact of physiological TNF-α neutralization on the above parameters with the use of a rat model of unilateral ureteral obstruction (UUO).

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MATERIALS AND METHODS

Animals, experimental groups, and operative techniques. The animal protocol was reviewed and accepted by the Animal Care and Research Committee of the Indiana University School of Medicine. Male Sprague-Dawley rats weighing 250–300 g were acclimated and maintained on a standard pellet diet for 1 wk before initiation of the experiment. After induction of isoflurane anesthesia, the left ureter in each rat was isolated and completely ligated via a midline laparotomy. Sham-operated animals underwent an identical surgical procedure without ureteral ligation. Obstructed animals were treated with either a pegylated form of soluble TNFR1 (PEG-sTNFR1; 0.5 mg/kg sc 24 h before surgery and every 8 h thereafter; Amgen, Thousand Oaks, CA) or vehicle in the same volume and dosing schedule. Sham-operated animals received vehicle. At the completion of the experiment, the animals were reanesthetized, the left kidneys were removed and snap frozen in liquid nitrogen, and the animals were subsequently euthanized.

The animals were divided into the following experimental groups (n = 5–8 per group): 1) 1 day of sham operation + vehicle; 2) 1 day of UUO + vehicle; 3) 1 day of UUO + PEG-sTNFR1; 4) 3 days of sham operation + vehicle; 5) 3 days of UUO + vehicle; 6) 3 days of UUO + PEG-sTNFR1; 7) 1 wk of sham operation + vehicle; 8) 1 wk of UUO + vehicle; and 9) 1 wk of UUO + PEG-sTNFR1. A second group of animals was exposed to a 1-wk course of UUO in the presence of PEG-sTNFR1 or vehicle, with glomerular filtration rate (GFR; insulin clearance) measurements taken as a means to quantify and compare renal function in these two treatment groups (n = 6–8 per group).

PEG-sTNFR1. TNF-α neutralization was achieved with a soluble, long-acting form of TNFR1. Recombinant sTNFR1 is an Escherichia coli-derived, two-domain, monomeric form of the four-domain sTNFR1. For prolonged half-life, a high-molecular-weight PEG molecule was attached at the NH2-terminal position. Preclinical studies to date demonstrate that subcutaneous administration of PEG-sTNFR1 has been effective in limiting the inflammatory reaction of rheumatoid arthritis in rat models at a dose of 0.3 mg/kg (2, 3).

Tissue homogenization. A portion of the renal cortex from each kidney was homogenized for analysis using a rat TNF-α ELISA. Homogenization was performed after the tissue samples had been diluted in 5 vol of homogenate buffer [10 mM HEPES (pH 7.9), 10 mM KCl, 0.1 mM EGTA, 1 mM DTT, and 0.5 mM PMSF] by a vertishear tissue homogenizer. Renal homogenates were centrifuged at 3,000 g for 15 min at 4°C. The supernatants were subsequently stored at −80°C until the TNF-α ELISA could be performed.

TNF-α and TGF-β1 protein expression. Renal cortical homogenous TNF-α and TGF-β1 protein content were determined by ELISA. The TNF-α ELISA was performed by adding 100 μl of each sample to wells in a 96-well plate of a commercially available rat TNF-α ELISA kit (BD Biosciences, San Diego, CA) according to the manufacturer’s instructions. The TGF-β1 ELISA was performed by activating latent TGF-β1 in each homogenized sample by adding 1 N HCl to each sample at a 1:25 dilution for 60 min at 4°C. The samples were then neutralized with 1 N NaOH and immediately tested. One hundred microliters of each sample were added to wells in a 96-well plate of a commercially available rat TGF-β1 ELISA kit (BD Pharmingen), and the assay was performed according to the manufacturer’s instructions. All samples were tested in duplicate. The ELISA results were expressed as picograms of TNF-α or TGF-β1 per milliliter.

