Expression of adhesion molecules in rat renal cortex during experimental hydronephrosis

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Expression of adhesion molecules in rat renal cortex during experimental hydronephrosis. Unilateral ureteral obstruction (UUO) is associated with an early and steadily increasing infiltration of macrophages into the renal cortical interstitium. As adhesion molecules may play an important role in macrophage recruitment following the mechanical disturbance after UUO, we delineated the time course of intercellular adhesion molecule (ICAM)-1 and vascular adhesion molecule (VCAM)-1 mRNA and protein expression. A significant 6.6- (P < 0.001), 2.6- (P < 0.025), 2.6- (P < 0.01), and 2.0-fold (P < 0.005) increase in ICAM-1 mRNA expression was observed at 12, 24, 48, and 96 hours after obstruction, respectively, in comparison to the contralateral unobstructed kidney (CUK). Despite an apparent relief of obstruction, four weeks following reversal of obstruction mRNA levels of ICAM-1 remained equivalent to the 96-hour obstructed kidney group. No significant difference in VCAM-1 mRNA expression was observed between the obstructed kidneys and CUK specimens. Immunohistochemistry revealed focal labeling of ICAM-1 on the apical and basolateral surface of the renal tubules, peritubular interstitium, and vessels of the renal cortex by 12 hours after UUO. In contrast, only faint staining for ICAM-1 protein was observed in the cortex from CUK specimens. The obstructed and CUK specimens exhibited diffuse immunolocalization of VCAM-1 in the cortical tubules and Bowman's capsular epithelium. In situ hybridization showed mRNA transcription for ICAM-1 localized in the peritubular interstitium and cortical tubules from obstructed kidneys. To lend mechanistic insight into the response of ICAM-1 to the mechanical disturbance after UUO, the expression of ICAM-1 mRNA was examined when freshly isolated proximal tubules were exposed to angiotensin II (1 to 1000 µM) immediately after preparation. Levels of ICAM-1 mRNA were elevated 1.4-, 7.1-, and 3.7-fold when exposed to 10 µM, 100 µM and 1000 µM of angiotensin II for one hour, respectively, when compared to control cultures. The addition of losartan to proximal tubules for one hour prior to angiotensin II stimulation decreased ICAM-1 levels to control values. In summary, this investigation demonstrates that ICAM-1 is important in the initiation of macrophage recruitment into the renal cortex of the obstructed kidney. These findings provide evidence that angiotensin II, produced after unilateral ureteral obstruction (UUO), is a potent chemoattractant for macrophages. Rovin et al [6] have demonstrated that acute ureteral obstruction results in the release of a specific chemokine that is a lipid.

Renal interstitial macrophage influx is one of the earliest responses of the kidney to ureteral obstruction. Schreiner et al [7] observed an initial macrophage infiltrate within four hours after unilateral ureteral obstruction (UUO), with a peak response by 24 hours and stabilizing thereafter. They found that the mononuclear cell infiltrate in the obstructed kidney consisted primarily of macrophages, with T lymphocytes of the cytotoxic suppressor cell subclass accounting for a substantial portion of the cell infiltrate [7]. Our studies on interstitial macrophage numbers show that the parenchymal infiltration develops early, but not until 12 hours after ureteral ligation, with marked serial increments in cortical interstitial macrophage number continuing to rise through to 96 hours. ED-1-positively labeled macrophages were observed almost exclusively in the peritubular cortical interstitial space in a ring-like pattern and with the majority of macrophage clusters around glomeruli [2, 3].

Recently, the role of adhesion molecules in the accumulation of inflammatory cells and matrix molecules in the kidney has garnered increasing attention. Intracellular adhesion molecule (ICAM)-1 (CD 54) is a cell surface glycoprotein that is constitutively expressed by endothelial cells, and its expression can be induced on other cell types such as mesangial cells and epithelial cells by pro-inflammatory cytokines such as interleukin (IL)-1, tumor necrosis factor (TNF), and γ-interferon (IFN) [8, 9]. ICAM-1 plays a major role in macrophage and T-lymphocyte adhesion to activated endothelium via binding to the ligand, lymphocyte function-associated antigen (LFA)-1 [9]. LFA-1 is a member of the β2 integrin family and is expressed on most leukocytes [10]. Vascular cell adhesion molecule (VCAM)-1 is...
constitutively expressed at low levels by endothelial cells and can also be induced on endothelial cells and other cell types by cytokines [11]. VCAM-1 supports adhesion of monocytes, eosinophils, basophils, and lymphocytes, but not neutrophils.

