Obstructive nephropathy and renal fibrosis

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Klahr, Saulo, and Jeremiah Morrissey. Obstructive nephropathy and renal fibrosis. Am J Physiol Renal Physiol 283: F861–F875, 2002; 10.1152/ajprenal.00362.2001.—Interstitial fibrosis has a major role in the progression of renal diseases. Several animal models are available for the study of renal fibrosis. The models of aminonucleoside-induced nephrotic syndrome, cyclosporin nephrotoxicity, and passive Heyman nephritis are characterized by molecular and cellular events similar to those that occur in obstructive nephropathy. Additionally, inhibition of angiotensin-converting enzyme exerts salutary effects on the progression of renal fibrosis in obstructive nephropathy. Unilateral ureteral obstruction (UUO) has emerged as an important model for the study of the mechanisms of renal fibrosis and also for the evaluation of the impact of potential therapeutic approaches to ameliorate renal disease. Many quantifiable pathophysiological events occur over the span of 1 wk of UUO, making this an attractive model for study. This paper reviews some of the ongoing studies that utilized a rodent model of UUO. Some of the findings of the animal model have been compared with observations made in patients with obstructive nephropathy. Most of the evidence suggests that the rodent model of UUO is reflective of human renal disease processes.

renal fibrosis; gene knockout models; apoptosis; tubular atrophy

INFLAMMATION OF THE TUBULOINTERSTITIAL compartment, leading to fibrosis, is a major factor in the progressive loss of renal function in patients with a wide variety of kidney diseases. About 80% of total kidney volume is composed of tubular epithelial cells and cells within the interstitial space. Most of the nonepithelial cells are associated with the rich vascular network of the kidney. There are also a small number of resident mononuclear cells and fibroblasts. A model of renal fibrosis that encompasses many aspects of other models of kidney disease is unilateral ureteral obstruction (UUO) (38, 39, 40, 62). Nephropathies induced by the administration of streptozotocin, cyclosporin, aminonucleoside, adriamycin, and ANG II and by the ischemia-reperfusion maneuver are associated with cellular infiltration, fibroblast differentiation/proliferation, increased extracellular matrix protein deposition, and tubule atrophy. In an attempt to maintain the integrity of tubules, there is an activation of proliferative pathways within the epithelial cells (30, 90). If the proliferative forces or homeostatic factors within the kidney dissipate, the apoptotic pathway(s) overwhelms the ability of tubular epithelial cells to survive and tubular atrophy ensues.

There are features of the UUO model that occur within 1 wk of the onset of ureteral ligation. There are many readily quantifiable cellular and molecular events during the initiation and progression of renal fibrosis that make UUO an increasingly good experimental model for study. Recently, more than 100 abstracts at the 2001 World Congress of Nephrology dealt with various aspects of the pathophysiological and molecular events elicited by ureteral obstruction. Table 1 describes factors, the expression of which is increased in kidneys with ureteral obstruction, and Table 2 depicts factors, the expression of which is decreased in kidneys with ureteral obstruction.

In this review, we highlight most of the key features of the pathophysiology of renal fibrosis in obstructive nephropathy that were known by 1997. In addition, we have emphasized recent studies using a rodent UUO model for the study of the mechanisms of renal fibrosis and also for the evaluation of the impact of potential therapeutic approaches to ameliorate renal disease.
Table 1. Factors with increased expression in kidneys with ureteral obstruction

<table>
<thead>
<tr>
<th>Protein</th>
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<tr>
<td>Transforming growth factor-β</td>
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<tr>
<td>Protein 53</td>
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<tr>
<td>Protein 21 (WAF1)</td>
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<tr>
<td>Tissue inhibitor of metalloproteinase-1</td>
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<tr>
<td>Decorin</td>
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<tr>
<td>Nuclear factor-xB</td>
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<tr>
<td>Tumor necrosis factor-α</td>
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<td>Vasoactive compounds</td>
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<tr>
<td>ANG II</td>
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<tr>
<td>Endothelin</td>
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<tr>
<td>Thromboxane A₂</td>
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<tr>
<td>Prostaglandin</td>
</tr>
<tr>
<td>Protonocytogenes</td>
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<tr>
<td>c-fos, c-jun, jun B, c-myc, cH-Ras</td>
</tr>
<tr>
<td>Growth factors</td>
</tr>
<tr>
<td>Interleukin-6</td>
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<tr>
<td>Platelet-activating factor</td>
</tr>
<tr>
<td>Basic fibroblast growth factor</td>
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<tr>
<td>Proteins involved in apoptosis</td>
</tr>
<tr>
<td>Clusterin (SCG-2)</td>
</tr>
<tr>
<td>Osteopontin</td>
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<tr>
<td>Chemotactants</td>
</tr>
<tr>
<td>Monocyte chemotactant peptide-1</td>
</tr>
<tr>
<td>Osteopontin</td>
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<tr>
<td>Adhesion proteins</td>
</tr>
<tr>
<td>Intercellular adhesion molecule-1</td>
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<tr>
<td>Vascular cell adhesion molecule-1</td>
</tr>
<tr>
<td>Fibronectin; alternate splice forms</td>
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<tr>
<td>Matrix/basement membrane proteins</td>
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<td>Collagen types I, III, and IV</td>
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Table 2. Factors with decreased expression in kidneys with ureteral obstruction

<table>
<thead>
<tr>
<th>Protein</th>
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<tr>
<td>Growth or homeostatic factors</td>
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<tr>
<td>Epidermal growth factor</td>
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<td>Hepatocyte growth factor</td>
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<tr>
<td>Bone morphogenetic protein-7</td>
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<tr>
<td>Redox state</td>
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<tr>
<td>Copper, zinc superoxide dismutase</td>
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<td>Catalase</td>
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<tr>
<td>Reduced glutathione</td>
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<tr>
<td>Others</td>
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<tr>
<td>Meparin</td>
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<td>Inducible nitric oxide synthase</td>
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Adapted from Ref. 41.

model that highlight the molecular and cellular pathways that culminate in renal fibrosis.

ROLE OF VASOACTIVE COMPOUNDS IN OBSTRUCTIVE NEPHROPATHY

A substantial vasoconstriction of the renal vascular bed is the predominant alteration observed after ureteral obstruction of 24-h duration or longer. The glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) are decreased, and mean arterial pressure is increased, after unilateral release of bilateral ureteral obstruction. An imbalance between vasoconstrictor and vasodilatory substances may explain the hemodynamic alterations observed in this setting.

In a rat model of UUO, Sweeney et al. (89) visualized changes in renal blood flow (RBF) using a radiolabeled

Table 2. Factors with decreased expression in kidneys with ureteral obstruction

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<tr>
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<td>Bone morphogenetic protein-7</td>
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<td>Inducible nitric oxide synthase</td>
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Adapted from Ref. 76 and 78 by permission.