RT-PCR. Semiquantitative RT-PCR was used to assess renal TGF-β1 gene expression. Renal tissue was obtained from sham-treated and obstructed kidneys with and without TNF-α neutralization (3 different samples per time point). Total RNA was extracted from the tissue by homogenization in Trizol (GIBCO BRL, Gaithersburg, MD) as previously described (19). PCR was performed by adding 2 μl of RT product to a commercial dual-PCR supernmix (Maxim Biotech, San Francisco, CA) containing Tag DNA polymerase and primers for both TGF-β1 and GAPDH. The amplified products were separated in a 2% agarose gel containing 1× Tris-borate-EDTA (pH 8.3). PCR amplification products were quantified by staining the gel with ethidium bromide; we determined the density of each band by NIH Image analysis software. The data are presented as the ratio of the densitometric units of the TGF-β1 mRNA band to the densitometric units of the GAPDH mRNA band.

Macrophage ED-1 immunolabeling. Macrophage infiltration into renal cortical tissue sections was evaluated by staining representative tissue sections with a monoclonal anti-ED-1 antibody. Transverse 6-μm sections were prepared, deparaffinized, hydrated, and washed in Tris-buffered saline (TBS). H2O2 (3%) was applied to the tissue sections, and the sections were washed, subjected to enzyme digestion with proteinase K (Dako, Carpinteria, CA), and washed again. Tissue sections were then incubated with diluted primary antibody (1:50; Chemicon, Temecula, CA) for 45 min, then washed with TBS, and incubated with peroxidase-conjugated secondary antibody (EnVision kit; Dako). The slides were developed, rinsed in TBS, dehydrated, and mounted.

Collagen I and IV expression. Protein extracts from homogenized samples (60 μg/well) were denatured and dotted onto a nitrocellulose membrane with the use of a vacuum manifold (MiniFold II; Schleicher & Schuell, Keene, NH). Immunoblotting was performed by incubating the membrane in 5% dry milk for 1 h, followed by incubation with an anti-collagen I or IV goat polyclonal antibody (1:100; Southern Biotechnology, Birmingham, AL) for 2 h. After membranes were washed twice in Tweek phosphate-buffered saline (T-PBS), the blots were incubated with peroxidase-conjugated secondary antibody (1:750; StressGen, Victoria, British Columbia, Canada) and developed with enhanced chemiluminescence (Amersham Pharmacia Biotech, Piscataway, NJ). We determined band density using NIH Image analysis software.

The volume of the renal interstitium was evaluated in each renal sample by staining representative tissue sections (3 per sample) using an anti-collagen IV goat polyclonal antibody (Southern Biotechnology). Transverse 6-μm cryosections were prepared and fixed for 10 min in 70% acetone-30% methanol at −20°C. Normal goat serum was applied as a blocking agent, and the slides were washed in PBS three times for 3 min. Sections were then incubated with diluted primary antibody [goat anti-collagen IV antibody (1:40); Southern Biotechnology] for 1 h and then washed with PBS and incubated with a FITC-conjugated secondary antibody (1:1,000; StressGen) for 45 min. The slides were mounted with an antiquenching agent, ProLong Antifade (Molecular Probes, Eugene, OR), and stored at −4°C. Tissue sections were photographed (×400) with a fluorescent microscope (Leica, Wetzlar, Germany).

α-SMA accumulation. Interstitial accumulation of α-SMA was evaluated by staining representative tissue sections with a monoclonal anti-α-SMA antibody (1A4 clone; Dako Cytomation, Carpinteria, CA). Transverse 6-μm sections were prepared, deparaffinized, and washed in TBS. The tissue sections were subjected to an avidin-biotin block for 10 min, rinsed in TBS, and then blocked with normal horse serum for 20 min. Sections were then incubated with diluted primary antibody (1:1,000) for 10 min and then washed with TBS and incubated with a biotinylated secondary antibody (1:500; Dako Cytomation LSAB2 kit) for 10 min. The sections were then incubated with streptavidin-horseradish peroxidase for 10 min, rinsed in TBS, dehydrated, and mounted.

