Nitric Oxide and Glomerulonephritis

By Howard Trachtman

Glomerulonephritis is a common clinical condition that is caused by immune-mediated injury to the kidney and is characterized by dysfunction of the glomerular capillary filtration barrier. Nitric oxide (NO), a ubiquitous molecule with many biological functions throughout the body, has been evaluated as an inflammatory mediator in these circumstances. NO may induce glomerular injury directly or may act via stimulation of a host of other inflammatory mediators. A variety of experimental models of glomerulonephritis have been studied including those induced by infusion of antibodies to the Thy1.1 antigen or glomerular basement membrane, Heymann nephritis, and autoimmune nephritis. In virtually all of these cases there is evidence of increased NO production. Excessive production of NO by inducible nitric oxide synthase (iNOS), derived from infiltrating immune cells or resident glomerular cells, nearly always is associated with increased glomerular injury. Interventions that inhibit this enzyme result in less proteinuria and diminished glomerular damage. In contrast, NO derived from endothelial nitric oxide synthase (eNOS) may limit glomerular disease by preserving endothelial cell integrity. There are only a limited number of studies that have evaluated the impact of NO in patients with glomerulonephritis. Although the bulk of evidence supports a role of NO as a pro-inflammatory mediator in glomerulonephritis, additional work is needed to show an association between altered NO production and the severity and outcome of disease in patients with this disease. It is hoped that better understanding of the role of NO in glomerulonephritis will lead to the development of therapies to ameliorate the disease.

AFTER THE original reports in 1987 of the role of an endothelial-derived factor that mediates vasodilatation and its identification as nitric oxide (NO), there has been an explosion of interest in this molecule. This culminated in the award of a Nobel Prize to the investigators who first called attention to the unique features of this ubiquitous signaling molecule. Within the nephrology community, the contribution of NO to normal renal physiology and kidney disease states has been the focus of numerous research meetings, colloquia, original investigations, and review articles, of which this issue of *Seminars in Nephrology* is the latest in a distinguished list of earlier publications.[1,2]

This article attempts to clarify the importance of NO in the pathogenesis of glomerulonephritis. The available information on this topic has been collected by using a wide range of disease models, experimental interventions, animal strains, and in vitro systems. This has lead to a wealth of data, some of which appears to be contradictory and internally inconsistent. In an effort to approach the problem of NO in glomerulonephritis from a different perspective, this article is divided into 3 sections. The first segment summarizes studies implicating NO as a mediator of renal injury in nephritis, the second details the reports in which NO is protective against inflammatory kidney disease, and the third presents those reports that evaluate the role of NO in clinical glomerulonephritis and includes studies that are equivocal or in which the effect of NO cannot be categorized definitively as pro- or anti-inflammatory. Finally, this is followed by a closing section in which an attempt is made to organize the current evidence and synthesize a coherent view of the role of NO in glomerulonephritis. The review focuses primarily on studies that have appeared since Cattell's[3] excellent summary that appeared in *Seminars in Nephrology* in 1999.

NO IN EXPERIMENTAL GLOMERULONEPHRITIS: PRO-INFLAMMATORY MEDIATOR

NO is synthesized by 3 isoforms of the enzyme nitric oxide synthase (NOS) that are expressed heterogeneously by a variety of distinct cell types within the kidney including endothelial, mesangial, and tubular epithelial cells. Moreover, NO has many actions that contribute to the inflammatory response including leukocyte adhesion to endothelial cells, platelet aggregation, stimulation of the release of other mediators, and modulation of the net accumulation of extracellular matrix proteins.