Table 3. Effects of vasoconstrictors and vasodilators on renal hemodynamics in rats after unilateral release of bilateral ureteral obstruction

<table>
<thead>
<tr>
<th>Maneuver</th>
<th>GFR, %</th>
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<tbody>
<tr>
<td>Before maneuver</td>
<td>After maneuver</td>
</tr>
<tr>
<td>Inhibition of synthesis or activity of</td>
<td></td>
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<tr>
<td>vasoconstrictors</td>
<td></td>
</tr>
<tr>
<td>RAS inhibition</td>
<td>20.7</td>
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<tr>
<td>TxA₂ inhibition</td>
<td>23.5</td>
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<tr>
<td>Prevention of macrophage infiltration</td>
<td>21.5</td>
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<tr>
<td>TxA₂ synthesis inhibition</td>
<td>17.0</td>
</tr>
<tr>
<td>LT synthesis inhibition</td>
<td>21.5</td>
</tr>
<tr>
<td>Inhibition of ADH V₁ receptors</td>
<td>22.3</td>
</tr>
<tr>
<td>Endothelin antibody</td>
<td>21.0</td>
</tr>
<tr>
<td>Deendothelialization of main renal arteries</td>
<td>21.0</td>
</tr>
<tr>
<td>Administration or activation of vasodilators</td>
<td></td>
</tr>
<tr>
<td>L-Arginine administration for NO synthesis</td>
<td>24.3</td>
</tr>
<tr>
<td>TxA₂ inhibition and PAF administration</td>
<td>17.0</td>
</tr>
<tr>
<td>ANP administration</td>
<td>24.8</td>
</tr>
</tbody>
</table>

GFR, glomerular filtration rate; RAS, renin-angiotensin system; TxA₂, thromboxane A₂; LT, leukotrienes; ADH, antidiuretic hormone; NO, nitric oxide; PAF, platelet-activating factor; ANP, atrial natriuretic peptide.

Table 3 summarizes the overall role of vasoconstrictors and vasodilators in the renal hemodynamics observed after unilateral release of bilateral ureteral obstruction. The overall role of endothelin in the altered hemodynamics of bilateral ureteral obstruction seems to be modest compared with compound and radioautography. They measured an initial vasodilation in the cortex within 10 min of obstruction. This was followed by a significant vasoconstriction in both the cortical and medullary beds (89). The changes in blood flow were mediated by prostanooid, ANG II, and nitric oxide (NO). We have identified a significant pathophysiological role of several vasoconstrictive substances during ureteral obstruction. These vasoconstrictors include ANG II, thromboxane A₂, and antidiuretic hormone (77). In each of these studies, the use of specific antagonists or inhibitors of the synthesis of vasoconstrictors resulted in significant increases in ERPF and GFR. We also found a vasoconstrictive effect of leukotrienes in the setting of bilateral ureteral obstruction (77). On the other hand, it is likely that vasodilatory substances are decreased in the setting of ureteral obstruction or are not increased to a sufficient degree to balance the increased activity of the vasoconstrictor compounds. Administration of vasodilators (prostaglandins E₂ and I₂) and platelet-activating factor (PAF) after inhibition of the synthesis of thromboxane A₂ increased GFR and ERPF significantly but not to normal levels. We also found that endothelin has an important role in the renal functional alterations observed in rats after unilateral release of 24-h duration bilateral ureteral ligation (76). Rats given a specific anti-endothelin antibody had significantly higher GFR and ERPF values than untreated rats subjected to bilateral ureteral obstruction. Table 3 summarizes the overall role of vasoconstrictors and vasodilators in the renal hemodynamics observed after unilateral release of bilateral ureteral obstruction.
that of other vasoconstrictors or the decreased activity of other vasodilators known to have a role in this setting. L-Arginine, PAF, and atrial natriuretic peptide (ANP) are among the vasodilators that have a role in the hemodynamics of bilateral ureteral ligation. The administration of L-arginine, the substrate for NO synthesis, increased GFR to ~50% of the values obtained for one kidney in sham-operated rats. Administration of PAF, in the setting of prior inhibition of thromboxane A2 synthesis, increased GFR to ~60% of values observed in one kidney in a normal rat. ANP administration increased GFR after unilateral release of bilateral ureteral ligation to ~40% of values observed in one kidney in sham-operated rats.

Inducible nitric oxide synthase (iNOS) has been identified by immunohistochemistry in the renal tubules after UUO (25). Vasodilation of the afferent arteriole during acute UUO (<5 h) may account for the increase in RBF and ureteral pressure. Several studies have explored the role of NO during ureteral obstruction. Intrarenal infusion of G-monomethyl-L-arginine attenuated the increase in RBF after acute ureteral obstruction (25). These effects of G-monomethyl-L-arginine were reversed by the intrarenal infusion of L-arginine.

The increased activity of the NO system after ureteral obstruction may be due to altered synthesis of l-arginine or increased utilization of l-arginine. Ureteral obstruction for >18 h results in marked decreases in both RBF and ureteral pressures. Constriction of the afferent arteriole may be the underlying cause. The vasoconstrictors ANG II, thromboxane A2, and endothelin predominate and counteract the effect of vasodilating substances, including NO. During late obstruction, either an abnormal synthesis of l-arginine or increased use of this amino acid results in decreased plasma levels of l-arginine and, presumably, decreased synthesis of NO.

NO plays an important role in the regulation of blood flow in the normal and the diseased kidney. In UUO, the renal vasculature remains responsive to the vasodilatory actions of NO, and blood flow changes associated with UUO involve impairment of the NO synthetic pathways in the kidney. Increased expression of both endothelial NOS (eNOS) and iNOS is seen with increasing duration of obstruction, but this probably does not correspond to a sufficient increase in enzyme activity. It should be kept in mind that these studies were of only a 24-h duration of obstruction. Substrate deficiency in the form of an l-arginine deficit is unlikely to play a major role, as serum and tissue levels show no dramatic change in UUO. This suggests that impairments at other sites in the NO production pathway are likely. Bypassing these metabolic processes, exogenous sources of NO provide a restorative effect on RBF and likely a protective effect to the kidney. Further efforts to elucidate the mechanisms underlying impaired endogenous NO production and how this might be enhanced warrant close attention (25).

**ANG II**

A number of kidney diseases, and their progression to end-stage renal failure, are driven by the intercrine, autocrine, paracrine, and endocrine effects of ANG II (38, 39, 62). All the components of the renin-angiotensin system (RAS), including substrate (angiotensinogen), enzymes involved in the synthesis and degradation of angiotensins, as well as receptors for angiotensins, are present in the kidney (41). Recent studies (84, 92) suggest that the interstitial concentration of ANG II may be 60- to 100-fold the plasma concentration. Regardless of whether the intrarenal ANG II level is 60- or 100-fold higher than in the circulation, an unexplored question is how a further increase in ANG II leads to renal fibrosis. Compelling evidence from Chevalier and co-workers (16) indicates that ~50–60% of the eventual fibrosis associated with a mouse model of UO is dependent on expression of the angiotensinogen gene. This important study utilized angiotensinogen knockout mice and compared the results with those from mice constructed to have one, two, three, or four copies of the angiotensinogen gene. Consistent with this study are others using angiotensin-converting enzyme (ACE) inhibition or ANG II type 1a (AT1a) receptor knockout mice (23), which indicate that ANG II generation and action result in at least half of the eventual fibrosis of obstructive nephropathy. An important question that remains to be deciphered is how an increase in intrarenal ANG II above the high basal level is sensed by the kidney to initiate molecular and cellular changes.