Western blot analysis. Protein extracts from homogenized samples (50 μg/lane) were electrophoresed into an 18% Tris-glycine gel and transferred to a nitrocellulose membrane. Immunoblotting was performed by incubating each membrane in 5% dry milk overnight at 4°C, followed by incubation with an anti-α-SMA antibody (1:100 for 2 h at room temperature; Abcam, Cambridge, MA) or an anti-angiotseninogen antibody (1:1,000 overnight at 4°C; Wwant, Bellinzona, Switzerland). After membranes were washed three times in T-PBS, each membrane was incubated for 1 h at room temperature with a peroxidase-
conjugated secondary antibody (1:5,000 for α-SMA and 1:2,000 for angiotensinogen). Equivalent protein loading for each lane was confirmed by stripping and reblotting each membrane for β-actin (Sigma, St. Louis, MO; primary 1:5,000 for 30 min at room temperature, secondary 1:5,000 for 30 min at room temperature). The membranes were developed by enhanced chemiluminescence (Amersham Pharmacia), and the density of each band was determined by NIH Image analysis software and expressed as a percentage of β-actin density.

GFR. Inulin clearance rates were used to measure total GFR in animals exposed to UUO as described by Qi et al. (36). FITC-labeled inulin was diluted in saline to a concentration of 20 mg/ml and heated to 45–50°C to dissolve in solution. FITC-inulin (7 mg) was then injected into the tail vein of each rat 48 h before the onset of obstruction (baseline) and 1 wk after the onset of obstruction. Blood samples were obtained from the tail vein of each rat 10, 30, 60, and 120 min after the injection. The quantity of FITC-inulin in the serum of each blood sample was determined with a fluorometer, and the GFR (μl/min) was calculated by measuring the rate at which FITC-inulin was cleared from the bloodstream.

Single-kidney GFR was measured by injecting FITC-inulin (7 mg) into the tail vein of each rat. Blood samples were obtained from each tail vein 10 min after injection. A nephrostomy tube was then inserted into the obstructed left kidney immediately after inulin injection, and urine production from the obstructed kidney was collected over 1 h. The concentration of FITC-inulin in the serum and urine samples was determined with a fluorometer. Single-kidney GFR (μl/min) was calculated by the following formula: [(urine inulin concentration × urine volume)/serum inulin concentration] ÷ time.

Statistical analysis. Data are presented as means values ± SE. Differences at the 95% confidence intervals were considered significant. The experimental groups were compared by ANOVA with post hoc Bonferroni-Dunn (JMP statistical software version 5.0, Berkeley, CA).

RESULTS

Kinetics of TNF-α production. Renal cortical TNF-α levels increased in response to ureteral obstruction, as shown in Fig. 1. Sham-operated animals demonstrated low levels of TNF-α at each time point. In contrast, renal cortical TNF-α levels increased significantly after 1 day of obstruction (109 ± 66 vs. 6 pg/ml in Sham; \( P < 0.05 \)), reached peak levels after 3 days of obstruction (215 ± 68 vs. 3 pg/ml in Sham; \( P < 0.01 \)), and remained elevated after 1 wk of obstruction (140 ± 29 vs. 65 ± 4 pg/ml in Sham; \( P < 0.05 \)). TNF-α protein levels after 1, 3, and 7 days of obstruction in the presence of PEG-STNFRI were reduced to 84 ± 6 vs. 109 ± 16 pg/ml.

Fig. 2. Macrophage infiltration during unilateral ureteral obstruction. Photographs depict macrophages (ED-1) in the tubulointerstitial space of the renal cortex (6-μm sections, ×400). Macrophages are stained black. T, tubule; G, glomerulus. A: 1-wk sham-operated animal. Few macrophages are visible. B: 1-wk obstructed kidney. A significant number of macrophages are visible throughout the interstitial space (blue arrows). C: 1-wk obstructed kidney after PEG-sTNFR1 administration. The macrophage infiltrate in the interstitial space remains prominent.