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The theoretical basis for considering NO as a factor that contributes to renal injury in glomerulonephritis includes: (1) activation of signaling cascades, cytokines, and chemokines that promote inflammation;[4] (2) the reaction of NO with superoxide to form the highly reactive nitrogen radical, peroxynitrite;[5] and (3) direct action on glomerular vascular permeability to enhance albumin leak.[6] For example, NO is involved in the modulation of gene expression, synthesis, and release of inflammatory mediators and chemokines, such as interleukin-1β, interleukin-8, monocyte chemoattractant protein-1, macrophage inflammatory protein-2, and RANTES.[7-9] Other targets of NO action include matrix metalloproteinases, plasminogen activators, and their endogenous inhibitors, which may influence the net accumulation of extracellular matrix in the inflamed glomerulus.[7] It has been shown that the relative rates of production of NO and superoxide radical by NOS and other enzymes such as the reduced form of nicotinamide-adenine dinucleotide phosphate oxidase and their rapid chemical reaction to form peroxynitrite is a critical determinant of the cellular response to immune injury (ie, proliferation versus apoptosis).[7] NO increases albumin permeability in isolated glomeruli in vitro by a tyrosine phosphorylation–dependent mechanism involving the epithelial cell podocyte.[6]

The presumption that NO is a pro-inflammatory mediator in glomerulonephritis first prompted investigations into the cellular source of NO under these circumstances. Originally, the data were most consistent with infiltrating cells, specifically polymorphonuclear leukocytes and macrophages, as the primary site of high-output NO synthesis in the nephritic glomerulus.[10-12] Moreover, iNOS activity, induced by cytokines and endotoxin, was thought to be the main enzymatic isoform involved in the stimulation of NO releases. However, it is now apparent that resident renal cells, specifically glomerular mesangial cells, contribute to the inflammatory response in nephritis. Thus, incubation of human mesangial cells with either immune globulin (Ig)A- or IgG-containing immune complexes triggers transcription of the inducible NOS (iNOS) gene, protein expression, and NO synthesis via a nuclear factor κ B (NF-κB)–dependent pathway. The capacity of Fc fragments to counteract the effect of immune complexes on iNOS expression and glomerular NO release in vitro and in an experimental model of nephritis in rats immunized with ovalbumin underscores the importance of the Fc receptor in this process.[13]

All of these pathophysiologic pathways and disease mechanisms have been addressed in various experimental types of renal disease. In the following sections, the following models are discussed: (1) anti-Thy1 antigen nephritis; (2) antiglomerular basement antibody nephritis; (3) Heymann nephritis; and (4) autoimmune nephritis in the NZB/W and MRL/lpr mouse models.

Anti-Thy1 (Antithymocyte Serum) Antibody Nephritis

Narita et al[14] first reported that after injection of antithymocyte serum (ATS), there was complement-mediated mesangial cell lysis, followed by a marked increase in proteinuria and urinary nitrite excretion within 1 to 3 days. Administration of the nonspecific NOS inhibitor L-N^G-monomethyl arginine (L-NMMA) 60 minutes before the infusion of ATS prevented proteinuria, glomerular accumulation of extracellular matrix, and expression of transforming growth factor β (TGF-β). Provision of a diet deficient in L-arginine for 6 days before induction of nephritis mimicked the effects of L-NMMA and also ameliorated glomerular injury. The notion that NO promotes glomerular injury in this form of nephritis has been confirmed by studies in which treatment with agmatine (50 mg/kg/d intraperitoneally), an inhibitor of NOS, also improved renal function.[15] In contrast, dietary supplementation with L-arginine (provided as 1% drinking water) for 1 week before induction of nephritis with ATS to increase intrarenal NO production augmented mesangial cell injury and renal fibrosis.[16]

Immunohistochemical studies revealed that infiltration of glomeruli by polymorphonuclear leukocytes in association with increased expression of iNOS within 1 hour of injection of ATS was the source of the NO that mediated mesangial cell lysis and injury in this model of nephritis.[17] In cultured rat mesangial cells, NO induces the transcription of macrophage inflammatory protein-2 and cytokine-induced neutrophil chemoattractant protein 2 genes, effects that were abolished by L-NMMA.[18] This finding paralleled the observation that administration of the specific iNOS inhibitor N^6-(1-iminoethyl)-L-lysine (L-NIL) markedly decreased macrophage inflammatory protein-2 messenger RNA expression and neutrophil infiltration in glo-

meruli of rats with anti-Thy1 antibody-induced nephritis.