The UUO model of obstructive nephropathy is associated with an early and steadily increasing renal cortical macrophage infiltrate [2]. We have delineated the time course of ICAM-1 and VCAM-1 mRNA expression, by Northern analysis, as well as the mRNA and immunohistochemical localization patterns of ICAM-1 and VCAM-1 by in situ hybridization and avidin biotinylated peroxidase staining, respectively, in an attempt to demonstrate that these particular adhesion molecules may play an important early role in macrophage recruitment into the tubulointerstitium in this typically-viewed nonimmune model of progressive tubulointerstitial fibrosis. We also utilized reversal of obstruction (R-UUO), a maneuver that reproducibly yields a reduction in the renal cortical macrophage infiltrate [3, 7], as an interventional maneuver to give additional insight into the role of ICAM-1 in the kidney’s response to the mechanical disturbance produced by UUO. In order to lend mechanistic insight into this pathobiologic process, we also chose to assess whether angiotensin II, which is up-regulated within hours in this model, can increase the mRNA expression of ICAM-1 in isolated proximal tubules.

Methods

Animals and tissue preparation

Male Sprague-Dawley rats (Charles River Breeding Laboratories, Wilmington, MA, USA), weighing 150 to 200 g were fed standard rat chow (5001; Purina Chows, St. Louis, MO, USA) and given water ad libitum. With Brevital (50 mg/kg body wt, intraperitoneal; E. Lilly, Indianapolis, IN, USA) anesthesia, animals underwent left proximal ureteral ligation via a midline abdominal incision.

Both the obstructed kidneys and the contralateral unobstructed kidney (CUK) specimens were harvested from animals (N = 6 animals/time point) at 12, 24, 48, and 96 hours after UUO. Total RNA was extracted from renal cortical tissue and was purified for Northern analysis of rat ICAM-1 and VCAM-1. Midcoronal sections of the kidneys were also taken for immunohistochemical labeling and in situ hybridization. For immunohistochemistry, specimens were initially fixed in periodate-lysine-paraformaldehyde (PLP) for three hours [12]. Serial 6 μ cryostat sections were cut and stained.

Other groups of rats (N = 6/time point) underwent a reversal of left ureteral obstruction (R-UUO) according to the methods of Schreiner et al [7]. Briefly, this maneuver was performed by tying a ligature over a piece of bisected polyethylene tubing (PE-50), 5 mm in length, that had been passed around the left ureter. The ureter was subsequently released by removing the PE tubing without damage to the ureter. R-UUO was performed at 96 hours after UUO. Our previous studies have revealed that this time interval corresponds to maximal interstitial macrophage number, cortical TGF-β1 [2], α-smooth muscle actin [13], and tissue inhibitor of metalloproteinase-1 [14] mRNA expression.

At 96 hours post-ureteral ligation, there was marked hydrouretonephrosis of the left kidney and collecting system with prominent pelviectasis. Beginning 96 hours to four weeks following a release of obstruction, there was a complete resolution of the left hydroureteronephrosis. The left ureter of rats with R-UUO was seen to have active peristalsis with only mild residual pelviectasis in the 96-hour post-R-UUO animals. Groups of rats with R-UUO were sacrificed at 24, 48, 72, and 96 hours and at 1, 2, and 4 weeks after this intervention to discern whether cortical adhesion molecule expression is modulated over time in response to this maneuver.

