

# Nitric Oxide and Tubulointerstitial Nephritides

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**As part of the exponential growth in our understanding of nitric oxide (NO) in health and disease over the past 2 decades, the kidney has become appreciated as a major site where NO may play a number of important roles. Although earlier work on the kidney focused more on effects of NO at the level of larger blood vessels and glomeruli, there has been a rapidly growing body of work showing critical roles for NO in tubulointerstitial disease. In this review we discuss some of the recent contributions to this important field.**

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**D**ESPITE ITS deceptively simple structure, nitric oxide (NO) is now known to exert physiologically important effects in virtually every organ system in the body. The kidney has stood out as one such organ system. Just as an intensive focus on tubulointerstitial disease appeared to lag a bit behind glomerular disease as an area of research, the study of NO and the kidney appears to show a similar sequence of investigative focus. In recent years, we have been fortunate to experience a rapid growth in research on NO and the tubulointerstitium and in this review we discuss some of these recent contributions.

NO has many complex effects because of its unique biochemical properties. NO has an extremely short half-life (on the order of seconds) in vivo because it is scavenged rapidly. It also easily penetrates cell membranes to modulate signaling cascades via covalent interactions with various targets. These unique biochemical characteristics make NO well suited as an autocrine or paracrine signaling mediator.

NO interacts with many targets. Perhaps the most well-defined interaction is with the heme group of soluble guanylate cyclase mediating the conversion of guanosine triphosphate to cyclic guanosine monophosphate and the subsequent downstream signaling events. NO also interacts with thiol groups on proteins forming S-nitrosothiols, Fe/S groups on hemoglobin,<sup>1</sup> and superoxide radical to form peroxynitrite, which causes cellular toxicity via posttranslational changes in tyrosine residues of proteins.<sup>2</sup>

NO is synthesized from L-arginine under enzymatic activation by NO synthase (NOS). Given NO's role as a paracrine mediator, the site of expression of NOS determines where it will exert its actions. There are 3 NOS isoforms. Neuronal NOS (nNOS) and endothelial NOS (eNOS) are constitutive enzymes that produce low levels of NO. nNOS resides in neurons throughout the body

and in the juxtaglomerular apparatus in the kidney where it is important in regulating tubuloglomerular feedback.<sup>3,4</sup> eNOS appears in endothelial cells where it is important in regulating systemic and renal vascular tone. There is also an inducible form of NOS (iNOS) that is induced transcriptionally by proinflammatory cytokines and produces large amounts of NO.<sup>5</sup> iNOS has been localized in the kidney in mesangial cells, in the afferent arteriole, in inner medullary collecting duct cells, as well as in infiltrating neutrophils and macrophages. iNOS also has been an important topic of research in tubulointerstitial nephritis and NOS inhibitors such as *N*<sup>G</sup>-monomethyl-L-arginine, *N*<sup>W</sup>-nitro-L-arginine-methyl ester (L-NAME), and the iNOS-specific L-*N*<sup>6</sup>-(1-iminoethyl) lysine (L-NIL) have been important tools in this work as is discussed later.

## NO PHYSIOLOGY

NO was first discovered because of its systemic vascular effects as endothelial-derived relaxing factor. NO also has important effects on the renal vasculature. Studies have shown that eNOS inhibition in rats leads to increased glomerular vascular resistances,<sup>6-10</sup> effects that may involve inhibitory effects of NO on angiotensin II as described later. Angiotensin II infusion in rats similarly increases glomerular vascular resistances.<sup>9,11,12</sup> Rats

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pretreated with losartan, an angiotensin II receptor antagonist, were largely protected from the effects of NOS inhibition. This provides importance evidence that NO antagonizes angiotensin II.

NO exerts many actions on tubular function. It inhibits Na entry into the cortical collecting duct, Na-H exchange in proximal tubules, and Na-K-adenosine triphosphatase activity, as well as decreasing collecting tubule responsiveness to anti-diuretic hormone.<sup>13-15</sup> These actions lead to enhanced renal sodium and water excretion. Also, increased salt intake and NO donors increase renal Na excretion. On the other hand, NOS inhibition decreases renal Na excretion.<sup>16</sup> Therefore, NO appears to play a major regulatory role on renal salt and water handling. This important function is discussed later in the discussion of how chronic tubulointerstitial injury may lead to the development of salt-sensitive hypertension.

NO, mostly via iNOS, interacts with the immune system on many levels, leading to beneficial (tumor cell and intracellular parasite destruction) as well as harmful (cellular toxicity) outcomes. iNOS has been shown to be induced by many compounds including CD40/CD40 ligand on T cells, lipopolysaccharide, tumor necrosis factor- $\alpha$ , interferon- $\gamma$ , interleukin (IL)-12, and IL-1- $\beta$ ; also, the iNOS promoter has nuclear factor  $\kappa$  B (NF- $\kappa$ B) and interferon- $\gamma$  response elements.<sup>5,17-21</sup> On the other hand, iNOS induction can be inhibited by IL-4, IL-5, and transforming growth factor- $\beta$ .<sup>5</sup> These interactions may play a role in tubulointerstitial nephritis.