Induction of iNOS in parallel with an increased number of glomerular macrophages also has been documented in both acute and chronic forms of anti-Thy1 nephritis.[19] The enhanced expression of iNOS in this model is mediated, in part, by a complement-dependent generation of reactive oxygen molecules because treatment with N-acetyl cysteine or soluble complement receptor 1 ameliorated the features of the acute disease in vivo and reduced inflammation in isolated nephritic glomeruli in vitro.[20] Although increased expression of neuronal NOS in the macula densa as well as iNOS in glomeruli have been observed in this model, no differences in endothelial NOS (eNOS) expression have been detected.[19] The transcription factor NF-κB is involved in the inflammatory response in anti-Thy 1.1 nephritis via its regulatory effect on iNOS gene expression. Thus, administration of the fungal metabolite gliotoxin or the plant extract, parthenolide, attenuates NF-κB activity, which in turn diminishes the renal expression of iNOS and monocyte chemoattractant protein-1, reduces glomerular proliferation, and decreases proteinuria and hematuria. The beneficial effect of these agents, namely inhibition of phosphorylation and degradation of the IκBα subunit and inhibition of NF-κB activation, was confirmed in cultured mesangial cells and monocytes.[21]

Antiglomerular Basement Antibody (Nephrotoxic Serum) Nephritis

In this widely used model of nephritis, animals may or may not be preimmunized or sensitized with heterologous antibody. They are then given antibody from the same species directed against antigens of the glomerular basement membrane. The proliferative glomerular lesion and early-phase injury are mediated by neutrophils in non-sensitized animals and by macrophages in pretreated animals.

In nephrotoxic serum nephritis, there is an abrupt increase in glomerular iNOS expression within 24 hours that is paralleled by increased urinary nitrite excretion, enhanced glomerular expression of intercellular adhesion molecule-1, and infiltration of glomeruli by macrophages.[22,23] The changes in iNOS expression are relatively short-lived because enzyme expression is reduced within 2 days of onset of disease and returns to normal

after 4 to 10 days. In addition, eNOS expression is suppressed below control levels in animals with anti–glomerular basement membrane (GBM) antibody–induced nephritis. It is important to note that excessive glomerular NO production in anti-GBM nephritis represents a balance between the activity of the enzymes NOS and arginase.[24] Thus, during the acute phase of the disease, when iNOS activity is stimulated and NO synthesis is enhanced, there is a parallel decrease in the enzymatic activity of arginase isoforms I and II. In contrast, as the glomerular injury resolves, there is a decrease in NOS activity with increased arginase to facilitate tissue repair.[25-27]

The importance of NO in this experimental setting is supported by the beneficial effects of treatment with inhibitors of NOS activity. For example, administration of the nonspecific antagonist N^G-nitro-L-arginine methyl ester (L-NAME) or the relatively selective iNOS inhibitor aminoguanidine decreased urinary nitrite excretion and NO levels within the renal tissue. Although provision of L-NAME reduced macrophage infiltration and intercellular adhesion molecule-1 expression more effectively than aminoguanidine, the later agent attenuated inflammation and resulted in greater preservation of glomerular structure.[23] Similarly, administration of all-trans-retinoic acid suppressed iNOS activity and reduced renal expression of TGF-β, leading to less severe glomerular damage in anti-GBM antibody nephritis.[28] The protective effect of NOS inhibitors in nephrotoxic serum nephritis also has been observed in Wistar-Kyoto mice given ONO-1714, a novel cyclic amidine analog that selectively blocks the activity of iNOS.[29] Selective inhibition of iNOS in the early neutrophil-dependent phase of nephrotoxic serum nephritis had no effect on proteinuria despite decreased NO release by isolated glomeruli, suggesting that attenuation of glomerular injury in this model is accomplished by interference with iNOS enzymatic activity in macrophages and not in neutrophils.[30] The effects of iNOS inhibition in anti-GBM nephritis may be partly the result of secondary alterations in the synthesis of the prostaglandins, prostaglandin E_2 and prostaglandin I_2, 15-hydroxyeicosatetraenoic acid, and leukotriene B4.[31] Finally, the beneficial impact of iNOS inhibition has been achieved by modulating the activity of endogenous regulators of the enzyme. There is a close interaction iNOS and heme oxygenase-1,