Cortical RNA extraction and Northern hybridization

The cortex was isolated from individual rats in all groups at 4°C and this tissue was minced and homogenized in cold phosphate-buffered saline. Total cellular RNA was extracted from the renal cortex by the acid guanidinium thiocyanate-phenol chloroform method [15]. Total RNA (20 μg/lane), was denatured and electrophoresed through 1.2% agarose gels containing 0.66 M formaldehyde and transferred to nylon filters (Nytran; Schleicher and Schuell, Keene, NH, USA) by capillary blotting. RNA was immobilized by baking at 80°C for 30 minutes. The blots were examined under ultraviolet light in the presence of ethidium bromide to determine the position of the 28 s and 18 s ribosomal RNA bands and to assess the integrity of the RNA. The blots were hybridized in a solution containing 5 mol/liter sodium chloride, 0.05 mol/liter Tris, pH 7.4, 20 × dextran sulphate solution, 0.1 mg/ml salmon sperm DNA, and 1.0% sodium dodecyl sulphate with the addition of the following cDNA probes: rat ICAM-1 [16], which yields a 0.9 kb mRNA transcript (kindly provided by Dr. T. Benveniste, University of Alabama, Birmingham, AL, USA), and rat VCAM-1 which yields a 2.2 kb mRNA transcript (kindly provided by Dr. T. Collins, Department of Pathology, Brigham and Women’s Hospital, Harvard Medical School, Boston, MA, USA). The cDNA probes were labeled with [32P]deoxyctydine triphosphate, with a random primer cDNA labeling kit (Boehringer-Mannheim, Indianapolis, IN, USA). After hybridization at 65°C for 20 hours, blots were washed and quantitative densitometry was evaluated with an IBM-AT compatible computer (Quantity One, PDI Incorporated, Huntington, NY, USA). The negative images of the photographs of the formaldehyde gels stained with ethidium bromide were also scanned for the density of the 28 s ribosomal band to quantify the amount of RNA loaded onto the gel, as previously reported [3], thereby correcting for variation in loading samples. The mRNA levels of rat ICAM-1 and VCAM-1 were expressed as a ratio of the optical density for the specific adhesion molecule mRNA to that of the optical density of 28 s ribosomal RNA from the ethidium bromide-stained formaldehyde gel. The peak optical density reading of each band on the autoradiograph is reported as densitometric units.

Immunohistochemical labeling

This technique has been previously described in detail [2, 3]. Briefly, midcoronal kidney sections obtained from both the obstructed kidney and CUK specimens, over the 4- to 96-hour study interval, had endogenous peroxidase activity quenched with 4:1 methanol-hydrogen peroxide solution and endogenous biotin blocked with avidin D and biotin blocking solutions (Vector Laboratories, Burlingame, CA, USA). With the use of an avidin-biotin complex immunoperoxidase system, a series of incubations were used. First, normal horse serum (1:20, Vector) incubation for 50 minutes at 25°C was performed. Then sections were incubated with a mouse monoclonal immunoglobulin G (IgG) antibody obtained from ascites fluid that recognizes rat ICAM-1 (1:50; Serotec, Oxford, UK) and a mouse monoclonal antibody to
rat VCAM-1 (1:3200; a gift from Biogen Corp., Cambridge, MA, USA). Next, a biotinylated horse anti-mouse (rat adsorbed) antibody (1:150, Vector) was used as the secondary antibody for one hour at 25°C. An avidin-biotinylated horseradish peroxidase complex (Vector) incubation was for one hour at 25°C. This was followed by color development with the chromagen, diaminobenzidine (DAB, Sigma), 1 mg/ml in 0.1 mol/liter Tris buffer, to which 0.075 ml of 3% hydrogen peroxide had been added. Sections were counterstained with 1% methyl green solution for 20 minutes. Negative controls for immunohistochemistry consisted of replacing each of primary antibodies with equivalent concentrations of an irrelevant murine monoclonal antibody.

**In situ hybridization**

The rat ICAM-1 plasmid was linearized with either Eco-RI and an antisense riboprobe generated with T7 polymerase in the presence of 35S-labeled UTP (New England Nuclear, Boston, MA, USA). The 565 base pair riboprobe was separated from unincorporated precursors using G-50 Quick Spin columns (Boehringer Mannheim, Indianapolis, IN, USA). Riboprobe in situ hybridization was performed on 10% neutral buffered formalin-fixed paraffin sections according to established protocols [17]. Sections were alcohol dehydrated and incubated with prehybridization solution containing, 5 M NaCl, 1 M Tris, 6% Ficoll, 6% HSA, 6% PVP, 0.5 M EDTA, 10% salmon sperm DNA, and 0.1 mg/ml of yeast tRNA. Tissue sections were hybridized at 50°C overnight in an identical solution, containing, 50% formamide, 1 M DTT, 20% SDS and the 35S-labeled cRNA probe at a specific activity of 4 x 10^4 cpm/µl. After hybridization, the slides were rinsed in a series of washes, including RNase post-treatment. The final wash was in 2 x SSC for two hours at 60°C. The slides were exposed to BioMax autoradiography film (Eastman Kodak Co., Rochester, NY, USA) and then dipped in diluted Kodak NTB-2 emulsion and stored at 4°C for one to two weeks. Exposure times were five days. Sections were developed and counterstained with hematoxylin. Controls included substituting an 35S sense probe for the antisense probe used and an incubation in 0.1 mg/ml RNase A for 90 minutes at 25°C.

