A protective role for endothelial nitric oxide synthase in glomerulonephritis

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A protective role for endothelial nitric oxide synthase in glomerulonephritis. In acute glomerulonephritis (GN), increased nitric oxide (NO) production occurs, suggesting a pathophysiological role for NO in the disease process. Although NO potentially could have both toxic as well as protective effects, its exact role in the pathophysiology of GN is unclear and may depend on the NOS isoform generating NO. The protective effects of NO such as prevention of leukocyte and platelet activation and adhesion have been attributed to NO generated by endothelial nitric oxide synthase (eNOS). Evidence for a beneficial role for eNOS includes the demonstration of reduced eNOS expression in experimental models of GN as well as human biopsy specimens that is mostly likely due to endothelial cell necrosis. Reduced NO production in GN also may occur through reaction of NO with superoxide anions or the myeloperoxidase (MPO)/hypochlorous acid (HOCL) system. Further evidence has been provided by the observation that in several experimental models of GN, glomerular injury is exacerbated following treatment with non-selective NO inhibitors. Finally, the development of GN is severely aggravated in mice lacking a functional gene for eNOS as compared to wild-type mice, providing direct support for a protective role of eNOS-derived NO in acute GN.

It is well established now that acute glomerulonephritis is associated with increased glomerular expression of nitric oxide (NO) [1]. In particular, this has been demonstrated in several well-defined animal models of glomerulonephritis such as nephrotoxic nephritis, active Heymann nephritis and anti-Thy-1 nephritis. In these models, high amounts of NO are generated by the inducible form of NOS expressed in infiltrating inflammatory cells such as neutrophils and monocytes. These observations have added NO to the list of mediators contributing to the development of tissue injury. Indeed, excessive amounts of NO can be directly cytostatic or cytotoxic by its reaction with iron sulfur groups and DNA. NO also may react with superoxide anions generating highly reactive radicals such as peroxynitrite that initiate lipid peroxidation and can damage proteins [2]. However, results from studies attempting to inhibit excessive NO production in acute models of GN using competitive NO inhibitors are inconclusive and sometimes conflicting [1]. In many of these studies exacerbation of the disease occurred suggesting that part of the NO response is protective. In general, these beneficial effects of NO have been attributed to the low levels of NO being generated by the constitutive isoform of nitric oxide synthase (NOS) expressed by the endothelium (eNOS).

Nitric oxide radicals generated by eNOS play an important role in the regulation of vascular homeostasis by inducing vasorelaxation and inhibiting platelet adhesion and aggregation to the endothelium [3–5]. In addition, several studies have demonstrated that eNOS-derived NO is a critical modulator of leukocyte-endothelial cell interactions. Inhibition of NO production has been shown to promote leukocyte rolling and firm adherence in the microcirculation and in several models of inflammation NO donating agents or supplementation with L-arginine has been found to reduce the extent of neutrophil-mediated tissue injury [1, 6]. Here, we will discuss the evidence that eNOS-derived NO radicals exhibit properties that are beneficial in limiting inflammatory reactions in acute glomerulonephritis.

EXPRESSION OF eNOS IN GLOMERULONEPHRITIS

In the kidney, eNOS is constitutively expressed by normal glomerular endothelium and interstitial vessels [7]. Over the past few years, several studies have been reported regarding the expression of eNOS in GN employing experimental animal models of GN as well as renal biopsies from patients with various forms of GN [8–13]. The first report studying the status of eNOS in experimental GN demonstrated a slight, biphasic, increase in glomerular eNOS mRNA during the course of anti-Thy-1 nephritis [8]. In rat as well as murine nephro-
toxic nephritis models, a significant focal loss of glomerular eNOS expression has been shown using immunogold electron microscopy and immunohistochemistry, respectively [12, 14]. Interestingly, the diminished eNOS expression in the rat model was reversed by treatment with a non-selective NO inhibitor [L-arginine methyl ester (L-NAME)]. Using immunohistochemistry, the loss of eNOS expression also has been observed in an experimental model of anti-myeloperoxidase (MPO) associated GN induced by immunizing Brown Norway rats with MPO followed by an unilateral perfusion of the left kidney with a neutrophil lysosomal extract containing proteolytic enzymes, MPO and H2O2 [11]. In this model, immunohistochemical detection of platelets demonstrated that, at sites where eNOS immunoreactivity was absent, massive adhesion and aggregation of platelets occurred, emphasizing the importance of eNOS-derived NO in inhibiting platelet adhesion and aggregation. Although other factors may be involved, the decrease of glomerular eNOS expression in this model is probably due to endothelial cell necrosis, since at these sites immunohistochemical staining for a rat endothelial cell marker (RECA-1) was also absent.