NO has important effects on T cells. Two rat phenotypes have been described based on T-cell response to mitogens: low responders and high responders.<sup>22,23</sup> Nonselective NOS inhibition can transform low responders to high responders.<sup>24</sup> In addition, iNOS  $-/-$  mice show enhanced T-cell proliferation.<sup>25</sup> On the other hand, IL-12 causes suppression of the cellular immune response via iNOS induction.<sup>21</sup> These findings suggest that NO is immunosuppressive to T cells. The immunosuppressive effects of NO appear to have relevance to interstitial nephritis as outlined later, although it should be noted that as discussed by Kelly and Gold,<sup>26</sup> NO may have important immunoregulatory effects in a variety of autoimmune diseases as they have reviewed in detail.

#### NO IN IMMUNE-MEDIATED TUBULOINTERSTITIAL NEPHRITIS

NO appears to have an important role in immune-mediated interstitial nephritis. An experimental model of T-cell-dependent autoimmune interstitial nephritis that can be induced in rats exposed to renal tubular antigen has served as a major system in which extensive insights have been gained into mechanisms of NO in tubulointerstitial nephritis.<sup>26,27</sup> In this model, Brown Norway rats are injected with renal tubular antigen in complete Freund's adjuvant. Initially, this results in polymorphonuclear leukocyte infiltration of the interstitium. Polymorphonuclear cells become replaced by monocytes, T lymphocytes, and B cells. Given NO's known effect on lymphocytes, Gabbai et al<sup>27</sup> examined the role of iNOS in this model of autoimmune interstitial nephritis. By using this model, these investigators examined the effects of oral administration of the iNOS inhibitors L-NAME and L-NIL on autoimmune interstitial nephritis. iNOS was shown to be markedly increased in cortical tubular epithelial cells of treated animals and this appeared to be specific to the kidney because despite the systemic administration of the combined renal tubular antigen/complete Freund's adjuvant other organs did not show increased iNOS immunostaining. There was also evidence of increased generation of NO as shown by enhanced levels of nitrate and nitrite in plasma and urine. The finding of enhanced iNOS expression and NO generation by itself does not point to an ameliorating or exacerbating effect on the course of the disease. One could envision that enhanced NO generation might promote tissue injury and exacerbate the course of interstitial nephritis while at the same time the immunosuppressive effect of NO as regards lymphocytes might predominate. In these studies, the investigators found that administration of L-NAME and L-NIL had deleterious effects on the course of disease. Renal function deteriorated faster in the L-NAME- and L-NIL-treated animals and treatment with these agents also resulted in worsened inflammatory infiltration of the interstitium. These results strongly suggest that in this model of autoimmune interstitial nephritis increased iNOS activity and NO generation occurs and has a beneficial impact on the course of disease.

The autoimmune model of interstitial nephritis also has allowed for the recent development of key insights into the previously poorly understood phenomenon of decreased glomerular filtration rate (GFR) in interstitial nephritis.<sup>28</sup> In contrast to glomerular disease, it has been unclear how an inflammatory process in the interstitium results in a pronounced decrease in the GFR. Several mechanisms for this have been debated including mechanical factors affecting filtration and altered levels of various vasoactive compounds. To evaluate the mechanism by which this takes place, Gabbai et al<sup>28</sup> performed micropuncture studies using the autoimmune interstitial nephritis model in the presence and absence of the iNOS blocker L-NIL. At day 7, despite there being only mild histologic changes in the kidney, there was a greater than 50% decrease in GFR and in the single-nephron GFR (the latter was associated with decreased single-nephron plasma flow and in the glomerular ultrafiltration coefficient<sup>28</sup>). By day 21 the kidneys showed extensive mononuclear cell infiltration and significant structural derangements of the interstitium though with normal glomeruli. The GFR rate was reduced further in these animals along with a reduction in mean arterial pressure while the single-nephron GFR was higher than in the rats at 7 days, although it only reached the level of control animals. As a final part of the study, the investigators infused L-NIL and performed micropuncture studies. Ten days after administration of renal tubular antigen and complete Freund's adjuvant, L-NIL infusion resulted in increased GFR, single-nephron GFR, and single-nephron plasma flow via a disproportionately greater decrease in afferent arteriolar resistance. Based on these findings, it appears that modulation of NO generation may have different consequences acutely and chronically. The acute effect of iNOS inhibition appears to result in enhanced GFR through its vascular effects. With sustained inhibition, however, immunologic effects as a result of iNOS inhibition may become of greater importance. Therefore, acute iNOS inhibition may be of benefit owing to its effects on the vasculature whereas chronic iNOS inhibition may be harmful owing to effects on the immune system.