ANG II is produced both systemically and locally in various tissues, including the heart and blood vessel walls. ANG II binds to two high-affinity receptors, designated AT1 and AT2. Signaling through the AT1 receptor results in vasoconstriction, stimulation of growth, and activation of fibroblasts and myocytes. Signaling through the AT2 receptor results in vasodilatation and antiproliferative responses, as well as an increase in apoptosis. Therefore, it appears that most of the damaging effects of ANG II are mediated by the AT1 receptor (81). Increasing levels of ANG II may, in turn, upregulate the expression of other factors, such as transforming growth factor-β1 (TGF-β1), tumor necrosis factor-α (TNF-α), osteopontin, vascular cell adhesion molecule-1 (VCAM-1), and nuclear factor-κB (NF-κB), among others (40). ANG II increases the expression of several proliferative factors, including platelet-derived growth factor (PDGF) and basic fibroblast growth factor.

ANG II also stimulates oxidative stress (95). Such stress potentiates the vasoconstrictive role of the peptide because of increased catabolism of NO. Also, the generation of reactive oxygen species by ANG II may promote atherogenesis by diverse mechanisms, including oxidation of low-density lipoprotein cholesterol. Pharmacological blockade of the AT1 receptor reverses endothelial dysfunction and catabolism of NO, leading to attenuation of atherosclerosis. Oxidative stress, fueled in part by ANG II, upregulates the expression of
adhesion molecules, chemoattractant compounds, and cytokines.

The angiotensinogen gene, which provides the precursor for angiotensin production, is stimulated by NF-κB activation (2, 46). Interestingly, NF-κB is activated by ANG II in the liver (46) and the kidney (61, 62). This provides an autocrine-reinforcing loop that upregulates ANG II production. We found that ANG II activates NF-κB through both the AT1 and AT2 receptors (39, 62). In addition, ACE inhibition markedly decreases NF-κB activation in the kidney in the setting of UUO (61). There are recent papers regarding the impact of NF-κB activation in renal disease in general (21, 50).

We also found that nuclear extracts obtained from the cortex of kidneys with ureteral obstruction contained proteins that bind to an NF-κB-like sequence contained in the rat TNF-α gene promoter (40). These proteins are not present in nuclear extracts prepared from the cortex of the contralateral kidney or kidneys of sham-operated animals. We found that ACE inhibition decreased the amount of NF-κB binding proteins within the nuclear extract at 4 h but not at longer time intervals. TNF-α itself stimulates NF-κB activation (3), which creates an autocrine-reinforcing loop for TNF-α formation (Fig. 1). Because the NF-κB family of transcription factors have many potential combinations, it is possible that different NF-κB isoforms are activated by ANG II at different phases in the progression of renal disease. ANG II stimulates NF-κB activation, leading to increased TNF-α synthesis, which, in turn, can further activate NF-κB. The 40% diminution in TNF-α mRNA expression observed with ACE inhibition in rats with renal disease may be attributable to the blunting of a subset of NF-κB homodimers or heterodimers. Other subsets of NF-κB dimers may perpetuate NF-κB induction, causing eventual escape from ACE inhibition.

Vasoactive prostanoid compounds (thromboxane A2 and prostaglandins) are also upregulated during the course of progressive chronic renal disease, in general, and in obstructive nephropathy in particular (38). Compared with ANG II, their role in the pathophysiology of renal insufficiency is not as well understood.

**TNF-α**

There are two members of the tumor necrosis factor family: TNF-α and TNF-β. These proteins are encoded by single 3-kb genes that contain four exons each and occur in tandem on human chromosome 6. Our discussion will be confined to TNF-α. Two TNF-α receptors have been described: one with a molecular mass of 55 kDa (TNFR1) and the other with a molecular mass of 75 kDa (TNFR2) (72). Binding of TNF-α to its receptors activates a number of signal transduction pathways that result in the expression of a variety of transcription factors, cytokines, growth factors, receptors, cell adhesion molecules, mediators of inflammatory processes, acute-phase proteins, and major histocompatibility complex proteins (72). Lipopolysaccharide (LPS)-induced renal injury is associated with increased expression of TNF-α by renal cells. Proximal tubular cells express TNF-α when stimulated with interleukin-1α or LPS (72). Also, mRNA transcripts of TNF-α are found in cortical tubules of mice injected with LPS. Thus resident renal cells (glomerular mesangial cell and tubular epithelial cells) are sources of TNF-α production in renal injury.

We found (33) that in normal rats, TNF-α mRNA was more abundant in glomeruli than in renal cortical tubules. We measured TNF-α mRNA in the renal cortex of rats at different times after the onset of UUO and determined whether ANG II inhibition or total body irradiation affected the mRNA levels of TNF-α (33). Cortical tubules obtained from kidneys with ureteral obstruction had a marked increase in TNF-α mRNA expression, whereas glomeruli obtained from the same kidneys did not. Thus upregulation of TNF-α expression was confined to renal tubular cells of obstructed kidneys. Administration of an ACE inhibitor before and during UUO decreased TNF-α mRNA levels in obstructed kidneys by ~40% at 4 h after the onset of obstruction. However, at 120 h there was no difference in TNF-α levels between the obstructed kidneys of rats given or not given an ACE inhibitor. Total body irradiation, which prevents the migration of macrophages to the obstructed kidney, did not affect the upregulation of TNF-α mRNA expression at 4 h after UUO.
Thus TNF-α may have a role in initiating tubulointerstitial injury in the obstructed kidney. Leukocytes, infiltrating the renal interstitium of the obstructed kidney, do not seem to contribute to the increased expression of TNF-α mRNA. ANG II seems to contribute, at least in part, to the early increase in expression of TNF-α mRNA in the obstructed kidney.

TNF-α has a role in the recruitment of inflammatory cells in animal models of glomerular injury. It stimulates the production of chemotactic factors by resident cells and upregulates macrophage chemotactic protein-1 (MCP-1) mRNA in human mesangial cells. Wolf et al. (97) found that TNF-α increases regulated on activation, normal T cell expressed, and presumably secreted (RANTES) mRNA in a murine mesangial cell line and in vivo in rat kidneys perfused with TNF-α. Mulligan et al. (65) reported that anti-TNF-α or soluble recombinant human TNFR1 blocked the upregulation of intercellular adhesion molecule 1, endothelial leukocyte adhesion molecule 1, and VCAM-1 in nephrotic nephritis. The above data support the concept that TNF-α contributes to the increased macrophage migration into the renal interstitium of the affected kidney. We found that macrophages infiltrated the interstitium of the obstructed kidney cortex as early as 4 h after the onset of UUO, and by 24 h the influx was at a level ~10-fold greater than normal. These observations suggest that an early increase in TNF-α after ureteral obstruction of the kidney upregulates the production of a chemoattractant(s) for monocytes and contributes to the infiltration of the obstructed kidney by leukocytes.

In another study, we examined the contribution of TNF-α to the pathophysiology of the interstitial fibrosis that occurs after UUO, using mice in which the known receptors for TNF-α had been individually deleted through genetic means (22, 23). This allowed us to determine whether TNF-α is a contributory factor to the pathophysiology of obstructive nephropathy. The vast majority of studies concerning tubulointerstitial fibrosis have utilized the rat model. The power of genetics and the ability to manipulate the genetics of the mouse cannot be ignored. Therefore, this study also served to provide a database for future experiments in the mouse.