**Isolation of kidney proximal tubules**

Sixteen male Sprague-Dawley rats (250 to 300 g) were anesthetized with ethyl ether (EM Science, Gibbstown, NJ, USA) and the kidneys perfused with Krebs saline buffer via an infrarenal aortic cannula for three to five minutes to remove circulating blood cells. Enzymatic digestion was initiated with a collagenase (type V; Sigma) perfusate (1750 U/ml). The kidneys were removed, decapsulated and the cortex dissected away from the medulla. Renal proximal tubules were isolated as previously described [18]. The renal cortex from the 16 rats were pooled and finely minced. The cortical tissue was incubated in 1% collagenase (type V) at 37°C for 30 to 40 minutes. The resulting homogenate was sieved with a tea strainer, centrifuged at 6000 RPM for one minute and washed with Krebs buffered saline. The pellet was centrifuged on a Percoll (Sigma) density gradient and the fourth band containing 96% proximal tubule cells [18] was collected. Isolated proximal tubules were incubated for one hour in Dulbecco's Modified Eagle's Medium/Hams F12 media containing angiotensin II (Sigma) at a concentration range of 1 to 1000 µM with or without pretreatment with losartan (10^-5 M). Total cellular RNA was extracted from the isolated proximal tubules for Northern hybridization and probed for ICAM-1. Experiments were done in triplicate.

**Analytical studies**

All values are expressed as means ± SEM. When multiple groups were compared, one-way analysis of variance was performed initially to confirm the presence of significant differences, then individual comparisons were performed with Student's t-test and multiple pairwise comparisons according to the method of Bonferroni [19] as appropriate. Statistically significant differences between groups were defined as P values < 0.05.

**Results**

**Northern analysis studies**

By Northern analysis, total cortical mRNA levels for ICAM-1 from the obstructed kidneys were significantly increased compared to the CUK specimens (Table 1). As early as 12 hours following obstruction, levels of ICAM-1 mRNA from the obstructed kidneys showed a significant 6.6-fold (0.33 ± 0.06 vs. 0.05 ± 0.01, P < 0.001) increase compared to the CUK specimens. Significant increases in ICAM-1 expression, relative to CUK values, were also observed 24 (0.51 ± 0.1 vs. 0.21 ± 0.04, P < 0.025), 48 (0.18 ± 0.03 vs. 0.07 ± 0.01, P < 0.01), and 96 hours (0.55 ± 0.06 vs. 0.28 ± 0.04, P < 0.005) after ureteral ligation where ICAM-1 mRNA levels showed a 2.6-, 2.6-, and 2-fold increase, respectively. Representative autoradiograms for ICAM-1 expression at 12 and 24 hours post-UUO are shown in Figures 1 and 2.

No significant difference in VCAM-1 expression was observed between the obstructed kidney and CUK specimens. Following UUO, there was no significant difference in renal cortical VCAM-1 mRNA levels at 12 (0.17 ± 0.04 vs. 0.16 ± 0.08, mean ± se), 24 (0.14 ± 0.08 vs. 0.19 ± 0.06), 48 (0.21 ± 0.04 vs. 0.27 ± 0.05), and 96 hours (0.26 ± 0.03 vs. 0.21 ± 0.05), in the obstructed kidney versus the CUK specimen.

**Effect of reversal of obstruction (R-UUO) on ICAM-1 steady-state mRNA levels**

Despite the apparent relief of obstruction, the renal cortical ICAM-1 mRNA levels at two weeks after R-UUO were equivalent to those in the 96 hours obstructed kidney control group (1.89 ± 0.47 vs. 1.40 ± 0.56). Equivalent mRNA levels were also