#### NO IN CHRONIC TUBULOINTERSTITIAL DISEASE AND HYPERTENSION

Another model of tubulointerstitial nephritis is one of chronic tubulointerstitial nephritis leading to salt-sensitive hypertension. This is an interesting model because it depicts how tubulointerstitial disease, however subtle, can lead to changes in tubular function. Angiotensin II and catecholamines have been shown to cause chronic tubulointerstitial injury and microvascular damage with resultant dysfunction of natriuresis and the development of salt-sensitive hypertension.<sup>29-31</sup> NOS inhibition would be expected to cause similar effects because NO acts as an inhibitor of angiotensin II and catecholamines. NOS inhibition has been shown to cause mild glomerulosclerosis, interstitial fibrosis, inflammatory infiltration into the tubulointerstitium with expression of adhesion molecules, and hypertension involving adrenergics and the renin-angiotensin system.<sup>32-35</sup> These findings suggest that NO appears to be important in preventing tubulointerstitial injury owing to angiotensin II. Because many of the earlier-described changes are T-cell mediated, measures to pharmacologically modulate T-cell proliferation might have an ameliorating effect on tubulointerstitial injury as well. Mycophenolate mofetil (MMF) represents one such agent that might be expected to impact favorably on tubulointerstitial disease via its immunologic effects. MMF inhibits inosine monophosphate dehydrogenase (de novo purine synthesis) and therefore inhibits T-cell proliferation, which is dependent on de novo purine synthesis.<sup>36</sup> Fujihara et al<sup>37</sup> evaluated the effect of MMF in rats subjected to chronic inhibition NOS. In these experiments, Munich-Wistar rats were placed on a high salt diet; a high salt diet plus L-NAME; and a high salt diet, L-NAME, and MMF. Hemodynamic and histologic studies then were performed. Compared with high salt alone, L-NAME treatment enhanced glomerulosclerosis, glomerular collapse, glomerular necrosis, interstitial area, as well as myointimal proliferation and fibrinoid necrosis. All of these variables with the exception of myointimal proliferation were diminished significantly by MMF treatment, although it did not ameliorate the hemodynamic abnormalities associated with this model. MMF had effects on cellular infiltration of the kidney as well. High salt treatment plus L-NAME increased infiltration of lymphocytes in the glo-

meruli, vasculature, and interstitium compared with high salt alone, but this was diminished by MMF treatment. Macrophage infiltration of the vasculature and interstitium was increased by high salt and L-NAME treatment as well. MMF treatment diminished macrophage infiltration of the interstitium but not the vasculature.<sup>37</sup>

#### NO IN PROTEINURIA-MEDIATED TUBULOINTERSTITIAL DISEASE

Although proteinuria was once viewed more as a consequence of kidney disease rather than as a factor in its progression, it is now well understood that proteinuria itself can have deleterious consequences for the tubulointerstitium. Tubulointerstitial damage as a consequence of glomerular proteinuria as well as from overflow proteinuria has been described in both humans and in animal models and has served as a productive area for investigation into mechanisms of progression of renal disease.<sup>38</sup> In the clinical setting, the presence of tubulointerstitial damage has been found to predict a worsened prognosis for a variety of glomerular diseases and therapeutic modalities directed at diminishing proteinuria such as angiotensin converting enzyme inhibition, angiotensin receptor blockade and dietary protein restriction may have beneficial effects by a consequent reduction in tubulointerstitial disease. The interaction of filtered proteins with tubular epithelial cells appears to initiate a cascade of signaling events that involves activation of the transcription factor NF- $\kappa$ B and secretion of various agents including cytokines and chemoattractant substances, which can recruit inflammatory cells into the interstitium and promote tubulointerstitial damage. NO is of potential interest in the area of proteinuria-mediated tubulointerstitial disease given that it does have effects on the transcription of NF- $\kappa$ B-dependent genes as noted earlier. As noted by Rangan et al,<sup>39</sup> the effects of NO on NF- $\kappa$ B appear to be divergent. Although NO appears to enhance activation of NF- $\kappa$ B in lymphocytes, NO appears to inhibit NF- $\kappa$ B activation in mesangial cells, endothelial cells, and in vascular smooth muscle cells. Given the ability of NO to modulate NF- $\kappa$ B activation and given the importance of NF- $\kappa$ B in proteinuria-mediated tubulointerstitial disease, these investigators performed studies to investigate the effects of the NO synthase inhibitors aminoguanidine and L-NIL in the doxorubicin model.<sup>39</sup> In this model, doxorubicin

hydrochloride administration resulted in the development of proteinuria with consequent tubulointerstitial damage manifested by tubular atrophy, cellular infiltration into the interstitium, and increased interstitial volume. In these studies, the investigators showed that administration of aminoguanidine and L-NIL exacerbated tubulointerstitial disease, suggesting that NO may exert a beneficial effect on the tubulointerstitium in the setting of proteinuria-mediated tubulointerstitial disease. Expression of the NF- $\kappa$ B-dependent genes monocyte chemoattractant protein-1 (MCP-1), IL-10, and osteopontin also were enhanced by aminoguanidine and L-NIL administration. These investigators also evaluated the effects of an NO donor, molsidomine (Mol), on tubulointerstitial disease in this model. Although one might have anticipated a beneficial effect of Mol in this model, it worsened the development of tubulointerstitial disease.<sup>39</sup> Given this plus the investigators' additional finding that renal cortical lipid peroxidation was increased by treatment by Mol, the investigators reasoned that in this setting Mol administration likely exerted a prooxidant effect associated with enhanced generation of peroxynitrite, leading to tissue injury. In contrast to this effect of a systemically administered NO donor, locally generated NO may instead exert beneficial effects on tubulointerstitial inflammation via mechanisms such as diminished proliferation of monocytes and T cells as outlined earlier, suggesting that the context and location in which NO is generated are important in determining its impact on tubulointerstitial disease. The report of Kang et al<sup>40</sup> showed that exacerbation of tubulointerstitial injury by NO inhibition in the remnant kidney model is consistent with this. Perhaps manipulation of NO generation may eventually represent a promising therapeutic strategy to diminish tubulointerstitial damage in the setting of glomerular disease and perhaps overflow proteinuria as well.