Mice in which individual TNF-α receptors TNFR1 or TNFR2 had been genetically knocked out were used, and results were compared with those from mice of C57BL/6 background after 5 days of UUO (22). The changes in interstitial volume (Vvint) of the contralateral unobstructed kidneys averaged 7% and were indistinguishable among the three genotypes of mice. Vvint of the UUO kidney of C57BL/6 mice averaged 33 ± 3.9% after 5 days of UUO. Vvint of the obstructed kidneys of TNFR1 mice was significantly reduced to 19.4 ± 3.1%, whereas that of TNFR2 mice was significantly decreased to 25.4 ± 4.8%. There was a modest but significant difference between Vvint of TNFR1 and TNFR2 mice (P < 0.047). Both collagen IV and α-smooth-muscle actin matrix scores were decreased significantly in the obstructed kidneys of TNFR1 mice compared with those of C57BL/6 and TNFR2 mice. Nuclear extracts prepared from kidney cortex had a significant increase in NF-κB binding activity in the obstructed kidneys compared with the contralateral kidneys. Individual knockout of the TNFR1 or TNFR2 genes resulted in significantly less NF-κB activation compared with wild-type, with TNFR1 knockout values being less than those in TNFR2 knockout mice (22). There was a significant increase in TNF-α mRNA in the kidneys with UUO in all three genotypes. TNFR1 knockout mice displayed a significant reduction in the amount of TNF-α mRNA induced compared with wild-type or TNFR2 knockout mice. Treatment of TNFR1 knockout (22) or TNFR1 and TNFR2 double knockout (23) mice with an ACE inhibitor further decreased Vvint and TNF-α mRNA induction, suggesting an interaction of the ANG II and TNF-α systems. These results suggest that TNF-α contributes, in part, to Vvint, myofibroblast differentiation, and NF-κB activation in the kidney during ureteral obstruction. These changes appear to be mediated through both TNFR1 and TNFR2 gene products, with effects through the TNFR1 receptor predominating. Furthermore, ANG II appears to stimulate TNF-α-related pathophysiological events leading to renal fibrosis, and together they can account for ~70–80% of the pathophysiological changes in obstructive nephropathy (23).

The stimulation of NF-κB activity by TNF-α raises an interesting dilemma with respect to the role of NF-κB in the overall pathophysiology of obstructive nephropathy in particular and renal disease in general. The activation of NF-κB in some cell types by TNF-α opposes the cytotoxicity of TNF-α (62). At this time, we do not know which NF-κB isotypes are associated with pathological effects such as tissue inflammation or with counterregulatory beneficial effects such as opposing apoptosis. Most, if not all, electrophoretic mobility shift assays performed to measure NF-κB activation utilize a consensus oligonucleotide sequence. The use of different oligonucleotide sequences found in the promoters of specific genes may help uncover the various detrimental and beneficial effects associated with generic NF-κB activation (62).

**Endothelin System**

A role for the endothelin system in obstructive nephropathy was suggested in a model of partial bladder outlet obstruction using rabbits (36). NADPH diaphorase activity was significantly decreased in the kidney cortex and medulla after obstruction. This diaphorase activity is a surrogate indicator of NOS activity. Endothelin concentration or activity was not measured; however, the number of endothelin ETα receptors was found to be significantly increased, whereas the number of ETβ receptors was decreased (36). In a rat model of UUO, the amount of ETα receptor and endothelin-converting enzyme-1 mRNA was increased (15). There was no change in the amount of ETβ receptor mRNA. In Sprague-Dawley rats transgenic for overexpression of human renin and angiotensinogen, a role of endothelin activation of NF-κB in the kidney was uncovered.
it is thought that the upregulation of ANG II that occurs in many organ systems of these animals subsequently increases the formation of endothelin. The ET<sub>A</sub>/ET<sub>B</sub>-receptor antagonist bosentan attenuated the increase in NF-κB activation that was normally seen in these animals (64). These three studies suggest that activation of endothelin production within the kidney may contribute to the pathophysiology of obstructive nephropathy. It should be noted that ANG II formation can only account for 50–60% (16, 22), whereas TNF-α accounts for ~20% (22), of the molecular and cellular changes that occur in ureteral obstruction. Additional extensive studies are needed to define the extent to which the endothelin system may contribute to the remaining 20–30% of the pathophysiology of obstructive nephropathy. That endothelin mRNA was elevated in patients was demonstrated by Knerr et al. (42). Twenty patients with pelviuretic junction obstruction were found to have significantly elevated endothelin-1 mRNA levels compared with 21 controls with a normal pelviuretic junction. This suggests that the endothelin system is active in the pathogenesis of congenital (42) hydronephrosis and animal studies are reflective of human pathophysiology.

**APOTOPSIS IN OBSTRUCTIVE NEPHROPATHY**

Apoptosis mediates the controlled deletion of unwanted cells. Apoptosis occurs when death is part of an organized tissue process, as in embryogenesis, metamorphosis, endocrine-dependent tissue atrophy, and the control of normalcy (19). The biological process involves a series of steps leading to the demise of cells by the activation of endogenous systems. Historically, apoptosis refers to a form of programmed cell death observed in animal tissues (93, 99).

Distinct patterns of cell proliferation and apoptosis have been described for tubular, interstitial, and glomerular cells in chronic obstructive nephropathy (91). In the kidney, apoptosis can affect resident cells (glomerular, tubular, fibroblast, endothelial) as well as infiltrating leukocytes. Apoptosis of tubular and interstitial cells is also thought to be at the origin of tubulointerstitial atrophy, secondary to obstructive nephropathy (6, 47).

Prolonged ureteral ligation induces marked hydronephrosis of the affected kidney and is accompanied by tissue loss (evident by a marked reduction in kidney weight), atrophy of tubular epithelial cells, and the development of interstitial inflammation and fibrosis. Truong et al. (91) have examined the effects of obstructive uropathy on cell proliferation and apoptosis. Rats with UUO were killed at several intervals between 1 and 90 days after the induction of obstruction and were compared with control sham-operated rats. Obstructed kidneys, contralateral kidneys, and kidneys from normal rats were subjected to in situ end-labeling of fragmented DNAs to detect apoptotic cells and were immunostained with monoclonal antibodies directed against the nuclear antigens associated with cell proliferation. Apoptosis of tubular cells from kidneys with chronic obstructive nephropathy increased rapidly, reaching 30-fold that of controls by 25 days of obstruction (91). This peak was followed by a rapid decrease to control levels. In the 25 days after the onset of obstruction, both kidney dry weight and mean tubular diameter decreased significantly. Thus apoptosis contributes to the tubular atrophy and renal weight loss observed in prolonged obstructive nephropathy. The rapid increase in tubular cell apoptosis was preceded by a 37% gain in kidney dry weight compared with controls; just before that increase, the proliferation rate of tubular cells (detected by immunostaining for proliferating cell nuclear antigen) increased ~60-fold. No apoptosis or proliferation of glomerular cells was observed during the course of this study. These observations suggest that tubular cell apoptosis might be pathogenetically related to the tubular atrophy and renal tissue loss that occur in animals or humans (38) with chronic obstructive nephropathy.