<table>
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<tr>
<th>Time</th>
<th>Group</th>
<th>Mean ICAM-1/28s mRNA ratios densitometric units</th>
<th>N</th>
<th>Magnitude of difference</th>
<th>P value</th>
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<tr>
<td>12</td>
<td>UUO</td>
<td>0.33 ± 0.06</td>
<td>6</td>
<td>6.6-fold</td>
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<td>12</td>
<td>CUK</td>
<td>0.05 ± 0.01</td>
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<td>2.6-fold</td>
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<td>24</td>
<td>UUO</td>
<td>0.51 ± 0.10</td>
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<td>2.6-fold</td>
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<td>24</td>
<td>CUK</td>
<td>0.21 ± 0.04</td>
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<td>48</td>
<td>UUO</td>
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<td>48</td>
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<td>96</td>
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<td>96</td>
<td>CUK</td>
<td>0.28 ± 0.04</td>
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Abbreviations are: UUO, unilateral ureteral obstruction; CUK, contralateral unobstructed kidney. Values are means ± SEM.
Fig. 1. Northern blot analysis of total cortical RNA probed for ICAM-1. The mRNA probed for ICAM-1 from obstructed kidneys (lanes 1 to 6) and the corresponding CUK specimens (lanes 7 to 12) at 12 hours after UUO are shown (top) in relation to the intact 28 s and 18 s ribosomal RNA bands (bottom). A single 0.9 kb transcript for ICAM-1 is apparent in all of the obstructed kidney specimens but not visible in the CUK specimens.

Fig. 2. Northern blot analysis of total cortical RNA from obstructed kidneys of rats undergoing UUO at 24 hours after ureteral ligation (lanes 1 to 6), and from the CUK specimens of rats undergoing UUO (lanes 7 to 12). Total cortical RNA was probed for ICAM-1 (top). Intact 28 s and 18 s ribosomal bands using ethidium bromide staining of agarose gel (bottom).

noted in the previously-obstructed kidneys at four weeks post-R-UUO compared to the 96-hour obstructed kidneys (0.41 ± 0.07 vs. 0.41 ± 0.21).

**Immunohistochemical studies**

Renal cortical ICAM-1 and VCAM-1 immunolabeling. The pattern of renal cortical ICAM-1 immunolocalization is shown in Figure 3. At 12, 24 and 96 hours after UUO, there was focal staining of ICAM-1 in the peritubular interstitial space and periglomerular region of the obstructed kidneys (Fig. 3 A, C). At 24 hours after UUO, the renal cortex exhibited an intense immunolocalization of ICAM-1 on the apical surface of the cytoplasm of the distal tubules (Fig. 3C). Immunolocalization was also observed in the perivascular adventitia and endothelial lining of the cortical arterioles from the obstructed kidneys (Fig. 3F). In the CUK specimens at this time point, there was only faint immunostaining for ICAM-1 in the peritubular region and parietal epithelium of Bowman’s capsule (Fig. 3 B, D).

At 12, 24 and 96 hours after obstruction, UUO and CUK specimens revealed a diffuse immunostaining pattern of VCAM-1 protein in the renal cortex in both tubular epithelium and Bowman’s capsule. Immunolocalization of the cortical interstitial cells was also noted. There was no difference in the pattern and intensity of VCAM-1 immunolocalization between the UUO and CUK specimens for any of the time points observed.

**In situ hybridization studies**

In situ hybridization showed 35S-labeled riboprobe transcription for ICAM-1 in the obstructed kidneys and CUK specimens. Following ureteral ligation there was an evident increase in the mRNA signal for ICAM-1 on qualitative assessment of the autoradiogram. Figure 4 shows a representative field from an autoradiogram of ICAM-1 expression at 96 hours following ureteral ligation. Lightfield microscopy showed ICAM-1 mRNA transcription in the proximal tubules and peritubular interstitium of the renal cortex of 96-hour obstructed kidneys (Fig. 4). The intensity of ICAM-1 mRNA transcription was higher in the obstructed kidneys compared to the CUK specimens upon qualitative examination (Fig. 4). The specificity of the antisense probe hybridization signal was demonstrated by the lack of label in sections hybridized to the sense probe, or following pre-digestion of tissue sections with RNase.