#### NO IN OBSTRUCTIVE TUBULOINTERSTITIAL DISEASE

Urinary tract obstruction results in injury to renal tubular cells, ultimately leading to tubulointerstitial damage, a process that precedes glomerular changes. Tubulointerstitial damage as a consequence of urinary tract obstruction represents another area in which NO may play a role in tubulointerstitial pathophysiology. Ureteral obstruction causes increased transforming growth factor  $\beta$

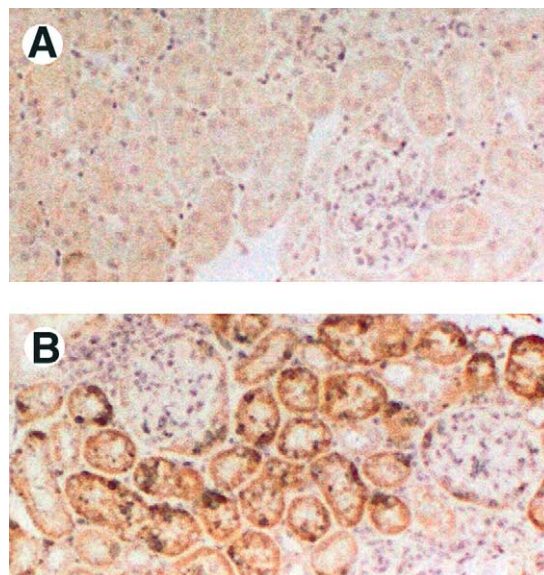
(TGF- $\beta$ ) expression and increased interstitial fibrosis as well as tubular cell apoptosis.<sup>41</sup> TGF- $\beta$  has a number of effects that could promote apoptosis in this setting including p53 up-regulation and inhibition of bcl-2.<sup>42,43</sup> After the demonstration that angiotensin converting enzyme inhibition diminishes interstitial fibrosis in ureteral obstruction, Morrissey et al<sup>44</sup> evaluated the role of NO in this process. Rats subjected to unilateral ureteral ligation were treated with or without the angiotensin converting enzyme inhibitor enalapril, enalapril plus L-NAME, or the NO precursor L-arginine. The decrease in tubulointerstitial damage by enalapril treatment, which was associated with increased urinary nitrate excretion, was antagonized by L-NAME treatment. L-arginine administration had a salutary effect on most of the injury parameters measured as well, suggesting that in the setting of ureteral obstruction NO may play an important role in attenuating the development of tubulointerstitial injury. Huang et al<sup>45</sup> also have reported more severe renal parenchymal injury in iNOS knockout mice after unilateral ureteral ligation. Because TGF- $\beta$   $-/-$  mice have increased iNOS expression<sup>46</sup> and other evidence suggesting that TGF- $\beta$  may diminish NO generation, TGF- $\beta$  may contribute to tubulointerstitial damage in obstructive uropathy in part via altered generation of NO. Administration of a monoclonal antibody to TGF- $\beta$ , 1D11, was found to diminish the development of tubular cell apoptosis and fibrosis in rats with unilateral ureteral obstruction.<sup>47</sup> The attenuation of fibrosis and tubular cell apoptosis in the obstructed kidney by 1D11 also was associated with an increase in iNOS activity.<sup>47</sup> These studies have provided important insights into how NO and its modulation by TGF- $\beta$  play roles in the pathogenesis of tubulointerstitial injury in obstructive uropathy.

#### NO IN ISCHEMIC, SEPTIC, AND DRUG-INDUCED TUBULOINTERSTITIAL DISEASE

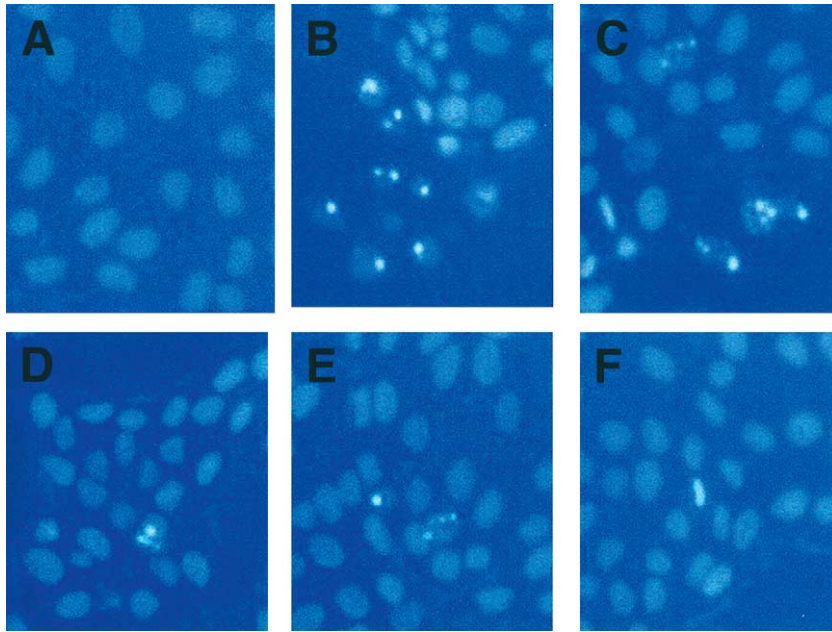
Tubulointerstitial damage as a consequence of ischemia, sepsis, and injury from drugs and toxins are a common clinical problem and NO appears to have a variety of roles in these settings as well. For example, Chatterjee et al<sup>48</sup> examined the impact of iNOS inhibition in an animal model of ischemia/reperfusion injury. In these studies, male Wistar rats were subjected to 45 minutes of bilateral renal ischemia followed by 6 hours of reperfusion in the