A large number of factors can initiate apoptosis, several of which may be pertinent to obstructive nephropathy, such as hypoxia, ischemia, cytokines, growth factors, ANG II, TNF-α, reactive oxygen species, and mechanical stretch.

These factors act on a family of cell membrane receptors that include the TNF receptor and Fas (also known as CD95 or APO-7). Members of this family share a common intracytoplasmic domain called the death domain (69). Stimulation of these receptors leads to conformational changes in the death domain, which initiates the activation of a cascade of intracytoplasmic molecules that include TRAD, FADD/MORT1, RIP, and RAIDD/CRADD (69). This leads to activation of a number of cytoplasmic signaling cascades, the best known among which is probably the mitogen-activated protein kinase pathway (20). The apoptotic signals can also act on cytokine receptors in the cell membrane or cause direct damage to the cell membrane with the release of several structural molecules including ceramide (20). The ligated cytokine receptor, in turn, activates several cytoplasmic signaling cascades (75). The converging point of the cytoplasmic signaling cascades is the activation of cystein-containing, aspartate-specific proteases (caspases) (44).

Kidneys with ureteral obstruction develop progressive tubulointerstitial damage. Several tubular changes occur, including dilatation, atrophy, and immature phenotype. In addition, apoptosis appears to play a major role in ureteral obstruction. It has been demonstrated that a marked increase in tubular and interstitial cell apoptosis is present in kidneys with ureteral obstruction. This increase in apoptosis is associated with an increase in p53 mRNA (57, 58). This finding suggests that p53 activation may have a role in the development of apoptosis after ureteral obstruction (11, 52).

Caspases are a family of cytoplasmic enzymes (caspases 1–9) that are normally present in the cytoplasm in inactive forms. Activated caspases represent the central effector molecules of apoptosis (11, 90) (see Fig. 2). Truong et al. (90) have demonstrated that
ureteral obstruction in mice induces apoptosis of both tubular and interstitial cells in the affected kidney in a distinctive pattern that parallels an increased expression of caspases. This correlation suggests that the caspases mediate renal cell apoptosis in obstructive nephropathy. Among the caspases evaluated, increased renal caspase-3 activity seems to play a central role in renal cell apoptosis associated with obstructive nephropathy.

In a recent study (11), genetically engineered mice were used to examine the role of p53 in renal cell apoptosis in obstructive nephropathy. Obstructed kidneys in p53+/+ and p53−/− mice were examined for apoptosis, members of the bcl-2 family, the death receptor family, and the common effectors of apoptosis. Obstructed kidneys in p53+/− and p53−/− mice exhibited equal attenuation of tubular and interstitial cell apoptosis (70 and 50%, respectively) compared with p53+/+ mice. However, p53 gene deficiency did not confer complete protection from apoptosis. These data suggest that apoptosis in obstructed kidneys involves p53-dependent as well as p53-independent pathways. The p53-dependent pathway may involve activation of caspases-1, -11, and -12, whereas the p53-independent pathway may involve activation of members of the bcl-2 and death receptor families.

Chronic administration of exogenous epidermal growth factor or insulin-like growth factor-1 reduces apoptosis and tubular atrophy in the obstructed kidney (8, 9). Chevalier et al. (10) have also reported an inverse correlation between the tubular production of the anti-apoptotic oncoprotein bcl-2 and regional apoptosis of renal tubular epithelial cells in the obstructed kidney.

Recently, the sphingolipid ceramide has been identified as a molecule that can act as a potent stimulus to apoptosis (79). When present in high concentrations, ceramide, one of the most hydrophobic molecules in the cell, can induce apoptosis and disrupt nephrogenesis (82). Ceramide synthesis has been described in glomerular endothelial cells, mesangial cells, and tubular epithelial cells. The developing kidney is an organ that normally undergoes extensive remodeling in fetal and early postnatal life, a process that involves widespread apoptosis. Malik et al. (49) have demonstrated that the very high prevalence of renal apoptosis in the fetal and neonatal rat is associated with similarly elevated levels of intrarenal ceramide, and both ceramide production and endogenous renal apoptosis decrease to adult levels during the first month of life. Another factor with which ceramide can interact, as a surrogate of LPS, is the CD14 receptor. We have found that CD14 is increased on the surface of renal epithelial cells during UUO (55). Mice that had a genetic incapacitation of the CD14 signaling system were found to have a significant reduction in epithelial cell apoptosis in UUO (55). The AT2 receptor is linked to sphingolipid synthesis, which then induces apoptosis (45).

In summary, in response to UUO in the neonatal rat, renal ceramide content (which normally decreases with maturation) increases progressively with 2–4 wk of persistent obstruction. This is associated with a parallel increase in apoptosis in the obstructed kidney. Relief of UUO after 5 days significantly reduces the ceramide content of the postobstructed kidney. In contrast, UUO has no effect on the ceramide content of the adult kidney. These results are consistent with a role for endogenous ceramide in the enhanced apoptotic response of the neonatal kidney subjected to prolonged UUO.

Stretch significantly induced apoptosis in NRK-52E cells, which was accompanied by an increased release

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**Fig. 2.** Major steps in apoptotic pathways and their molecular control. MAP, mitogen-activated protein; JAK/STAT, Janus kinase-signal transducer and activator of transcription. Reprinted from Ref. 91 with permission.
of TGF-β; 1D11 (10 mg/ml), an antibody to TGF-β, totally inhibited stretch-induced apoptosis (52). The obstructed kidneys contained 20-fold higher TGF-β compared with the unobstructed kidneys; 1D11 neutralized tissue TGF-β in the obstructed kidneys. The obstructed kidneys exhibited significantly more fibrosis and tubular apoptosis than their unobstructed counterparts. This was blunted by the administration of 1D11. In contrast, 1D11 significantly increased tubular proliferation. Immunostaining for p53 was localized to renal tubular nuclei of control obstructed kidneys and was diminished by 1D11. In contrast, bcl-2 was upregulated in the 1D11-treated obstructed kidneys. Total NOS activity and iNOS activity in the obstructed kidneys were increased by 1D11 treatment. This study strongly suggests that an antibody to TGF-β is a promising agent for the prevention of renal tubular fibrosis and apoptosis in UUO (52).

CONTRIBUTION OF OTHER PATHWAYS TO OBSTRUCTIVE NEPHROPATHY

As outlined above, the contributions of ANG II, TNF-α, and endothelin-1 to the pathophysiology of obstructive nephropathy have been extensively studied (ANG II and TNF-α) or are beginning to be studied (endothelin-1). Several recent studies have appeared that indicate that other systems are activated in the renal model of obstructive nephropathy; however, their exact contributions or importance has not been determined. These reports suggest a role of IL-1 (100), tissue factor (96), heparin-binding EGF (66), and heat shock proteins (48) in the process of renal fibrosis. An increase in heat shock protein-25 induction during 24 h of ureteral obstruction protected the kidney against ischemia-reperfusion injury 1 wk later (73). This is due to the downregulation of postischemic inflammation.