**Effect of angiotensin II-stimulated proximal tubules on ICAM-1 expression**

To examine the gene expression of ICAM-1 in response to vasoactive products of UUO, Northern analysis was performed on RNA isolated from proximal tubules in response to angiotensin II. Following stimulation with 10 μM of angiotensin II for one hour, there was a 1.4-fold increase in ICAM-1 mRNA levels (Fig. 5). ICAM-1 mRNA expression was further elevated 7.1-fold in isolated proximal tubules cultured with 100 μM angiotensin II for one hour. Isolated proximal tubules stimulated with 1000 μM of angiotensin II had a 3.7-fold increase in ICAM-1 mRNA in comparison to control cultures of isolated proximal tubules (Fig. 5). The addition of losartan to proximal tubules for one hour prior to angiotensin II stimulation decreased ICAM-1 mRNA levels compared to control levels (Fig. 6). Isolated proximal tubules stimulated with angiotensin II that were pre-treated with losartan (10−5 M) had ICAM-1 mRNA levels decreased 2.3-, 1.9-, and 1.9-fold, when compared to proximal tubules stimulated with 1 μM, 10 μM, and 100 μM of angiotensin II, respectively.
pattern for ICAM-1 in the peritubular and periglomerular region 
the present study, immunohistochemistry showed a focal staining 
epithelial cells of large vessels or peritubular capillaries [23, 24]. In 
capsule, parts of the proximal tubules and occasionally on endo-
normally expressed on parietal epithelial cells of Bowman’s 
cells of the Bowman’s capsule, and 
constitutively expressed in normal kidney in renal vascular endo-
proximal tubules [201 and murine mesangial cells [21]. ICAM-1 is 
control values.
mal tubules with losartan decreased ICAM-1 mRNA expression 
UUO and CUK specimens. Levels of ICAM-1 mRNA were found 
apparent relief of obstruction. No significant difference in 
compared with the 96-hour obstructed kidney specimens despite an 
R-UUO, levels of ICAM-1 mRNA were not significantly reduced 
up-regulated ICAM-1 mRNA expression and increased protein 
in the renal cortex of obstructed kidneys from UUO rats when 
the 96-hour obstructed kidney specimens despite an 
levels of ICAM-1 mRNA were observed in the corresponding 
immunolocalization was noted in the peritubular interstitium. The arrowheads in F 
cells, and in the apical cytoplasm of the distal tubules 
basolateral surface of renal 
immune injury [reviewed in 23, 25]. Marked changes 
in ICAM-1 expression have been reported in allograft rejection 
glomerulonephritis [29—32], and tubulointerstitial inflamma-
animals develop progressive and ultimately lethal tubulointerstitial nephritis [33]. This disease, where 
immune injury beginning at about four weeks, is associated with 
up-regulation of ICAM-1 expression in the renal interstitium, on 
Discussion

The present study demonstrates an early pattern (within 12 hr) 
of up-regulated ICAM-1 mRNA expression and increased protein 
in the renal cortex of obstructed kidneys from UUO rats when 
compared with the CUK specimens. Following four weeks of 
levels of ICAM-1 mRNA were not significantly reduced 
compared to the 96-hour obstructed kidney specimens despite an 
no significant difference in 
M. Pretreatment of angiotensin II-stimulated prox-
mal tubules with losartan decreased ICAM-1 mRNA expression 
to control values.
ICAM-1 is expressed in vitro in primary cultures of human 
proximal tubules [20] and murine mesangial cells [21]. ICAM-1 is 
constitutively expressed in normal kidney in renal vascular endo-
thelium, some parietal epithelial cells of Bowman’s capsule, and 
occasional interstitial cells [22, 23]. In contrast, VCAM-1 is 
normally expressed on parietal epithelial cells of Bowman’s 
capsule, parts of the proximal tubules and occasionally on endothelial cells of large vessels or peritubular capillaries [23, 24]. In the present study, immunohistochemistry showed a focal staining pattern for ICAM-1 in the peritubular and periglomerular region 
of the renal cortex of obstructed kidneys. Immunolabeling was 
also prominent on the luminal surface of the distal tubules, 
adventitia and endothelial lining cells of the cortical vessels. In situ 
hybridization revealed ICAM-1 mRNA transcription localized to 
the renal tubules and interstitium of the renal cortex.

