Activation and potential interactions between the arachidonic acid and L-arginine:nitric oxide pathways in glomerulonephritis

ELIAS A. LIANOS

Nephrology Division, Department of Medicine, Medical College of Wisconsin, Milwaukee, Wisconsin, USA

The arachidonic acid metabolites, collectively referred to as eicosanoids, and nitric oxide (NO) originating from the amino acid L-arginine following activation of nitric oxide synthase (NOS), have recently become the focus of intense investigation insofar as the biology of glomerulonephritis is concerned. Three key reasons are: (a) it was realized that certain eicosanoids (those of the arachidonate cyclooxygenase pathway) and NO can be synthesized in a constitutive or inducible manner; (b) while their constitutive release plays a homeostatic role in the kidney, their inducible synthesis can promote cell injury; and (c) regulatory interactions may occur between NO and certain eicosanoids and result in amplification of injury. A number of recent reviews have dealt with the synthesis and origin of eicosanoids and of NO in the normal and diseased kidney [1, 2]. This review emphasizes regulatory interactions that may occur between eicosanoids and NO in glomerulonephritis, and addresses issues regarding the cytotoxic potential of such interactions.

PRESENCE AND PHYSIOLOGIC ROLE OF THE L-ARGININE:NITRIC OXIDE PATHWAY IN THE KIDNEY

Whereas the role of eicosanoids in renal physiology and pathology has been extensively explored in the last decade, the presence and significance of the L-arginine:NO pathway in the kidney has only recently become apparent. Nitric oxide is a reactive radical gas formed from the terminal guanidino nitrogen atom of the amino acid L-arginine. This reaction is catalyzed by the enzyme nitric oxide synthase (NOS). At least three NOS enzymes have been described. The endothelial and neuronal isoforms are constitutively expressed and are quiescent until activated by increased Ca$^{2+}$ levels in a calmodulin-dependent manner. The inducible NOS (iNOS) is expressed following transcriptional activation by cytokines or lipopolysaccharides (LPS) [3]. Following its induction, iNOS remains active for sustained periods in a Ca$^{2+}$-independent manner. Production of NO from the constitutive forms of NOS is short-lived and regulates a number of homeostatic functions including modulation of renovascular tone, renin secretion, the tubuloglomerular feedback response, and sodium excretion [1, 4].

The topography of NOS synthase isoforms in the nephron has recently become elucidated. Terada et al demonstrated the presence of constitutive NOS in the glomerulus where they also found abundant expression of soluble guanylate cyclase, the well established acceptor of NO [5]. Mohaupt and co-investigators have recently reported the expression and induction of mRNAs encoding two inducible nitric oxide synthases in the rat nephron and found that endotoxemia and cytokines differentially induce their expression [6]. Nitric oxide originating from constitutive NOS plays an important homeostatic role in the glomerular microcirculation. Thus, inhibition of NOS in the normal rat results in glomerular arteriolar vasoconstriction and glomerular hypertension. Efferent arteriolar resistance is disproportionately increased resulting in glomerular capillary hypertension. In addition, the glomerular capillary ultrafiltration coefficient, Kf, drops [7]. These observations have established NO as a basal tonic dilator of the glomerular microcirculation. This effect is further supported by the observation that cultured glomerular endothelial cells release NO [8].

COACTIVATION OF THE ARACHIDONIC ACID AND THE L-ARGININE:NITRIC OXIDE PATHWAYS IN GLOMERULONEPHRITIS

Using experimental models of glomerulonephritis investigators have characterized conversion of arachidonic acid to various eicosanoids via the cyclooxygenase and lipoxygenase pathways, and generation of nitric oxide from L-arginine via nitric oxide synthase.