Epithelial-Mesenchymal Transdifferentiation

A contributing factor to renal fibrosis is the proliferation of fibroblasts in the interstitium. The origin of these fibroblasts is controversial, but the theories are mutually exclusive. One mechanism concerns the basic belief that the occasional interstitial fibroblast present in the normal kidney starts dividing due to the production of ANG II, TGF-β, and other growth factors by injured renal epithelial cells. Another mechanism centers around the idea of epithelial-mesenchymal transdifferentiation (24). This latter idea is rooted in the observation that renal epithelial cells dedifferentiate to a degree during various forms of renal disease. These dedifferentiated cells can then penetrate the basement membrane and redifferentiate into a myofibroblastic cell expressing α-smooth muscle actin and collagen I. Another marker for fibroblasts is fibroblast-specific protein-1 (FSP-1), which was characterized by Strutz et al. (87). FSP-1 also has a couple of aliases because the FSP-1 gene, the metastasis-associated protein (mts-1) gene, and the calcium binding protein S100A4 gene are all expressed from the same gene locus (1). Renal interstitial fibroblasts in a kidney with an obstructed ureter express FSP-1 (87). TGF-β is thought to be a driving force in this transdifferentiation process (85). The contribution of each mechanism to fibroblast proliferation in the kidney with an obstructed ureter awaits precise quantitation.

GENE KNOCKOUT MICE

Previous studies, using rat models of UUO, have suggested changes in the activity of many genes in the pathogenesis of obstructive nephropathy. With the advances in genetic manipulation and an increased understanding of mouse genetics, unique experimental animals that lack specific genes are available for study. This section summarizes recent evidence using genetically altered mice in evaluating the contribution of a number of genes to the renal fibrosis that occurs in the model of UUO.

ANG II Receptor Knockout

An increase in ANG II is thought to be a major factor in the initiation and progression of obstructive nephropathy (7, 38). The increase in ANG II is brought about by the action of ACE. The AT2 receptor appears to tonically downregulate ACE expression (86). Mice in which the AT2 receptor has been knocked out display more parenchymal damage during UUO, as the increase in ACE activity is thought to predispose them to injury (86). A previous study using a pharmacological AT2-receptor antagonist found an exacerbation of renal damage in the rat model of UUO (60). Mice in which the AT1a receptor was knocked out displayed blunted macrophage infiltration and TGF-β1 mRNA and collagen III expression early in the course of the disease (80). The interstitial volume expansion was decreased at 2 and 5 days of UUO; however, it was indistinguishable from that in wild-type mice by 10 days of UUO. This suggest that factors other than ANG II or systems operating through other pathways contribute to obstructive nephropathy.

Tissue Inhibitor of Metalloproteinase-1 and Plasminogen Activator Inhibitor-1 Knockout

One of the factors contributing to tubulointerstitial fibrosis in a kidney with an obstructed ureter is the deposition of matrix proteins in the renal interstitium. This increased deposition is multifunctional, including, but not limited to, a decrease in the degradation of matrix proteins. It was known that both the tissue inhibitor of metalloproteinase (TIMP-1) and plasminogen activator inhibitor-1 (PAI-1) were involved in this decrease in matrix degradation. What was not known, however, was the precise cellular localization of gene activation. Recent studies by Duymelinck et al. (13) focused on the location in the kidney with an obstructed ureter. These studies in the rat model of UUO suggest that TIMP-1 is increased in the interstitial cells by 24 h after the onset of UUO. These interstitial cells were not positive for α-smooth muscle actin nor were they ED-1-positive mononuclear cells. At a later time, interstitial cells positive for α-smooth muscle actin expressed S100A4, a marker for fibroblasts.
actin and ED-1-positive mononuclear cells expressed TIMP-1 (13). The expression of PAI-1, however, was found to be delayed in mice with UUO until 5 days after onset. This expression was associated with injured tubules and eventually with interstitial cells (13). The authors suggested that PAI-1 activation was associated with the progression phase of renal fibrosis (13). These studies would support roles for both TIMP-1 and PAI-1 in the overall process of renal fibrosis.

The laboratory of Eddy et al. utilized mice with UUO in which either the TIMP-1 (37) or the PAI-1 (68) gene had been knocked out. Many of the quantifiable fibrogenesis responses, such as tubulointerstitial area, collagen matrix, myofibroblast area, and interstitial macrophage number were indistinguishable between wild-type and TIMP-1 knockout mice (37). Although the number of apoptotic tubular epithelial cells was the same between the two mouse genotypes, there was a significant increase in the number of apoptotic interstitial cells in the kidneys of TIMP-1 knockout mice (37). When TIMP-1 expression was eliminated, however, there was a compensatory increase in TIMP-3 and PAI-1 expression. These results led the authors to suggest that inhibition of matrix metalloproteinase activity may not be a dominant profibrotic force by itself (37).

During the course of UUO, PAI-1 mRNA increased 8- to 10-fold. In another series of experiments, Oda et al. (68) utilized mice in which PAI-1 genes were genetically incapacitated. Interestingly, the expression of collagen I, collagen III, and TGF-β1 mRNA was found to be significantly decreased at 3 and 7 days of UUO, but not at 14 days of UUO, in the PAI-1 knockout mice compared with wild-type mice. Furthermore, F4/80 interstitial macrophage numbers were lower at 3 and 7 days of UUO in these mice but indistinguishable from wild-type levels by 14 days of UUO (68). Also of interest in this study was the observation that purified PAI-1 is a chemotactic for macrophages at concentrations ranging between 10^{-10} and 10^{-11} M. Because there are many chemotactic molecules expressed in the course of obstructive nephropathy, the initial decrease in F4/80 cells at 3 and 7 days of UUO was probably compensated for by other attractants such as MCP-1 by 14 days of UUO.

**iNOS Knockout**

Previous studies have demonstrated that iNOS was constitutively expressed in the kidney of normal animals (63). This constitutive expression may be due to the maintenance of tonic influences within the kidney that allow for a steady-state expression of inducible proteins. It was anticipated that iNOS would be further induced during UUO because of the activation of NF-κB. Mice in which the iNOS gene was incapacitated were studied 2 wk after UUO (26). These mice displayed increased interstitial volume, interstitial macrophages, and TGF-β1 expression compared with wild-type mice. The course of fibrosis was exacerbated in these iNOS-deficient mice, leading Hochberg et al. (26) to suggest that iNOS may serve a protective role in this setting.

In a follow-up study, Miyajima et al. (53) found a biphasic increase (by 3 days of UUO) and then a decrease (by 14 days of UUO) in iNOS expression. In parallel studies, the authors found that the iNOS knockout mice had more apoptotic cells and proliferating nuclear antigen-positive cells than did wild-type mice. Additional experiments with NRK-52E cells in culture on flexible surfaces suggested that stretching of renal cells induces apoptosis that was enhanced by generic NOS inhibition or diminished by L-arginine supplementation (26, 53). When combined, these two studies led the authors to suggest that in ureteral obstruction iNOS activity is crucial to maintain tubule integrity because of an antiapoptotic and antiproliferative effect due to NO generation. This supports a previous study, which indicated that a beneficial effect of ACE inhibitors was the generation of NO (59). The more recent investigation utilized the rat model of UUO and also demonstrated that generic inhibition of NOS was detrimental to the course of renal fibrosis (59). The source of the NO was not identified in the prior study (59); however, the work of Felsen et al. (26, 52, 53) is consistent with the notion of iNOS performing a protective role in renal epithelial cells in the setting of ureteral obstruction.