A number of studies suggest that ICAM-1 plays an important 
role in renal immune injury [reviewed in 23, 25]. Marked changes 
in ICAM-1 expression have been reported in allograft rejection 
glomerulonephritis [29—32], and tubulointerstitial inflammation [33]. Renal biopsy specimens from patients with 
allograft rejection frequently reveal a dramatically induced expression of ICAM-1 on the luminal surface of proximal tubules, 
some distal tubules and collecting ducts, and infiltrating leuko-
cytes [26—28]. Tubular localization of ICAM-1 is usually most 
intense in areas of marked leukocyte infiltration [26]. In an 
allogenic murine model of acute, lethal graft-versus-host disease, 
pretreatment with anti-ICAM-1 and LFA-1 antibodies enhanced 
survival and reduced renal injury [34].

Up-regulation of ICAM-1 expression on proximal tubules and 
the cortical interstitium has been reported in a murine model of 
hereditary tubulointerstitial nephritis [33]. This disease, where 
animals develop progressive and ultimately lethal tubulointerstitial 
nephritis beginning at about four weeks, is associated with 
up-regulation of ICAM-1 expression in the renal interstitium, on 
infiltrating leukocytes, and on the basolateral surface of renal 
hybridization revealed ICAM-1 mRNA transcription localized to 
the renal tubules and interstitium of the renal cortex. A. Immunostaining for ICAM-1 antibody in a representative midcoronal section from a UUO specimen, 24 hours after obstruction. ICAM-1 immunolabeling was typically observed in the peritubular interstitial space in the renal cortex. B. Only faint ICAM-1 labeling was observed in the corresponding CUK specimen, 24 hours after ureteral ligation. C. Focal pattern of ICAM-1 immunolocalization in an obstructed kidney, 24 hours after UUO. Immunolabeling was 
observed in both the parietal epithelial cells of the Bowman’s capsule (small arrowheads), and 
in the apical cytoplasm of the distal tubules (arrowheads). D. Only weak immunostaining 
was observed in the interstitium and 
periglomerular regions of the renal cortex in 
the CUK specimens, 24 hours after UUO. E. 
and F. Immunostaining with anti-ICAM-1 antibody on a 24-hour UUO specimen. E. 
Intense immunolocalization was noted in the 
peritubular interstitium. The arrowheads in F 
demonstrate the diffuse staining for ICAM-1 in 
the endothelium lining of the cortical vessels 
(arrowheads). Immunostaining for ICAM-1 was 
also observed in the periadventitial region of 
the renal vasculature. Publication of this figure 
in color was made possible by a grant from 
Merck & Co.
tubules. Administration of anti-ICAM-1 antibody to mice with established disease resulted in significant reductions in infiltrating leukocytes and tubular epithelial damage as assessed by light microscopy [33].

In an experimental model for active or passive anti-glomerular basement membrane disease in rats, recent investigations revealed a central role for ICAM/LFA-1 interactions in the accumulation of leukocytes into the glomerulus and tubulointerstitial
region [29–31]. The simultaneous administration of anti-ICAM-1 and anti-LFA-1 monoclonal antibodies prevented the development of proteinuria, influx of inflammatory cells into the glomerulus, and subsequent development of cellular crescents [29]. Recently, Nikolic-Paterson et al [32] demonstrated that the suppression of experimental glomerulonephritis by the IL-1 receptor antagonist is mediated by inhibition of ICAM-1 expression.

The initial cellular and molecular events leading to interstitial macrophage infiltration following UUO is fundamental in the development of tubulointerstitial injury and fibrosis. An increased cell-surface expression of ICAM-1 may be important in facilitating macrophage accumulation in the renal interstitium. We have previously observed [2] an increase in the interstitial macrophage number in the renal cortical interstitium by 12 hours after UUO, which continues to increase rapidly thereafter. A dramatic early up-regulation of ICAM-1 mRNA expression and increased protein are evident 12 hours after ureteral ligation, suggesting that increased ICAM-1 expression may be important in directing macrophage accumulation in the renal cortex. Intertial macrophage numbers are maximally increased at 96 hours after UUO, decreasing to control kidney levels by four weeks post-release of obstruction [3]. Following a four week R-UUO, levels of ICAM-1 mRNA were equivalent to the 96-hour left kidney control group despite the demonstrable decompression of the renal pelvis and calycyeal system. These data support the contention that a number of factors play a role in the stimulation of ICAM-1 expression in the renal cortex following ureteral obstruction. ICAM-1 mRNA expression does not decrease with infiltrating macrophage number following a release of obstruction, implicating a role for ICAM-1 in macrophage recruitment into the renal interstitium following ureteral ligation rather than serving as a marker for macrophage infiltration.