an enzyme that contributes to cell defense against NO-mediated injury, in anti-GBM nephritis. The primary increase in iNOS in this disease up-regulates heme oxygenase-1, which in turn attenuates iNOS activity in a negative feedback loop. Pretreatment with hemin (30 μmol/kg/d), significantly reduced glomerular iNOS expression in nephrotoxic serum nephritis, diminished macrophage infiltration, and lowered proteinuria.[32,33] In contrast, dietary supplementation with L-arginine augmented proteinuria and worsened the histopathologic damage to glomeruli in nephrotoxic serum nephritis.[34]

Heymann Nephritis

This form of nephritis, provoked by in situ immune complex formation, is used as an analogue of membranous nephropathy. It is characterized by markedly lower levels of intraglomerular inflammation and a reduced number of infiltrating cells. Nonetheless, in a unilateral disease model induced by perfusion of cationized human IgG in preimmunized rats, there was enhanced nitrite synthesis in isolated glomeruli that peaked at 4 days after the onset of injury. Moreover, administration of L-NAME or dexamethasone suppressed the cytokine-induced release of NO by the isolated glomeruli in vitro.[35] However, because of the relatively benign renal histopathology, there have been no further studies evaluating the pro-inflammatory effect of NO in this specific model and the impact of treatment with NOS inhibitors on the course of disease.

Autoimmune Nephritis: NZB/W and MRL/lpr Mice

MRL/lpr mice show overexpression of iNOS and overproduction of NO in an age-dependent manner that parallels the gradual worsening of arthritis, nephritis, and vasculitis.[36] This disturbance in NO production may represent in intrinsic abnormality in the modulation of the mediator. Thus, glomerular mesangial cells isolated from these mice manifest a defect in prostaglandin J2 production, which may prevent the normal tonic suppression of iNOS within the glomerulus.[37] In addition, MRL/lpr mice have diminished glomerular expression of the enzyme, catalase. The adverse effects of excessive NO production in combination with diminished and inadequate antioxidant defenses was highlighted by increased ni-

trotyrosine levels in kidney extracts from these animals.[38]

Long-term treatment of MRL/lpr mice with selective inhibitors of iNOS for 12 weeks effectively blocked NO production, decreased proteinuria, and reduced glomerular histopathology injury scores. The beneficial effects of iNOS inhibitor therapy were achieved despite unaltered production of autoantibodies, deposition within the glomerulus, or complement activation.[39] Similar results were obtained when this strain of mice was treated with the immunosuppressive drug mycophenolate mofetil 100 mg/kg/d for 12 weeks.[40] Administration of this novel drug to 3-month-old MRL/lpr mice for 3 months led to a 30% reduction in renal cortical iNOS messenger RNA expression, decreased urinary nitrite excretion, and decreased glomerulosclerosis.[41] Similar findings have been made in the NZB/W F1 mouse model of autoimmune glomerulonephritis. Thus, administration of the iNOS inhibitor aminoguanidine for 4 months, beginning at 2 months of age and before the onset of nephritis, decreased glomerular immunohistochemical staining for iNOS and TGF-β, decreased proteinuria, and protected against the development of glomerulosclerosis.[42] Finally, replicating their previous studies in ATS nephritis,[16] Peters et al[43] recently showed that L-arginine supplementation for 40 days, provided as a 1% drinking water solution, increased mortality by 50% in MRL/lpr mice. This dietary maneuver resulted in increased urinary nitrite and albumin excretion, more pronounced glomerular matrix accumulation and TGF-β expression, and more severe azotemia. A low-protein diet and reduced L-arginine intake were renoprotective, in conjunction with lower renal iNOS messenger RNA expression.