presence or absence of the iNOS inhibitors L-NIL or aminoethyl-isothiourrea. Treatment with both of these iNOS inhibitors reduced tubulointerstitial injury in this model. The investigators also showed decreased plasma levels of nitrite/nitrate as well as decreased tissue nitrotyrosine content. Other investigators have shown that NO donors can impair adhesion of tubular epithelial cells to matrix, an event that is consistent with increased cell death.<sup>49</sup> These findings suggest a deleterious effect of NO in the setting of acute ischemic injury.

Ischemic injury to tubular cells occurs in sickle cell disease as well. Using transgenic sickle cell mice, Bank et al<sup>50</sup> have shown enhanced expression of iNOS and the endothelial cell isoform of NOS as well as increased apoptosis and immunostaining for nitrotyrosine in tubular cells in areas staining for iNOS.<sup>51</sup> These investigators subsequently performed studies in transgenic sickle cell mice using mercaptoethylguanidine, which inhibits iNOS and functions as a peroxynitrite scavenger. These investigators found that administration of mercaptoethylguanidine markedly diminished iNOS



**Fig 1.** Mice transgenic for HIV-1 genes, in groups of 6, were administered normal saline or normal saline containing morphine daily for 24 weeks. Subsequently, kidneys were isolated, renal cortices were dissected, and immunohistochemical studies were performed to label AT1 receptors. Representative micrographs of (A) control and (B) morphine-treated mouse are shown. (B) Morphine-treated mouse showed enhanced tubular cell expression of AT1 receptors (brown staining). Magnification  $\times 150$ .



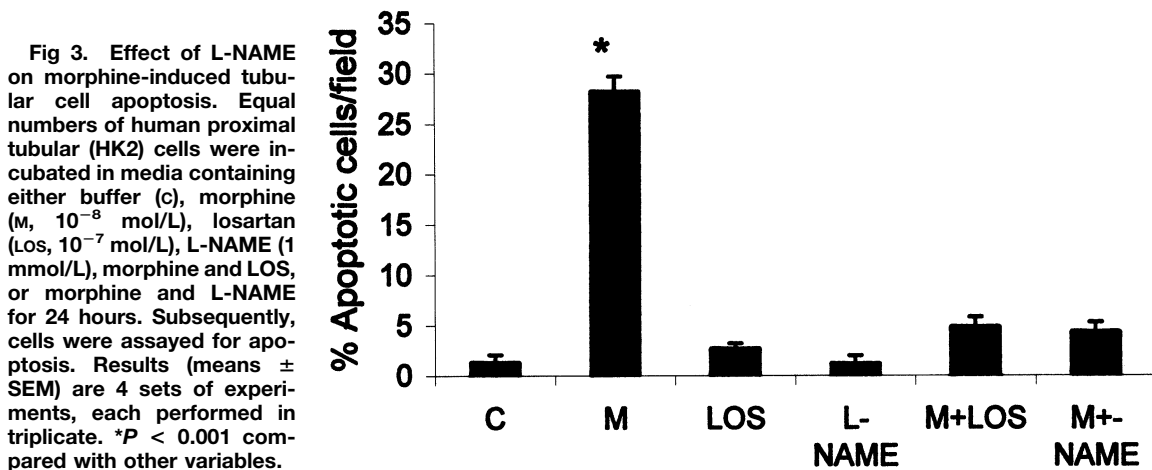
**Fig 2.** Equal numbers of HK2 cells were incubated in media containing either buffer, morphine ( $10^{-8}$  mol/L), Ang II ( $10^{-7}$  mol/L), morphine and losartan ( $10^{-7}$  mol/L), morphine and L-NAME (1 mmol/L), or Ang II and L-NAME for 24 hours followed by staining with H-33342. (A) Control cells. (B and C) Cells treated with morphine and Ang II, respectively. (D) Cells treated with morphine and losartan. (E and F) Cells treated with morphine and L-NAME and Ang II and L-NAME, respectively. Apoptotic cells are indicated by bright fluorescence by condensed/fragmented nuclei.

immunostaining and nitrotyrosine deposition as well as apoptosis of tubular epithelial cells.<sup>52</sup> These studies have suggested that tubulointerstitial injury in sickle cell disease may be associated with enhanced NO generation.