Studies employing immunohistochemistry to investigate eNOS expression in human renal biopsy specimens have demonstrated a loss of glomerular eNOS expression in IgA nephropathy, lupus nephritis and Wegener’s granulomatosis that was most prominent in areas of severe glomerular injury [10, 13]. Moreover, an inverse correlation between the extent of eNOS expression and the degree of glomerular injury was found in IgA nephropathy and lupus nephritis [10].

Taken together, the above-mentioned studies indicate that the development of glomerulonephritis is associated with a reduction in overall eNOS expression that could potentially exacerbate the inflammatory response.

EFFECTS OF PHARMACOLOGICAL INHIBITION OF eNOS ACTIVITY ON GLOMERULONEPHRITIS

Until recently, studies examining the effects of NO in GN relied on the use of structural variants of L-arginine that compete with L-arginine for binding to the active site of NOS. So far, results from these studies have been inconclusive and in some cases conflicting data exist. In many of these studies, the use of L-arginine analogs such as L-NAME has been found to worsen disease. For example, in rat nephrotoxic nephritis as well as in models of autoimmune Heymann nephritis treatment with L-NAME exacerbated proteinuria [15–17]. Furthermore, depletion of arginine levels through infusion of arginase resulted in more glomerular thrombosis and aggravated glomerular disease in rat nephrotoxic nephritis [15]. In the Thy-1 model of mesangioproliferative GN in the PVG/c rat, we have recently observed that treatment with L-NAME exacerbated proteinuria and increased systolic blood pressure [18]. Compared to non-treated rats, a marked increase in platelet aggregation was found whereas no differences in leukocyte accumulation were observed.

In contrast to these detrimental consequences, beneficial effects of NO inhibition in experimental GN have also been reported. In the rat anti-Thy-1 model of GN, Narita and colleagues have shown that infusion of a single dose of the L-arginine analog, L-NMMA, reduces proteinuria and inhibits mesangiolysis to a large extent [19]. In these studies, similar results were obtained using a low protein diet or a diet depleted of L-arginine. Furthermore, in spontaneous murine models of chronic autoimmune GN such as the MRL/lpr/lpr and New Zealand Black/White (NZB/W) mice, treatment with L-NMMA has been shown to ameliorate proteinuria and the development of proliferative GN [20, 21]. In contrast, however, no significant effect on GN was found when iNOS deficient mice were backcrossed to MRL/lpr/lpr mice, although renal vasculitis and arthritis were reduced [22].

Based on the studies using pharmacological inhibition of NO production described above, the exact role of NO in the pathogenesis in GN remains unclear. In particular, the use of L-arginine analogs that lack NOS isoform specificity complicates interpretation of the results. Nevertheless, these studies have provided indirect evidence that at least part of the NO response in GN, most likely generated by eNOS, is protective.

EFFECTS OF eNOS DEFICIENCY ON GLOMERULONEPHRITIS

Because most conventional NO inhibitors do not distinguish between the NOS isoforms, separate lines of mice have been generated with targeted disruption of the genes for inducible NOS (iNOS), neuronal NOS (nNOS) as well as eNOS [23]. These gene-targeted mice have demonstrated a loss of glomerular eNOS expression with a reduction in overall eNOS expression that could potentially exacerbate the inflammatory response.
NO in GN, we studied the course of murine accelerated anti-glomerular basement membrane (anti-GBM) in eNOS \(-/-\) mice and C57BL/6j WT mice [12]. In this model, GN was induced by preimmunization with sheep immunoglobulin G followed by intravenous administration of a subnephritogenic dose of sheep anti-mouse GBM antibody seven days later. In WT mice, GN developed characterized by early (24 hours) glomerular neutrophil accumulation and glomerular thrombosis that persisted until day 10. In addition, at day 10 glomerular hypercellularity and crescent formation in 17\% of glomeruli were observed. In eNOS \(-/-\) mice, GN was severely aggravated. In comparison to WT mice, increased morbidity was observed and serum blood urea nitrogen (BUN) levels were elevated indicating severe renal impairment in these mice. The most prominent feature of the disease in eNOS \(-/-\) mice was extensive glomerular capillary thrombosis often occluding all capillaries. At day 10, a modest increase in glomerular neutrophil influx was observed. Overall antibody depositions, glomerular endocapillary hypercellularity and crescent formation, however, were similar to that in WT mice.