![Fig. 2.](image-url)
Kinetics of TGF-β1 production. Renal cortical tissue obtained from sham-operated animals revealed minimal TGF-β1 mRNA at each time point (Fig. 3A). Steady-state TGF-β1 mRNA levels increased significantly after 3 days of renal obstruction (59 ± 1% vs. 26 ± 4% in Sham; P < 0.01) and reached peak levels after 1 wk of obstruction (92 ± 8% vs. 46 ± 5% in Sham; P < 0.01). Animals treated with 0.5 mg/kg PEG-sTNFR1 during the time period of renal obstruction demonstrated a significant reduction in TGF-β1 expression. After 3 days and 1 wk of obstruction, steady-state TGF-β1 mRNA levels were reduced to 42 ± 2% vs. 59 ± 1% of GAPDH mRNA (P < 0.01) and 58 ± 5% vs. 92 ± 8% of GAPDH mRNA in vehicle-treated rats (P < 0.05), respectively.

Renal cortical TGF-β1 protein levels were similarly elevated in response to ureteral obstruction, as shown in Fig. 3B. Although sham-operated animals demonstrated low levels of TGF-β1, renal cortical TGF-β1 levels increased significantly after 1 day (186 ± 17 vs. 102 ± 9 pg/ml in Sham; P < 0.01) and 3 days (317 ± 56 vs. 94 ± 10 pg/ml in Sham; P < 0.01) of renal obstruction, and peak levels of TGF-β1 occurred after 1 wk of renal obstruction (444 ± 57 vs. 130 ± 7 pg/ml in Sham; P < 0.01). The administration of PEG-sTNFR1 significantly reduced TGF-β1 protein levels after 1, 3, and 7 days of obstruction to 106 ± 5 vs. 186 ± 17 pg/ml (P < 0.01), 133 ± 23 vs. 317 ± 56 pg/ml (P < 0.01), and 160 ± 35 vs. 444 ± 57 pg/ml in vehicle-treated rats (P < 0.01), respectively.

Collagen I and IV expression. Protein extracts from homogenized sham and obstructed renal samples were analyzed for collagen I and IV content. Samples from sham-operated animals demonstrated low levels of collagen I and IV at each time point. In contrast, collagen I expression increased significantly after 3 days of renal obstruction (1.4 ± 0.2 vs. 0.3 ± 0.1 optical density (OD)/mm² in Sham; P < 0.05) and was maximal after 1 wk of obstruction (2.8 ± 0.7 vs. 0.5 ± 0.1 OD/mm² in Sham; P < 0.05; Fig. 4). Similarly, collagen IV expression increased significantly 3 days after the onset of obstruction (1.9 ± 0.3 vs. 0.8 ± 0.3 OD/mm² in Sham; P < 0.05) and 60 ± 8 vs. 215 ± 10 pg/ml (P < 0.01), and 44 ± 10 vs. 140 ± 29 pg/ml in vehicle-treated rats (P < 0.01), respectively.

Macrophage infiltration. Renal cortical tissue sections were stained for ED-1 to assess the degree of macrophage infiltration during renal obstruction (Fig. 2). Although sham-treated samples exhibited minimal macrophage staining (Fig. 2A), renal samples exposed to 1 wk of obstruction demonstrated a significant accumulation of macrophages within the interstitial space (Fig. 2B). The degree of macrophage infiltration was not affected by PEG-sTNFR1 treatment (Fig. 2C).
reached peak levels after 1 wk (2.4 ± 0.4 vs. 0.9 ± 0.3 OD/mm² in Sham; *P* < 0.05) of renal obstruction (Fig. 5).

TNF-α neutralization significantly reduced collagen I and IV activity in the renal cortex. After 1 day, 3 days, and 1 wk of renal obstruction, collagen I expression was reduced (vs. vehicle) to 0.6 ± 0.1 vs. 1.2 ± 0.2 OD/mm² (*P* < 0.05), 0.5 ± 0.1 vs. 1.4 ± 0.2 OD/mm² (*P* < 0.01), and 0.7 ± 0.1 vs. 2.8 ± 0.7 OD/mm² (*P* < 0.05), respectively. After 3 days and 1 wk of obstruction, collagen IV expression was reduced (vs. vehicle) to 0.9 ± 0.3 vs. 1.9 ± 0.3 OD/mm² (*P* < 0.05) and 0.8 ± 0.1 vs. 2.4 ± 0.4 OD/mm² (*P* < 0.05), respectively.