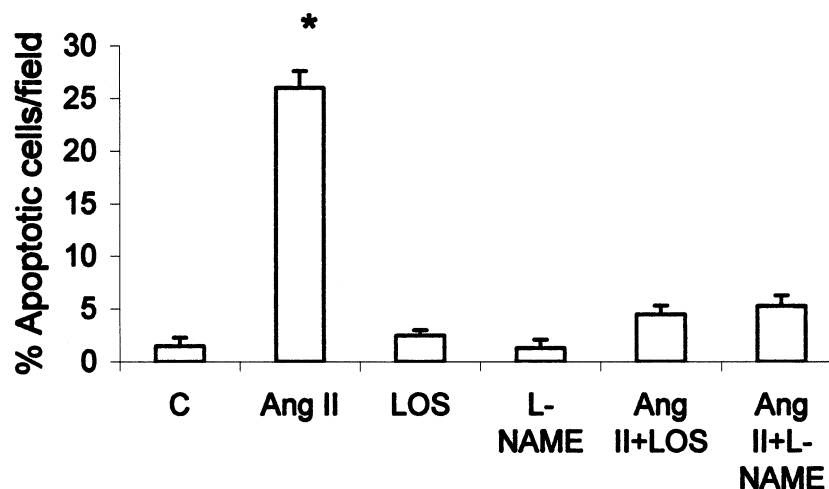
NO may participate in tubulointerstitial injury resulting from septic insults as well. Glynn et al<sup>53</sup> evaluated the effects of tumor necrosis factor- $\alpha$ , IL-1  $\alpha$ , and interferon- $\gamma$  in vitro on proximal tubular epithelial cell morphology, actin immunostaining, apoptosis, necrosis, integrin localization, and iNOS immunostaining. These investigators showed cell detachment related to NO generation

by iNOS and found co-expression of iNOS with integrins that had lost their basolateral localization. These findings suggest that the cytokine derangements characteristic of sepsis may in part promote tubular injury via NO, by a mechanism that might be related to altered localization of integrins and consequent disrupted adhesion to basement membrane, thereby resulting in cell death.

Drugs and toxins represent common sources of tubulointerstitial injury. For example, cyclosporine and tacrolimus are 2 such drugs for which we have gained substantial mechanistic insight into the role of NO in mediating injury. Amore et al<sup>54</sup> showed



**Fig 4. Effect of L-NAME on Ang II-induced tubular cell apoptosis.** Equal numbers of HK2 cells were incubated in media containing either buffer (c), Ang II ( $10^{-7}$  mol/L), losartan (Los,  $10^{-7}$  mol/L), L-NAME (1 mmol/L), Ang II and LOS, or Ang II and L-NAME for 24 hours. Subsequently, cells were assayed for apoptosis. Results (means  $\pm$  SEM) are 4 series of experiments, each performed in triplicate. \* $P < .001$  compared with other variables.



enhanced apoptosis of human renal tubular cells as well as of human mesangial cells, human umbilical vein endothelial cells, and murine endothelial cells in the presence of cyclosporine in a concentration-dependent manner. They found that apoptosis was decreased by L-NAME and worsened by the NO donor sodium nitroprusside,<sup>54</sup> suggesting a role for NO in tubular toxicity associated with the use of this drug.

Angiotensin II has been shown to play an important role in the progression of tubulointerstitial fibrosis. Tubulointerstitial lesions have been reported to be an important hallmark of human immunodeficiency virus (HIV)- and heroin-associated nephropathies. Heroin addiction is also an independent risk factor for the development of HIV-associated nephropathy. Recently, one of us observed that morphine (an active metabolite of heroin) increased expression of tubular cell angiotensin II type-1 (AT1) receptors as well as tubular cell apoptosis in a mouse model of HIV-associated nephropathy (Fig 1; and Singhal et al, unpublished data). In *in vitro* studies, morphine also promoted tubular cell apoptosis. This effect of morphine was inhibited both by an AT1 receptor inhibitor, losartan, and a NOS inhibitor, L-NAME (Figs 2 and 3, and Singhal et al, unpublished data). Interestingly, angiotensin II also promoted proximal tubular cell apoptosis, which was inhibited partially both by losartan and L-NAME (Figs 2 and 4, and Singhal et al, unpublished data). These findings suggest that drugs such as morphine may be inducing tubular cell injury through the generation of angio-

tensin II. The latter seems to mediate its effect through NO.

#### CONCLUSIONS

In recent years, our understanding of tubulointerstitial disease has increased dramatically and in parallel with this it is apparent that NO plays a number of important roles. Studies examining the role of NO in tubulointerstitial disease suggest that the setting in which NO is produced, including cell types that are exposed to it and the presence of other agents such as oxygen radicals, can result in somewhat divergent outcomes and that a deeper mechanistic understanding of NO in tubulointerstitial disease over the coming years may eventually allow for manipulation of its production and action to be used as a therapeutic tool.