The stimulus for induction of ICAM-1 on tubular epithelial cells in the renal cortex after UUO remains unclear. ICAM-1 expression can be up-regulated by pro-inflammatory cytokines and oxidants. IL-1, TNF-α and γ-IFN have been found to enhance ICAM-1 synthesis by glomerular endothelial cells, mesangial cells and renal tubular epithelial cells in vitro [10]. Generation of reactive oxygen species can activate ICAM-1 mRNA expression and other chemoattractants and adhesion molecules, including MCP-1 and RANTES, in cultured mesangial cells [35].

The present study provides evidence that angiotensin II may be involved in the activation of cell-surface expression of ICAM-1 on cortical proximal tubules following ureteral obstruction. Acute ureteral obstruction causes an initial increase in renal blood flow due to vasodilator prostaglandins, followed by progressive vasoconstriction of glomerular arterioles resulting in a marked reduction in renal blood flow and glomerular filtration rate (GFR). It is apparent that the renal cortical and medullary vasoconstriction that occurs after only two hours of UUO is due, at least in part, to enhanced intrarenal renin-angiotensin system activation, resulting in excessive production of angiotensin II [36]. Pimental et al [36] observed up-regulated renin mRNA in the obstructed kidney as early as one hour after UUO in rats. This increase in renin mRNA coincided with elevations in renal renin content, angiotensin I converting enzyme (ACE) activity and renal angiotensin II concentration [36]. This early time course of angiotensin II up-regulation after UUO indicates angiotensin II as a possible stimulus for the expression of ICAM-1, leading to recruitment of macrophages into the renal interstitium of the obstructed kidney.

The vasoactive effects of intrarenal angiotensin II have been demonstrated in a variety of models. Increasing support is being given to proximal tubular angiotensin II generation in the mediation of renal perturbations. Angiotensinogen is synthesized by proximal tubule cells. Radiolabeled renin injected intra-aortically accumulates in the renal cortex, principally the apical portion of proximal tubules. ACE is found in greatest concentrations in the kidney in the proximal tubules. Therefore, the proximal tubule has

Fig. 6. Northern blot analysis of RNA extracted from isolated proximal tubules probed for ICAM-1. Shown are mRNA from a control one hour proximal tubular suspension (lane 1), proximal tubules exposed to angiotensin II at concentrations of 1 μM, 10 μM, and 100 μM, in Lanes 2, 3, and 4, respectively, and proximal tubules stimulated with angiotensin II (100 μM) pretreated with losartan (10^{-7} M; lane 5). The mRNA probed for ICAM-1 (top) in relation to the 28 s and 18 s ribosomal RNA bands (bottom) are shown.
all the components of a local angiotensin-generating system [37, 38], which may be the stimulus for ICAM-1 up-regulated expression in the rat UUO model. Klahr and colleagues [39] demonstrated that administration of an angiotensin II receptor antagonist and an ACE inhibitor, enalapril, to UUO rats resulted in amelioration of the expansion of the renal cortical interstitium and decreased mRNA expression of TGF-β1 and collagen IV. Interstitial macrophage infiltration was not altered by the angiotensin II receptor antagonist, but was greatly reduced in the UUO rats administered enalapril [39]. These studies indicate that the renin-angiotensin system plays a major role in the development of tubulointerstitial injury after ureteral obstruction.

In conclusion, our studies document an early up-regulated ICAM-1 mRNA expression in the renal cortex of obstructed kidneys, temporally coinciding with interstitial macrophage accumulation. Our immunohistochemical labeling patterns for ICAM-1 were most prominent in the peritubular interstitium, periglomerular region and the luminal surface of distal tubule epithelial cells. Immunolocalization of ICAM-1 protein was also evident in the adventitia and endothelial lining cells of the renal vasculature. ICAM-1 mRNA transcription was localized to the peritubular interstitium and cortical tubules of the obstructed kidneys. The present study presents evidence that angiotensin II may play a central role in the activation of ICAM-1 from proximal tubule epithelial cells. The mechanical injury to the renal proximal tubules produced by urinary tract obstruction may induce locally-generated angiotensin II-mediated cell-surface expression of ICAM-1. This activation of ICAM-1 may be important in facilitating macrophage accumulation in the renal interstitium, thereby contributing to the cascade of pro-inflammatory events leading to the development of interstitial fibrosis.

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