**Osteopontin Knockout**

In many forms of renal disease, there is an infiltration of the interstitium by monocytes, some of which differentiate to tissue macrophages. This is also true for obstructive nephropathy (38). MCP-1 has been previously described as an inducible chemoattractant that is upregulated in the kidney with an obstructed ureter (58). Osteopontin is also considered a macrophage chemoattractant and has been shown to be induced in the UUO model (12). Mice in which the osteopontin gene had been incapacitated displayed an initial decline in macrophage infiltration (71). In later stages, however, macrophage infiltration was essentially indistinguishable from that seen in wild-type mice. There was also a measurable increase in both tubular cell and interstitial cell apoptosis in the osteopontin knockout mice (71).

The initial decline in macrophage infiltration followed by equivalent infiltration seen in the osteopontin knockout mouse can be possibly explained by redundant functional genes. In addition to osteopontin, MCP-1 (41) RANTES (12, 94), and CC-chemokine receptors (94) are induced in the kidney with an obstructed ureter. The induction of several chemoattractant genes during UUO would have a tendency to eventually overwhelm the gene knockout model with time.

**Cyclin-Dependent Kinase Inhibitor Knockouts**

During the course of UUO, there is an initial phase of tubular epithelial and interstitial cell proliferation (30, 91). There is a decline in this proliferative phase,
with the subsequent apoptotic loss of tubular and interstitial cells, resulting in tubular atrophy and interstitial fibrosis. The transit of all cells through the proliferative cycle is regulated at several checkpoints by two families of cyclin-dependent kinase inhibitors (83). One family includes p21WAF1/CIP1, p27KIP1, and p57KIP2. Both p21 and p27 are increased during ureteral obstruction, with p21 induction occurring in an ANG II-dependent manner (57, 58), whereas p27 induction is ANG II independent (18). The contribution of p57 has not been studied. One member of this family, p21, is interesting from the point of view that its levels are regulated through p53- and TGF-β-dependent mechanisms (83). Additionally, p21 is an inhibitor of proliferating cell nuclear antigen, which is used by many investigators as a marker for quantifying cell proliferation. A previous study using the rat model of UUO had documented that both p21 and p53 mRNA were increased in kidneys with obstructed ureters (57, 58). Latter studies from different groups have confirmed that p53 is induced in the UUO mouse model (11, 52).

The laboratory of Shankland et al. has utilized p27 knockout (70) and p21 knockout (28) mice in the UUO model. Interestingly, targeted disruption of the p27 gene led to increased tubular cell proliferation and apoptosis during UUO (70). In contrast, disruption of the p21 gene led to increased interstitial myofibroblast proliferation early in the disease process (28). There was no effect on interstitial cell apoptosis. Effects of p21 or p27 gene incapacitation were seen early (3–7 days) in the course of the disease, with no quantifiable differences at 14 days of UUO. The authors make two significant points from these observations. First, there are different effects of cyclin-dependent kinase inhibitors in different cell types. Second, with redundant functional proteins, effects may be seen early in a disease process but be masked at a later point. The issue of redundant functional genes masking changes in gene activity in the UUO model has been previously raised (55–57).

**Conditional Cell Knockout**

A component of the expanded interstitium in many models of renal disease is the fibroblast or myofibroblast (14, 62). Transgenic mice were prepared that expressed thymidine kinase under the control of the FSP-1 promoter (31). When sections of the kidneys from these animals were immunostained for thymidine kinase, an occasional fibroblast-like cell was seen in the interstitium. These conditional knockout mice were treated with vehicle or with ganciclovir and subjected to UUO for 10 days. There was a significant reduction in the histological score and collagen I mRNA in the kidneys of ganciclovir-treated animals compared with those receiving vehicle (31). The cell number within the interstitium was significantly less. This strategy of knockout is based on lethality of dividing cells. The authors could not distinguish between proliferation of endogenous resident fibroblasts or a process of epithelial-mesenchymal transdifferentiation as the source of the interstitial fibroblasts (31). This study provides a novel approach to targeting a specific cell type and deriving information concerning mechanisms of renal fibrosis.

**GROWTH AND HOMEOSTATIC FACTORS**

Mechanisms that contribute to the initiation and progression of renal fibrosis usually involve the upregulation of cytokines and growth factors such as TGF-β, TNF-α, and PDGF. Another important mechanism to consider is a decrease in the production of growth and homeostatic factors. These factors are normally endogenously produced by the kidney, and their absence may accelerate the progression of fibrosis. Conversely, treatment with purified growth and/or homeostatic factors could blunt the progression of disease or possibly reverse the loss of renal function.

The expression of preproepidermal growth factor is suppressed in kidneys with obstructed ureters in both neonatal (9) and adult (25) rats. Treatment of both adult (35) and neonatal (9) rats with UUO with epidermal growth factor significantly reduces tubular cell apoptosis. This would blunt tubular atrophy and tend to preserve renal function in cases where the eventual release of the obstruction is achieved.

Insulin-like growth factor-1 (IGF-1) has been used as a treatment of acute renal failure due to ischemia-reperfusion. Interestingly, endogenous IGF-1 expression was not changed during UUO in neonatal rats (8). Treatment of neonatal rats with UUO did not affect the suppression of nephrogenesis or tubular cell proliferation seen in UUO. However, treatment with exogenous IGF-1 did significantly blunt tubular cell apoptosis, tubular atrophy, and interstitial collagen deposition (8). The authors suggest that IGF-1 treatment offers another means of preserving the capacity of renal function once flow is reestablished.

Another aid in the recovery from acute ischemic renal injury is treatment with hepatocyte growth factor (HGF) (51). Indeed, a decrease in HGF levels induced by either an increase in ANG II or TGF-β was hypothesized to be a factor in the development of glomerulonephritis (102). HGF has been described as a substance produced by mesenchymal cells that maintains epithelial homeostasis (43). Mizuno et al. (54) used a mouse model of UUO and treated the animals with vehicle, a neutralizing antibody to HGF, or recombinant human HGF. Treatment with the HGF-neutralizing antibody was found to increase TGF-β expression, decrease tubular cell proliferation, and accelerate apoptosis. Treatment with exogenous HGF attenuated apoptosis and TGF-β expression (54). The authors suggested that the reduction in endogenous HGF can account for the progression of renal fibrosis in tubulointerstitial-based disease (54). This was underscored by a study in which HFG was administered by tail-vein injection every 12 h for the 7 days of UUO (101). The injection of HGF blunted the expected increase in TGF-β1 mRNA and various histological indices of fibro-
sis (101). The expression of α-smooth muscle actin and vimentin was decreased whereas the expression of E-cadherin was maintained. The authors suggest that HGF treatment prevents tubular epithelial cells from converting to myofibroblasts (101). This would be an important therapeutic consideration.