Collagen IV expression was further assessed by immunohistochemical techniques in sham-treated and obstructed renal cortical tissue sections. Collagen IV was detected in the basement membrane of renal tubules and capillaries in sham-treated animals; however, the interstitial space between the tubules demonstrated minimal staining (Fig. 6A). In contrast, obstructed kidneys demonstrated a marked increase in interstitial volume, in addition to massive tubular dilation (Fig. 6B), which was more pronounced as the duration of obstruction increased. A marked decrease in renal cortical interstitial volume, however, was detected in 1-wk obstructed rats exposed to TNF-α neutralization (Fig. 6C).

**α-SMA accumulation.** Increased interstitial accumulation of α-SMA is an indicator of renal fibrosis during renal obstruction (13, 14). Although sham-treated samples only exhibited

**Fig. 5.** Collagen IV activity after unilateral ureteral obstruction. Slot blot demonstrates collagen IV activity at various time points of renal obstruction after vehicle (obstruction) or PEG-sTNFR1 (obstruction + TBP) administration.

**Fig. 6.** Immunolocalization of collagen IV after unilateral ureteral obstruction. Photographs demonstrate collagen IV staining in response to renal obstruction and the effects of PEG-sTNFR1 (6-μm sections, ×400). Collagen IV is stained green. T, tubule; IS, interstitial space. A: 1-wk sham-operated animal. Collagen IV is detected in the basement membrane of tubules, but the interstitial space demonstrates minimal staining. B: 1-wk obstructed kidney. A significant increase in tubular diameter and interstitial volume are visible. C: 1-wk obstructed kidney after PEG-sTNFR1 administration. Renal tubules remain dilated; however, the interstitial volume is significantly reduced compared with vehicle-treated animals.
α-SMA staining in the wall of blood vessels (Fig. 7A), an increase in renal cortical staining for α-SMA and an increase in the size of the interstitial space was detected in renal samples exposed to 1 wk of obstruction (Fig. 7B). In contrast, obstructed renal samples exposed to TNF-α neutralization demonstrated a marked reduction in interstitial α-SMA accumulation (Fig. 7C).

These observations were confirmed with Western blot analysis (Fig. 8). A significant increase in the expression of α-SMA was detected in renal samples exposed to 1 wk of obstruction compared with sham-treated kidneys (0.23 ± 0.03 vs. 0.07 ± 0.04 OD/mm² in Sham; P < 0.05), whereas a significant decrease in renal cortical α-SMA expression was evident in obstructed rats exposed to TNF-α neutralization (0.13 ± 0.02 vs. 0.23 ± 0.03 OD/mm² in vehicle; P < 0.05).

Angiotensinogen expression. The impact of renal obstruction on renin-angiotensin axis signaling was evaluated by examining angiotensinogen expression in homogenized renal cortical samples. A significant increase in angiotensinogen expression was detected in renal samples exposed to 1 wk of obstruction compared with sham-treated kidneys (0.91 ± 0.19 vs. 0.18 ± 0.06 OD/mm² in Sham; P < 0.01; Fig. 9). The administration of PEG-sTNFR1 during renal obstruction markedly reduced angiotensinogen expression compared with vehicle-treated animals (0.45 ± 0.06 vs. 0.92 ± 0.19 OD/mm² in vehicle; P < 0.05).

Renal function. The impact of UUO on renal function was determined by measuring total and single-kidney GFR in animals subjected to 1 wk of UUO. As expected, total GFR was significantly reduced in the presence of UUO (44 ± 19 vs. 118 ± 10 µl/min in baseline; P < 0.05; Fig. 10A); however, total GFRs were significantly improved in PEG-sTNFR1-treated rats compared with vehicle-treated rats (97 ± 13 vs. 44 ± 19 µl/min in vehicle; P < 0.05). To further clarify the impact of PEG-sTNFR1, single-kidney GFR was measured in 1-wk obstructed kidneys exposed to either vehicle or TNF

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**Fig. 7.** Immunolocalization of α-smooth muscle actin (α-SMA) after unilateral ureteral obstruction. Photographs demonstrate α-SMA staining in response to renal obstruction and the effects of PEG-sTNFR1 (6-µm sections, ×400). α-SMA is stained brown, and renal tubular cells are stained blue. Arrowheads indicate areas of marked α-SMA accumulation. A: 1-wk sham-operated animal. α-SMA is only detected in the wall of blood vessels. B: 1-wk obstructed kidney. A significant increase in α-SMA deposition and interstitial volume are visible. C: 1-wk obstructed kidney after PEG-TNFR1 administration. α-SMA deposition and interstitial volume are significantly reduced compared with vehicle-treated animals.