To extend these observations, we recently studied the effect of eNOS deficiency on heterologous nephrotoxic nephritis induced by a single dose of rabbit anti-mouse GBM antibodies (E. Steenbergen, unpublished results). Compared to WT mice, the glomerular neutrophil influx was markedly enhanced in eNOS \(-/-\) mice at two hours \([5.4 \pm 2.6 (N = 7) \text{ vs. } 2.4 \pm 1.6 (N = 8), P < 0.02\] in eNOS \(-/-\) and WT mice, respectively). Also, at 3 days after induction of the disease, elevated serum BUN levels were detected whereas an increase in overall glomerular capillary thrombosis was observed. Taken together, these results provide further evidence for a protective role for eNOS-derived NO production in glomerulonephritis.

**FACTORS THAT MAY AFFECT eNOS EXPRESSION AND ACTIVITY IN GLOMERULONEPHRITIS**

Although eNOS expression is constitutive, in vitro studies have demonstrated that its expression can be subject to modulation. Several factors have been identified that either increase (such as, shear stress) or decrease [for example, hypoxia, endotoxin and tumor necrosis factor-\(\alpha\) (TNF-\(\alpha\))] eNOS expression [27]. Thus, pathophysiological mechanisms may be operative in GN that alter glomerular eNOS expression either at the transcriptional or post-transcriptional level. As discussed before, studies in experimental models of GN and human renal biopsies indicate that the development of glomerulonephritis is associated with a decrease in overall glomerular eNOS expression. In most cases, this loss of eNOS protein expression appears to be secondary to severe glomerular inflammation causing endothelial cell necrosis. However, other potential pathophysiological mechanisms may be operative in GN that could affect eNOS expression and activity.

In vitro studies have demonstrated that proinflammatory cytokines such as TNF-\(\alpha\) lower eNOS expression in cultured endothelial cells [27]. Furthermore, eNOS-derived NO may react with other free radicals such as superoxide leading to oxidative inactivation of NO [27]. Both TNF-\(\alpha\) and superoxide production have been documented in GN and could potentially alter bioactivity of NO [13, 28, 29].

More recently, an important role for myeloperoxidase (MPO) in modulating NO availability has been postulated. The heme protein MPO is the most abundant enzyme in neutrophils where it is contained in the azurophilic granules [30]. However, upon neutrophil activation MPO can be readily released into phagocytic vacuoles as well as the extracellular space. MPO catalyzes the oxidation of chloride by hydrogen peroxide resulting in the formation of hypochlorous acid (HOCL) that can react with a variety of cellular substrates including thiols, nucleotides and amines [30]. As such, MPO catalyzed HOCL formation contributes to the microbial killing function of neutrophils. However, when generated extracellularly it also may induce tissue injury.

The interaction between MPO and NO has recently been investigated [31]. This study demonstrated that NO can serve as a substrate for MPO in the presence of H\(_2\)O\(_2\), raising the possibility that MPO functions as a catalytic sink for NO in vivo. Furthermore, Zhang and colleagues have demonstrated that HOCL can react with \(\text{L-arginine}\) to form novel chlorinated metabolites [31, 32]. Interestingly, these chlorinated \(\text{L-arginine}\) compounds exhibited inhibitory effects on endothelium-dependent vasorelaxation in vivo as well as in vitro, displaying similar pharmacological properties as conventional NOS inhibitors such as L-NAME. From these results the authors postulated that chlorinated \(\text{L-arginine}\) metabolites represent endogenous competitive inhibitors of eNOS. These studies indicate that activation of the MPO/HOCL system may limit the bioavailability of NO in vivo, an effect that would be especially relevant at sites of inflammation where MPO, NO and H\(_2\)O\(_2\) are present. Whether these MPO related mechanisms play a role in GN remains to be determined. In this respect, it is interesting to note that catalytically active MPO has been detected in GN in conjunction with the occurrence of HOCL-modified proteins [28, 33].

**CONCLUSIONS**

This article summarizes the evidence supporting a protective role for eNOS-derived NO in the development of GN. This evidence includes the demonstration of reduced endothelial NO production in GN that may occur
due to loss of eNOS expression caused by endothelial cell necrosis or through the reaction of NO with superoxide anions or the MPO/HOCL system. In GN, eNOS-derived NO production could be protective through its inhibitory effects on platelet aggregation and leukocyte adhesion. The exacerbating effects of non-selective inhibition and selective eNOS deficiency in experimental models of GN appear to confirm this contention.

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APPENDIX

Abbreviations used in this article are: BUN, blood urea nitrogen; eNOS, endothelial nitric oxide synthase; GBM, glomerular basement membrane; GN, glomerulonephritis; HOCL, hypochlorous acid; L-NAME, l-arginine methylster; MPO, myeloperoxidase; NO, nitric oxide; NOS, nitric oxide synthase; RECA-1, rat endothelial cell marker; WT, wild-type.

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