In models of glomerulonephritis induced by antibody against the glomerular basement membrane, the glomerular epithelial cell and the glomerular mesangial cell, it was demonstrated that the synthesis of arachidonate cyclooxygenation and lipoxygenation eicosanoids in isolated glomeruli is enhanced [9]. The most abundant arachidonate cyclooxygenation eicosanoids are the prostaglandins (PG)E2 and PGF2α, and thromboxane (Tx)A2 [10]. The most abundant arachidonate lipoxygenation eicosanoids are leukotriene (LT)B4 and 5- and 12-hydroxyeicosatetraenoic acid (HETE) [11]. In the same models of injury it was demonstrated that generation of nitric oxide from L-arginine in isolated glomeruli is also increased [12]. The temporal relationship between glomerular eicosanoid synthesis and NO generation in the course of glomerular injury induced by anti-GBM antibody is diagramatically shown in Figure 1. In the same figure, changes in glomerular filtration rate (GFR), infiltration of glomeruli by platelets and leukocytes, and proliferation and accumulation of extracellular...
matrix are also shown. The following points are emphasized: (a) Enhanced glomerular synthesis of both eicosanoids and NO occurs and peaks at very early stages of the immune injury (within the first 24 hr). (b) The peak of eicosanoid synthesis coincides with a marked drop in GFR. Glomerular filtration rate subsequently recovers partially despite continued synthesis of vasoconstrictor eicosanoids (TxA2). This recovery is coincidental with peak generation of NO. (c) At stages of glomerulonephritis beyond the early phase (24 hr), a dichotomy in glomerular eicosanoid synthesis occurs; whereas synthesis of PGs, TxA2 and 12-HETE remains enhanced, and that of arachidonate 5-lipoxygenation eicosanoids (5-HETE and LTB4) becomes impaired. Following its peak, glomerular generation of NO is sustained and parallels that of TxA2 and 12-HETE.

In glomerulonephritis, eicosanoids and NO may originate either from intrinsic glomerular cells or from infiltrating platelets and leukocytes. Using platelet depletion and leukocyte depletion studies, it was shown that activated infiltrating macrophages account to a large extent for the glomerular origin of 5-HETE and LTB4, whereas infiltrating platelets entirely account for the origin of 12-HETE and to a lesser extent for that of TxA2 [13]. Using the same approach it was shown that infiltrating macrophages are the source of NO in glomeruli isolated from rats with experimental membranous nephropathy [14] and mesangioproliferative nephropathy [15]. Thus, the infiltrating activated macrophage has been identified as a key site where synthesis of both eicosanoids and NO occurs in glomerulonephritis. However, a possible contribution of mesangial cells should not be ignored [16].

An important aspect of the arachidonic acid and of the L-arginine:NO pathways is that cyclooxygenase activity. Evidence that this may indeed occur has emerged recently. The initial observations pointing to up-regulation of

sustained synthesis of proinflammatory prostaglandins and in sustained high-output generation of NO. Macrophages infiltrating glomeruli in the course of glomerulonephritis are likely to express inducible COX and NOS as a result of their activation by proinflammatory cytokines following adherence to the glomerular capillary endothelium. Indeed, in forms of experimental glomerulonephritis in which glomerular infiltration by macrophages is a prominent feature, iNOS was immunocytochemically localized in these cells [17]. In the same forms, enhanced expression of inducible cyclooxygenase [also referred to as COX-II or prostaglandin H synthase (PGHS-II) ] was also demonstrated [18]. Cytoxygenase (COX)-II expression has not only been detected in isolated glomeruli but also in mesangial cells after exposure to cytokines [19–21].