#### REFERENCES

1. Gow AJ, Stamler JS: Reactions between nitric oxide and haemoglobin under physiological conditions. *Nature* 391:169-173, 1998
2. Ronson RS, Nakamura M, Vinten-Johansen J: The cardiovascular effects and implications of peroxynitrite. *Cardiovasc Res* 44:47-59, 1999
3. Thomson SC, Bachmann S, Bostanjoglo M, et al: Temporal adjustment of the juxtaglomerular apparatus during sustained inhibition of proximal reabsorption. *J Clin Invest* 104:1149-1158, 1999
4. Ichihara A, Inscho EW, Imig JD, et al: Neuronal nitric oxide synthase modulates rat renal microvascular function. *Am J Physiol* 274:F516-F524, 1998
5. MacMicking J, Xie Q-W, Nathan C: Nitric oxide and macrophage function. *Annu Rev Immunol* 15:323-350, 1997
6. Baylis C, Mitruka B, Deng A: Chronic blockade of nitric

oxide synthesis in the rat produces systemic hypertension and glomerular damage. *J Clin Invest* 90:278-281, 1992

7. Baylis C, Harton P, Engels K: Endothelium-derived relaxing factor controls renal hemodynamics in the normal rat kidney. *J Am Soc Nephrol* 1:875-881, 1990

8. Ferrario R, Takahashi K, Fogo A, et al: Consequences of acute nitric oxide synthesis inhibition in experimental glomerulonephritis. *J Am Soc Nephrol* 4:1847-1854, 1994

9. Nicola LD, Blantz RC, Gabbai FB: Nitric oxide and angiotensin II: Glomerular and tubular interactions in the rat. *J Clin Invest* 89:1248-1256, 1988

10. Baylis C, Qiu C: Importance of nitric oxide in the control of renal hemodynamics. *Kidney Int* 49:1727-1731, 1996

11. Zatz R, De Nucci G: Effects of acute nitric oxide inhibition on rat glomerular microcirculation. *Am J Physiol* 261:F360-F363, 1991

12. Blantz RC, Konnen KS, Tucker BJ: Angiotensin II effects upon glomerular microcirculation and ultrafiltration coefficient of the rat. *J Clin Invest* 57:419-434, 1976

13. Stoos BA, Garcia NH, Garvin JL: Nitric oxide inhibits sodium reabsorption in the isolated perfused cortical collecting duct. *J Am Soc Nephrol* 6:89-94, 1995

14. Kang DG, Kim JW, Lee J: Effects of nitric oxide synthesis inhibition on the NaK-ATPase activity in the kidney. *Pharmacol Res* 41:121-125, 2000

15. Garcia NH, Pomposiello SI, Garvin JL: Nitric oxide inhibits ADH-stimulated osmotic water permeability in cortical collecting ducts. *Am J Physiol* 270:F206-F210, 1996

16. Bech JN, Nielsen CB, Ivarsen P, et al: Dietary sodium affects systemic and renal hemodynamic response to NO inhibition in healthy humans. *Am J Physiol* 274:F914-F923, 1998

17. Stout R, Suttles J, Xu J, et al: Impaired T cell-mediated macrophage activation in CD40 ligand-deficient mice. *J Immunol* 156:8-11, 1996

18. Xie Q-W, Cho H, Calaycay J, et al: Cloning and characterization of inducible nitric oxide synthase from mouse macrophages. *Science* 256:225-228, 1992

19. Xie Q-W, Whisnant R, Nathan C: Promoter of the mouse gene encoding calcium independent nitric oxide synthase confers inducibility by interferon-gamma and bacterial lipopolysaccharide. *J Exp Med* 177:1779-1784, 1993

20. Xie Q-W, Kashiwabara Y, Nathan C: Role of transcription factor NFkB/Rel in the induction of nitric oxide synthase. *J Biol Chem* 269:4705-4708, 1994

21. Koblisch H, Hunter C, Wysocka M, et al: Immune suppression by recombinant interleukin 12 involves interferon-gamma induction of nitric oxide synthase 2 (iNOS) activity: Inhibitors of no generation reveal the extent of rIL-12 vaccine adjuvant effect. *J Exp Med* 188:1603-1610, 1998

22. Cole BC, Griffiths MM, Sullivan GJ, et al: Role of non-RT1 genes in the response of rat lymphocytes to mycoplasma arthritis T cell mitogen, concanavalin A And phytohemagglutinin. *J Immunol* 136:2364-2369, 1986

23. Newlin CM, Gasser DL: Genetic control of the in vitro responses of rat peripheral blood lymphocytes to PHA and Con A. *J Immunol* 110:622-628, 1973

24. Fu Y, Blankenhorn E: Nitric oxide-induced anti-mitogenic effects in high and low responder rat strains. *J Immunol* 148:2217-2222, 1992

25. Wei X-Q, Charles IG, Smith A, et al: Altered immune

responses in mice lacking inducible nitric oxide synthase. *Nature* 375:408-411, 1995

26. Kelly CJ, Gold DP: Nitric oxide in interstitial nephritis and other autoimmune diseases. *Semin Nephrol* 19:288-295, 1999

27. Gabbai FB, Boggiano C, Peter T, et al: Inhibition of inducible nitric oxide synthase intensifies injury and functional deterioration in autoimmune interstitial nephritis. *J Immunol* 159:6266-6275, 1997

28. Gabbai FB, Hammond TC, Thomson SC, et al: Effect of acute iNOS inhibition on glomerular function in tubulointerstitial nephritis. *Kidney Int* 61:851-854, 2002

29. Johnson RJ, Gordon KL, Suga S, et al: Renal injury and salt-sensitive hypertension after exposure to catecholamines. *Hypertension* 34:151-159, 2000