NO IN EXPERIMENTAL GLOMERULONEPHRITIS: ANTI-INFLAMMATORY MEDIATOR OR NONACTIVE FACTOR

Anti-Thy1 (ATS) Antibody Nephritis

There are a few reports that suggest that NO may protect against glomerular injury in anti-Thy1 antibody nephritis. For example, intraglomerular platelet aggregation and fibrin deposition were increased for up to 3 days in rats with ATS nephritis that were treated with L-NAME. In addition, proteinuria was worsened by the administration of L-NAME. Interestingly, although this regimen reduced urinary nitrite excretion, it did not alter

glomerular accumulation of leukocytes.[44] Similarly, in a Lewis rat substrain (LEW/Maa) with ATS nephritis, animals given the selective iNOS inhibitor L-NIL, 20 mg/kg for 7 days, had reduced urinary nitrite excretion and decreased levels of NO release by isolated glomeruli ex vivo. However, these changes were associated with increased proteinuria and enhanced deposition of platelets and fibrin within glomeruli.[45] These findings suggest that beneficial effects of NO in this model may be indirect and related to hemodynamic and vascular effects rather than caused by actions exerted by NO derived from inflammatory cells.

Antiglomerular Basement Antibody (Nephrotoxic Serum) Nephritis

One of the original studies that evaluated the effect of NOS inhibitors in nephrotoxic serum nephritis indicated that administration of L-NMMA increased proteinuria without modulating leukocyte infiltration within glomeruli.[46] The reduction in NO production was associated with a marked increase in the net transcapillary hydraulic pressure gradient, suggesting that the NO was involved in the maintenance of the baseline vascular resistance in the glomerular microcirculation and prevention of hemodynamically mediated increases in urinary protein excretion.

Data in support of such a proposal, namely, a protective effect of NO released by eNOS, have been derived from a related model of necrotizing crescentic nephritis induced by antimyeloperoxidase antibodies. Immunohistochemical studies showed that although iNOS expression was detectable from 1 to 10 days after the onset of disease, eNOS staining was reduced greatly in glomeruli and the interstitium after 24 hours. At later time points, eNOS was virtually absent in severely damaged glomeruli.[47] These findings imply that failure to maintain glomerular perfusion augmented the renal damage. In normal animals with ATS nephritis, eNOS-mediated synthesis may be compromised directly by the structural damage to the glomerulus and inactivation of enzyme-derived NO by rapid interaction with superoxide radicals and products of the myeloperoxidase/hypochlorous acid system.[48] In contrast to these reports, Wagner et al[49] described reduced urinary nitrite excretion in animals with anti-GBM nephritis that they attributed to glomerular damage and circulating inhibitors of NOS such as asymmetric dimethylarginine. Moreover, renal eNOS activity was unchanged. However, these discrepant data may relate to the prolonged observation period in the animals (ie, 12–20 weeks after the onset of disease), in the study by Wagner et al.[49] A protective role of eNOS has been substantiated in experiments using an accelerated model of anti-GBM antibody nephritis in eNOS knockout mice. In these animals, glomerular injury and renal disease were more severe. Thus, at 10 days, the genetically modified mice had worse azotemia, more severe glomerular thrombosis, and a greater influx of neutrophils.[50] In contrast to these findings with eNOS knockout mice, Cattell et al[51] found no difference in albuminuria or glomerular inflammation in wild-type versus iNOS knockout animals. In genetically intact mice, L-arginine depletion, accomplished by a single intravenous dose of arginase, inhibited glomerular NO synthesis, promoted intravascular thrombosis, increased blood pressure levels, and exacerbated the histopathologic injury.[11] Taken together, these investigations generally support the notion that eNOS-derived NO is beneficial in nephrotoxic serum nephritis by maintaining glomerular perfusion and preventing vascular injury and intraglomerular thrombosis. This NOS isoform does not appear to act by attenuating the inflammatory response, namely neutrophil and macrophage influx into the injured glomerulus.