**Fig. 8.** α-SMA expression after unilateral ureteral obstruction. Shown are Western blot analysis (left) demonstrating α-SMA activity and the corresponding densitometric analysis (right) of α-SMA bands (represented as the α-SMA density percentage of β-actin density) after 1 wk of renal obstruction following vehicle (obstruction) or PEG-sTNFR1 (obstruction + TBP) administration.
neutralization. Interestingly, single-kidney GFR was found to be significantly higher in obstructed kidneys exposed to PEG-sTNFR1 than in kidneys exposed to vehicle alone (28 ± 2.7 vs. 16.5 ± 3.2 μl/min in vehicle; P < 0.05; Fig. 10B).

DISCUSSION

Tubulointerstitial fibrosis is a major pathological component of obstructive renal injury, contributing to obstruction-induced renal damage and renal insufficiency. The pathophysiology of obstruction-induced renal fibrosis involves fibroblast proliferation, macrophage infiltration, the elaboration of cytokines and other proinflammatory mediators, and an imbalance in ECM deposition and degradation. ANG II has widely been considered the primary initiating factor in this complex pathophysiological process (14, 16, 17, 21, 33); however, our results demonstrate that TNF-α also has a significant role in obstruction-induced fibrosis and subsequent renal dysfunction.

TNF-α is a potent proinflammatory cytokine implicated in the pathophysiology of a wide variety of renal diseases (10, 14, 22, 26, 32). TNF-α upregulates its own expression and the expression of other inflammatory mediators, recruits and stimulates a variety of immunologically active cells, and induces apoptotic renal tubular cell death (25, 26, 29, 32a). Renal cortical TNF-α mRNA expression and protein production increase in response to obstruction (13, 14, 18, 28), a finding that is corroborated by our demonstration of increased TNF-α levels after 1 day and peak TNF-α levels after 3 days of renal obstruction. Interestingly, we found that TNF-α neutralization did not appreciably alter obstruction-induced macrophage infiltration into the kidney, suggesting that the effects of TNF-α on renal fibrosis were independent of macrophage accumulation. TNF-α production is stimulated by ANG II (18, 21); however, ANG II inhibition only has a partial effect on TNF-α production. Although Kaneto et al. (18) demonstrated a 40% reduction in TNF-α mRNA after 4 h of renal obstruction with the administration of enalapril (angiotensin-converting enzyme inhibitor), no reduction in TNF-α mRNA was evident after 5 days (18). In light of these findings and of previous work demonstrating renal tubular cell TNF-α production independent of inflammatory cell infiltration (18, 28), it is clear that resident renal tubular cells produce significant levels of TNF-α independent of the renin-angiotensin signaling axis.

To evaluate the role of TNF-α in obstruction-induced renal fibrosis, obstructed renal samples were analyzed for TGF-β1, collagen I and collagen IV expression, and α-SMA accumulation in the presence and absence of TNF-α neutralization. Our results demonstrate a significant reduction in TGF-β1 mRNA expression and protein content, approaching sham levels, in response to TNF-α neutralization. This is contrary to observa-
tions made by Guo et al. (13, 14), in which obstructed kidneys from TNFR1 and TNFR2 double knockout mice exhibited only a modest reduction in TGF-β1 protein levels and no reduction in steady-state TGF-β1 mRNA levels. The authors did demonstrate a marked reduction in both parameters, however, with the addition enalapril and therefore concluded that ANG II is the primary mediator of obstruction-induced renal injury (14). Interestingly, in this same study, Guo et al. (13, 14) observed a greater reduction in collagen IV staining and interstitial volume in TNFR1 and TNFR2 double knockout mice than they observed in ANG II receptor knockout mice. Our results similarly demonstrate a significant reduction in collagen I and collagen IV expression, a significant reduction in interstitial α-SMA expression and accumulation, and a marked reduction in interstitial volume in obstructed kidneys exposed to TNF-α neutralization.