30. Johnson RJ, Schreiner G: Hypothesis: Is essential hypertension an acquired tubulointerstitial renal disease? *Kidney Int* 52:1169-1179, 1997

31. Lombardi D, Gordon KI, Polinsky P, et al: Salt-sensitive hypertension develops after short-term exposure to angiotensin II. *Hypertension* 33:1013-1019, 1999

32. Baylis C, Mitruka B, Deng A: Chronic blockade of nitric oxide synthesis in the rat produces systemic hypertension and glomerular damage. *J Clin Invest* 90:278-281, 1992

33. Kashiwagi M, Shinozaki M, Hirakata H, et al: Locally activated renin-angiotensin system associated with TGF-beta1 as a major factor for renal injury induced by chronic inhibition of nitric oxide synthase in rats. *J Am Soc Nephrol* 11:616-624, 2000

34. Melaragno MG, Fink GD: Role of ANG II in hypertension produced by chronic inhibition of nitric oxide synthase in conscious rats. *Am J Physiol* 271:H806-H811, 1996

35. Spiecker M, Darius H, Kaboth K, et al: Differential regulation of endothelial cell adhesion molecule expression by nitric oxide donors and antioxidants. *J Leukoc Biol* 63:732-739, 1998

36. Allison AC, Eugui EM: Mycophenolate mofetil and its mechanism of action. *Immunopharmacology* 47:85-118, 2000

37. Fujihara CK, Avancini Costa Malheiros DM, de Lourdes Noronha IL, et al: Mycophenolate mofetil reduces renal injury in the chronic nitric oxide synthase inhibition model. *Hypertension* 37:170-175, 2001

38. Abbate M, Remuzzi G: Proteinuria as a mediator of tubulointerstitial injury. *Kidney Blood Press Res* 22:37-46, 1999

39. Rangan GK, Wang Y, Harris DCH: Pharmacologic modulators of nitric oxide exacerbate tubulointerstitial inflammation in proteinuric rats. *J Am Soc Nephrol* 12:1696-1705, 2001

40. Kang DH, Nakagawa T, Feng L, et al: Nitric oxide modulates vascular disease in the remnant kidney model. *Am J Pathol* 161:239-248, 2002

41. Truong LD, Petrusevska G, Yang G, et al: Cell apoptosis and proliferation in experimental chronic obstructive uropathy. *Kidney Int* 50:200-207, 1996

42. Teramoto T, Kiss A, Thorgeirsson SS: Induction of p53 and Bax during TGF-beta-1 initiated apoptosis in rat liver epithelial cells. *Biochem Biophys Res Commun* 251:56-60, 1998

43. Basile DP, Liapis H, Hammerman MR: Expression of Bcl-2 and Bax in regenerating rat renal tubules following ischemic injury. *Am J Physiol* 272:F640-F647, 1997



44. Morrissey JJ, Ishidoya S, McCracken R, et al: Nitric oxide generation ameliorates the tubulointerstitial fibrosis of obstructive uropathy. *J Am Soc Nephrol* 7:2202-2212, 1996
45. Huang A, Palmer LS, Hom D, et al: The role of nitric oxide in obstructive nephropathy. *J Urol* 163:1276-1281, 2000
46. Vodovotz Y, Geiser AG, Chesler L, et al: Spontaneously increased production of nitric oxide and aberrant expression of the inducible nitric oxide synthase in vivo in the transforming growth factor beta 1 null mouse. *J Exp Med* 183:2337-2342, 1996
47. Miyajima A, Chen J, Lawrence C, et al: Antibody to transforming growth factor-beta ameliorates tubular apoptosis in unilateral ureteral obstruction. *Kidney Int* 58:2301-2313, 2000
48. Chatterjee PK, Patel NSA, Kvale EO, et al: Inhibition of inducible nitric oxide synthase reduces renal ischemia/reperfusion injury. *Kidney Int* 61:862-871, 2002
49. Wangsiripaisan A, Gengaro PE, Nemenoff RA, et al: Effect of nitric oxide donors on renal tubular epithelia cell-matrix adhesion. *Kidney Int* 55:2281-2288, 1999
50. Bank N, Aynedjian HS, Qui J-H, et al: Renal nitric oxide synthases in transgenic sickle cell mice. *Kidney Int* 50:184-189, 1996
51. Bank N, Kiroycheva M, Ahmed F, et al: Peroxynitrite formation and apoptosis in transgenic sickle cell mouse kidneys. *Kidney Int* 54:1520-1528, 1998
52. Bank N, Kiroycheva M, Singhal PC, et al: Inhibition of nitric oxide synthase ameliorates cellular injury in sickle cell mouse kidneys. *Kidney Int* 58:82-89, 2000
53. Glynne PA, Picot J, Evans TJ: Coexpressed nitric oxide synthase and apical beta(1) integrins influence tubule cell adhesion after cytokine-induced injury. *J Am Soc Nephrol* 12:2370-2383, 2001
54. Amore A, Emancipator SN, Cirina P, et al: Nitric oxide mediates cyclosporine-induced apoptosis in cultured renal cells. *Kidney Int* 57:1549-1559, 2000