Heymann Nephritis

In this autoimmune model of glomerulonephritis, there is decreased urinary excretion of cyclic guanosine monophosphate (cGMP), an indirect index of intrarenal NO activity. Administration of L-NAME for 12 weeks enhanced peritubular infiltration by macrophages, worsened hypertension, and augmented proteinuria. Interestingly, treatment with the angiotensin converting enzyme inhibitor captopril limited the extent of disease noted in the L-NAME animals in association with increased urinary cGMP excretion.[52,53] In another model of glomerular injury characterized primarily by nephrotic syndrome, namely that induced by the injection of puromycin aminonucleoside, inhibition of iNOS activity with aminoguanidine reduced urinary nitrite excretion but did not alter the temporal pattern or magnitude of proteinuria.[54] These findings imply that NO plays a less critical role in mediating renal injury and glomerular barrier dysfunction in models of disease associated with minimal degrees of cellular inflammation.

NO IN GLOMERULONEPHRITIS: A HUMAN PERSPECTIVE

In comparison with the amount of work that has been performed in experimental models of nephritis, there are far fewer investigations into the role of NO in human nephritis. Similar to the animals experiments described earlier, immunohistochemical studies have confirmed an increase in glomerular iNOS staining localized to mesangial cells in patients with IgA nephropathy and lupus nephritis. Moreover, there was an inverse relationship between eNOS staining and disease activity.[55] In addition, intraglomerular expression of the chemokines CXCL9 and CXCL10 is increased in patients with crescentic glomerulonephritis. This finding was confirmed in cultured mesangial cells obtained from renal biopsy specimens of these patients. Addition of NO donors suppressed chemokine expression in vitro, suggesting that NO deficiency leads to glomerular infiltration by leukocytes in these conditions.[56]

By using the NO content in exhaled air as an index of the production of the mediator, Kakoki et al[57] documented increased net NO synthesis in patients with chronic glomerulonephritis. In contrast, Duan et al[58] assayed endothelin-1 and nitrite in the urine of 27 patients with biopsy examination–proven glomerulonephritis and found increased levels of the vasoconstrictor peptide and reduced excretion of nitrite. These divergent results may be explained by differences in systemic versus intrarenal NO synthesis during the course of clinical disease.

The 2 forms of glomerular disease that have been studied the most and that have been linked consistently to abnormal NO metabolism are lupus nephritis and IgA nephropathy. Thus, plasma NO concentrations are higher in patients with systemic lupus erythematosus, regardless of whether or not they have renal disease. Moreover, the levels correlate with the circulating concentrations of soluble thrombomodulin and soluble vascular cell adhesion molecule-1.[59] The increased plasma NO levels in patients with systemic lupus erythematosus nephritis correlate with the plasma concentration of interleukin-18, suggesting that this cytokine may be involved in the increased production of NO in lupus renal disease.[60] These changes were accompanied by increased expression of iNOS in the vascular endothelium in skin biopsy specimens obtained from these patients.[61] In addition, enhanced iNOS expression has been observed in renal tissue of adults with lupus nephritis and this change correlated with the extent of apoptosis within the glomeruli.[62] Serum nitrite and 3-nitrotyrosine levels, a more chronic indicator of NO-mediated protein modification, correlate with acute disease activity scores, especially nephritis, in adults with systemic lupus erythematosus.[63] A long-term cohort study showed that serum 3-nitrotyrosine levels were also useful markers of the chronic severity of lupus nephritis, especially in African Americans.[64] It is important to note that not all investigators have confirmed the prognostic value of monitoring serum and urinary nitrite levels as markers of renal disease activity in patients with systemic lupus erythematosus.[65]

The clinical information that is available in patients with IgA nephropathy is more preliminary than the data in lupus nephritis. Roccatello et al[66] studied 36 patients with biopsy examination–proven IgA nephropathy and detected reduced urinary cGMP levels in those patients with impaired renal function. Importantly, the urinary cGMP levels were not correlated with plasma levels of atrial natriuretic peptide. This same group then showed that administration of the NO donor isosorbide 5-mononitrate to patients with IgA nephropathy who had reduced urinary cGMP excretion increased the plasma NO levels and lowered proteinuria.[67] Moreover, incubation of human mesangial cells with glycosylated forms of IgA1 present in the circulation of patients with IgA nephropathy stimulated NO synthesis.[68] However, not all groups have detected a difference in urinary nitrite excretion between patients with IgA nephropathy and healthy controls.[69,70] Finally, in children with Henoch Schoenlein purpura, a disease that may be related to IgA nephropathy, serum and urinary nitrite levels were increased compared with healthy controls.[71] It is clear that a great deal more work needs to be performed to elucidate the role of NO in the initiation and progression of human glomerular disease.