Recently, another protein has emerged as a potential renotrophic factor: bone morphogenetic protein-7 (BMP-7). In a preventative protocol, BMP-7 treatment was found to significantly decrease renal injury in a rat model of UUO when treatment was initiated at the time of injury (27). Subsequent studies suggested that BMP-7 treatment will also attenuate renal fibrosis when administered after renal fibrosis has begun (56). This treatment protocol was also found to significantly increase renal function from the levels measured in the vehicle-treated group (56). These studies underscore the value of histological parameters as an indicator of renal function and the potential of renal homeostatic factors in the treatment of kidney disease.

TGF-β

For almost a decade, many of the cellular and molecular changes in the pathophysiology of obstructive nephropathy have been attributed to an increase in TGF-β expression (see Fig. 3). The increase in TGF-β expression was found to occur in the renal tubules, with no change in glomerular expression (32). This observation was recently corroborated using microdissected nephron segments and by in situ hybridization, with the major increase in TGF-β mRNA seen in the proximal convoluted tubules (17). There was no difference in TGF-β expression in glomeruli or collecting ducts and only sporadic expression in ED-1-positive monocyte/macrophage cells.

Two recent studies suggest that the increase in TGF-β expression is indeed linked to renal fibrosis (29, 52). In a rat model of UUO, retrograde infusion of HVJ liposome-encased TGF-β1 antisense oligonucleotide attenuated the increase in interstitial volume (29). This was due to a decrease in macrophage infiltration, myofibroblast formation, and collagen I deposition. In another study using a rat model of UUO, Miyajima et al. (52) treated rats with a neutralizing antibody to TGF-β. There was less tubular cell apoptosis with consequently less tubular atrophy. The induction of p53 was blunted whereas Bcl2 and iNOS expressions were increased. This is consistent with the authors’ hypothesis that iNOS serves a protective role in obstructive nephropathy (26, 52). Overall, these two studies indicate that TGF-β alters the fibrotic forces within the kidney and is not an epiphenomenon when pathological changes are observed in the kidney with an obstructed ureter.

Finally, all animal models of UUO suggest that TGF-β1 is increased in kidneys with obstructed ureters. Kaneto et al. (34) show that this is true in the kidneys of humans with ureteral obstruction. Material from kidneys of nine patients with carcinoma of the ureter was compared with the normal portion of kidneys of eight patients with renal cell carcinoma. The patients were basically age and gender matched. There was a significant increase in TGF-β1 mRNA in the kidneys of patients with an obstructed ureter (34). There was a positive and significant correlation between the amount of TGF-β1 mRNA and the increased interstitial volume found in the patients’ kidneys. The amount of collagen IV and fibronectin mRNA was also found to be increased (34). This study suggests that animal models of UUO are reflective of the molecular changes seen in human obstructive nephropathy.

INTERPRETATION OF MOUSE MODELS

The reader should be cautioned about overinterpretating observations related to renal disease in general and UUO in particular without considering the genetic
background of mice. There are many comments in the literature suggesting that certain mouse strains may be more susceptible to the development and progression of renal disease. Furthermore, due to the redundancy of many biological systems such as chemotransmitters, adhesion molecules, TIMPs, cyclin-dependent kinase inhibitors, etc., knockout of one gene may have effects early but not later in a disease model. This could well explain many of the studies listed above, in which marginal but significant \( (P > 0.05) \) effects were observed at 3 or 5 days of UUO but which then became insignificant, with wide SDs by 7–14 days of UUO. These cautions need to be kept in mind when one compares observations in different studies. Short- and long-term time courses are needed to determine the appropriate biological windows for effects to be quantitated. Similarly, effects seen in Balb/c mice may not hold true for C57BL/6 mice.

**PEDIATRIC AND ADULT OBSTRUCTIVE NEPHROPATHY**

A significant cause of renal failure in children and infants is congenital obstructive nephropathy (5). Fetal, compared with adult, obstructive nephropathy is particularly devastating because renal growth and continued nephron development are impaired by the progression of fibrosis. Several studies (4–10, 16, 103) have examined many aspects of obstructive nephropathy in the newborn using a neonatal rat model of UUO. At the time of birth, the rodent kidney is not fully developed and is representative of human renal development at about the mid-trimester.

A major feature of the neonatal kidney that is responsible for pathophysiological differences is the preponderance of AT2 receptors in the developing kidney compared with a preponderance of AT1 receptors in the mature kidney (4, 5). The neonatal kidney with an obstructed ureter displays blunted tubular cell proliferation but enhanced apoptosis (4, 5, 9). This leads to maturational changes that severely inhibit subsequent renal development even if a brief obstruction is relieved. In a study of 15 cases of fetal obstructive nephropathy, there was a significant increase in mesenchymal and tubule cell apoptosis compared with that in gestational age-matched controls. Again, this suggests that animal models tend to depict the pathophysiological events of human obstructive nephropathy.

The transformation of renal mesenchyme to epithelium is crucial for renal development. A study by Nguyen et al. (67) indicates that members of the Wnt gene family are important mediators of the transformation in neonatal and adult rats models of UUO. Their study suggests that ureteral obstruction disrupts the normal pattern of Wnt-4, Wnt-7b, and Wnt-11 gene activity and maintains the cell in a mesenchymal state. The expression of Wnt-4 in particular was maintained rather than decreased if the kidney of newborn rats became obstructed, and the expression of Wnt-4 re-emerged if the kidney of adult rats was obstructed (67). The latter observation was coincident with the reexpression of the mesenchymal marker vimentin by epithelial cells (67). This study suggests that members of the Wnt gene family are important in renal development and in the dedifferentiation of renal epithelial cells during obstructive nephropathy. In a study focusing on a role for Wnt-4 in renal fibrosis in general but UUO in particular, Surendran et al. (88) localized Wnt-4 expression to collecting duct epithelial cells and surrounding mesenchymal cells. In addition to UUO, Wnt-4 expression was also increased during folic and induced nephropathy, genetic polycystic kidney disease, and needle-puncture wounding (88). The highest level of Wnt-4 expression was found in interstitial fibroblasts also expressing collagen I (\( \alpha 1 \)) and \( \alpha \)-smooth muscle actin. Other genes involved in development will undoubtedly be linked to renal cell differentiation and dedifferentiation in disease.

**CONCLUSION**

There are a growing number of studies that now utilize the rodent model of UUO. Obstructive nephropathy is useful to study the cellular and molecular changes that occur in renal epithelial cells and cells within the interstitium of the kidney. In additional to being an aid in developing a fundamental understanding of the pathophysiology of renal fibrosis, the UUO model can be used to determine the efficiency of treatments to resolve fibrosis. Use of the mouse model of UUO takes advantage of the ability to genetically manipulate this species. This has built on the studies conducted in the 1980s and early 1990s, which were largely confined to the rat. Studies are required to compare the cellular and molecular events of obstructive nephropathy between different strains of mice. This may lead to insights regarding racial differences in human renal disease and/or help uncover susceptibility genes that modulate the disease process in patients. The use of neonatal rats and mice should provide a better understanding of fetal obstructive nephropathy and, it is hoped, yield information for in utero treatments to minimize renal problems after birth. In sum, obstructive nephropathy is an increasingly popular model of renal disease. It has the potential to help develop treatments designed to halt or reverse the progression of kidney disease.

The assistance of Monica Waller in the preparation of this manuscript is gratefully acknowledged.

This work was supported by National Institute of Diabetes and Digestive and Kidney Diseases Grant DK-09976.

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