In many organ systems, TNF-α has been shown to promote interstitial remodeling and ECM degradation by stimulating the production of stromal collagenases and matrix metalloproteinases and simultaneously inhibiting the synthesis of structural components of ECM, such as elastin and collagen (12, 34, 38, 39). In cultured dermal fibroblasts, TNF-α has also been shown to interfere with TGF-β downstream signaling, resulting in decreased collagen gene synthesis and matrix deposition (42, 43). Chou et al. (7), however, discovered that the low concentrations of TNF-α found in chronic inflammatory conditions actually inhibits collagen phagocytosis and promotes tissue fibrosis. Indeed, in an animal model of crescentic glomerulonephritis, TNF-α inhibition reduced tubulointerstitial fibrosis, α-SMA and collagen deposition, and renal dysfunction (20). Our data support these findings and provide further evidence for the observation of Chou et al. that the end organ effect of TNF-α is dependent on its local and/or systemic concentration.

The renin-angiotensin signaling axis has a prominent role in obstruction-induced renal fibrosis, and the effect of TNF-α neutralization on angiotensinogen expression was therefore evaluated. Angiotensinogen is the precursor to ANG I, and its gene expression is regulated, in part, by NF-κB (24). It has previously been shown that ANG II stimulates TNF-α production (18, 21); however, in this investigation, we demonstrate that TNF-α also influences the renin-angiotensin signaling axis by increasing angiotensinogen expression. TNF-α induces NF-κB activation during UUO (27), and it is likely through this mechanism that TNF-α increases angiotensinogen expression. Although the interrelationship and relative contribution of TNF-α and ANG II to obstruction-induced renal fibrosis warrants further investigation, it is clear, based on our observations in a physiological model of TNF-α inhibition, that TNF-α is an important mediator of obstruction-induced fibrosis.

Significantly, this is the first demonstration that a reduction in obstruction-induced TGF-β, collagen expression, α-SMA accumulation, and interstitial volume following TNF-α neutralization correlates to an improvement in renal function. After 1 wk of renal obstruction, GFRs were measured in animals exposed to either PEG-8TNFRI or vehicle. TNF-α neutralization markedly improved renal function in these animals compared with vehicle-treated rats, providing evidence that the beneficial effects of TNF-α neutralization on cytokine production and ECM deposition translate into protection against obstruction-induced renal dysfunction. At high circulating concentrations, TNF-α is directly injurious to the kidney, inducing glomerular endothelial damage, polymorphonuclear cell accumulation, and acute tubular necrosis (4, 41), and it is unclear from this study whether TNF-α impairs renal function directly during UUO or whether its effect on renal function is mediated through an exacerbation of tubulointerstitial fibrosis. Given the lack of glomerular injury, tubular necrosis, and significant polymorphonuclear neutrophil infiltration, however, it seems most plausible that the low concentrations of TNF-α generated during this chronic insult contribute to renal dysfunction via stimulation of profibrotic signaling pathways.

Upper urinary tract obstruction is an important clinical problem in both children and adults. Obstructive renal injury stimulates a cascade of events culminating in tubulointerstitial fibrosis, programmed cell death, and, eventually, a permanent decline in renal function. This study identifies TNF-α as an important mediator of obstruction-induced renal fibrosis. Physiological neutralization of TNF-α activity with PEG-sTNFR1 during renal obstruction not only reduces TGF-β1 expression, collagen I and IV expression, α-SMA accumulation, angiotensinogen expression, and interstitial volume, it improves renal function after obstructive injury. As the role of TNF-α in obstruction-induced renal injury becomes more clearly defined, new therapeutic strategies aimed at ameliorating renal fibrosis may be realized.

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REFERENCES

10. Donnahoo KK, Meng X, Ayala A, Cain MP, Harken AH, Meldrum DR. Early kidney TNF-α expression mediates neutrophil infiltration and