NO IN EXPERIMENTAL GLOMERULONEPHRITIS: A PROVISIONAL VERDICT

Glomerulonephritis is a complex kidney disease. There is a wide range of experimental models that are available for investigation and they vary sig-

nificantly in the individual immune components that are involved in mediating glomerular inflammation, severity, and duration of disease. In addition, the glomerular disease process in nephritis has initiation, amplification, maintenance, and resolution phases and NO may contribute differently to each stage of the illness.[4] Moreover, NO is a ubiquitous molecule with multiple functions within each cell and body organ. Thus, it is no surprise that no incontrovertible statement can be made about the role of NO in this disease setting. Nonetheless, based on the number of pages devoted to the first 2 sections of this review, it seems reasonable to conclude that NO plays primarily a pro-inflammatory role in the initiation and potentiation of glomerular injury in experimental nephritis. Although the initial impression was that the source of the increased intrarenal levels of NO in glomerulonephritis were infiltrating immunoeffector cells, after much investigation it is now apparent that resident glomerular cells, including mesangial cells, also overproduce this mediator and contribute to the cell injury. It is likely that variations in iNOS activity in response to cytokines and other stimuli that are activated during the disease process and temporal differences in NO production among different immune and renal cell populations contributes to the lack of uniformity of response to iNOS inhibitors in experimental models of glomerulonephritis.

There is both direct glomerular cytotoxicity and endothelial dysfunction in all models of nephritis. NO contributes to injury in all glomerular cell types including glomerular epithelial and mesangial cells and endothelial cells. However, although iNOS-mediated NO synthesis may directly cause injury to the vasculature and glomerular cells, lack of eNOS-derived NO may cause endothelial damage that exacerbates glomerular damage. Thus, activation of iNOS in glomerulonephritis probably always is associated with inflammatory injury. In contrast, eNOS may reduce glomerular damage in nephritis by limiting the disruption of the endothelium and intraglomerular thrombosis. Unfortunately, none of the available NOS inhibitors that are available for in vivo studies are pure antagonists of a single NOS isoform. Therefore, it is plausible that differences in the degree of inhibition of iNOS versus eNOS in specific experimental circumstances also may account for the discrepant reports about the role of NO in glomerulonephritis.

Finally, NO is only one among a broad spectrum of inflammatory mediators that are involved in the pathogenesis of glomerulonephritis. It can react with superoxide radicals and other products of activated leukocytes. It may induce some mediators or inactivate others. Even in the face of defined interventions to alter NO production, the impact of the change may not be solely owing to the change in NO synthesis but instead may be the consequence of altered reactions between NO and other mediators. These complex interactions between NO and a host of other inflammatory molecules render it difficult to draw up an unequivocal picture of the role of NO in glomerulonephritis.

Nonetheless, at the end of the day, I think it is fair to conclude that iNOS-derived NO promotes the development glomerulonephritis whereas eNOS-derived NO protects the vasculature, maintains glomerular perfusion, and protects against further renal injury in experimental models of disease.

There is a profound shortage of data about the role of NO in patients with glomerulonephritis. There is an urgent need for further work in this area and better delineation of the correlations between changes in the NO synthesis, severity of disease, extent of glomerular histopathologic injury, and course of disease in this setting. Noninvasive markers of renal NO production are needed to monitor glomerular NO production during the course of disease and to assess the impact of targeted therapeutic interventions on the course of disease. However, if the conclusions derived from the experimental models of glomerulonephritis are valid, then it is only a matter of time until specific safe and well-tolerated iNOS inhibitors are introduced into clinical practice that ameliorate the course of glomerulonephritis.

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