PUTATIVE INTERACTIONS BETWEEN THE ARACHIDONIC ACID AND THE L-ARGININE: NITRIC OXIDE PATHWAYS

In its capacity as a reactive radical gas, NO binds avidly to iron and iron-containing enzymes. A multitude of effects mediated by NO have been attributed to its high affinity for iron-containing substrates. For example, the ability of NO to inhibit platelet aggregation and to relax vascular smooth muscle has been attributed to NO binding to the heme-Fe$^{3+}$ of the soluble guanylate cyclase leading to stimulation of this enzyme and subsequent increase in the levels of cGMP [22]. Similarly, NO interacts with hemoglobin and with iron-sulfur centers in key enzymes of the respiratory cycle and DNA synthesis [23]. Arachidonate cyclooxygenase may become a target for NO because it contains an iron-heme center at the site that confirms its hydroperoxidase function [24]. Binding of NO to this center may enhance cyclooxygenase activity. Evidence that this may indeed occur has emerged recently.
cyclooxygenase activity by NO were based on macrophages stimulated with endotoxin. In these cells, endotoxin stimulated iNOS as well as the inducible isofrom of cyclooxygenase (COX-II) resulting in large production of NO and of prostaglandins, respectively. Pharmacologic inhibition of iNOS not only reduced the release of NO but also that of prostaglandins [25]. In these studies iNOS inhibitors employed were specific in that they did not have an effect on COX activity, nor did they alter the level of induction of COX-II. To further explore the role of NO in up-regulating COX-II activity, interleukin (IL-1β) stimulated human fibroblast cells that did not possess an endogenous L-arginine:NO pathway were exposed to exogenous NO. Exposure of IL-1β stimulated fibroblasts to either NO or to two NO donors, sodium nitroprusside and glyceryltrinitrite, increased COX activity by at least fourfold and production of prostaglandins [25]. This was independent of an effect of NO on the soluble guanylate cyclase. A likely mechanism by which NO activates COX is direct stimulation of the enzyme. Indeed, NO directly increases COX activity of microsomal sheep seminal vessels as well as the activity of murine recombinant COX-I and COX-II [25]. The exact molecular mechanism by which NO activates COX remains to be identified. In addition to binding to the heme-iron center of COX, NO interacts with oxygen-derived free radicals such as the superoxide ion (O\textsuperscript{2–}), which is also released at sites of inflammatory injury. Interaction between NO and O\textsuperscript{2–} leads to the formation of peroxynitrite (ONOO\textsuperscript{−}), which can then decompose to form the hydroxyl radical, OH\textsuperscript{−}. Thus, NO may augment COX activity by acting as an anti-oxidant (removal of O\textsuperscript{2–}) or by generating ONOO\textsuperscript{−} and OH\textsuperscript{−} that can subsequently activate COX. Indeed, it was shown that O\textsuperscript{2–} and OH\textsuperscript{−} can modulate the COX pathway [26]. Finally, it was recently shown that NO nitrosylates cysteine residues in the catalytic domain of COX enzymes [27]. This points to an additional/alternative mechanism whereby NO can stimulate COX.

In vivo studies supporting regulatory interactions between the arachidonic acid and the L-arginine:NO pathways are also emerging. In endotoxin-induced septic shock in rats, inhibition of NOS using aminoguanidine reduces the release of prostacycline (PGI\textsubscript{2}) by approximately 85% in plasma and urine [28]. Rat islet cells or vascular smooth muscle cells stimulated with IL-1β release NO and prostaglandins; inhibition of NO production blocks PGE\textsubscript{2} release [29, 30]. In bovine coronary microvascular endothelial cells NO activates constitutive cyclooxygenase leading to increased production of prostacyclin [31]. In a rat model of experimental glomerulonephritis resembling human forms of rapidly progressive crescentic nephritis, iNOS inhibition using a relatively selective pharmacologic inhibitor results in reduced synthesis of the arachidonate cyclooxygenation products, PGE\textsubscript{2} and PGI\textsubscript{2}, in isolated glomeruli without an effect on TXA\textsubscript{2} [this issue, 65].

Arachidonate lipoxigenases may also become NO targets because they contain non-heme iron [32]. Indeed, bond formation between NO and the non-heme iron of lipoxigenase was demonstrated using purified soybean 15-lipoxigenase and methods of electron spin resonance spectroscopy [33]. Whether this binding results in up- or down-regulation of lipoxigenase activity is of apparent importance in forms of glomerulonephritis characterized by enhanced arachidonate lipoxigenation to hydroxyeicosatetraenoic acids (HETE) and leukotrienes and enhanced generation of iNOS-derived NO. Indeed, in experimental rapidly progressive nephritis, pharmacologic inhibition of iNOS results in reduced synthesis of 5-HETE and 15-HETE and of LTB\textsubscript{4} [65].

Evidence that eicosanoids may regulate generation of NO is also emerging. Arachidonic acid and the leukotrienes (LTB\textsubscript{4}) stimulate generation of NO in neutrophils and platelets in amounts sufficient to inhibit platelet aggregation [34]. On the other hand, eicosanoids can modulate NOS expression. In this regard, PGE\textsubscript{2} negatively modulates iNOS induction in glomerular mesangial cells in response to interleukin-1 [35]. In experimental rapidly progressive glomerulonephritis inhibition of cyclooxygenase or of 5-lipoxigenase enhance expression of iNOS in isolated glomeruli [65].

It becomes clear from the evidence reviewed above that regulatory interactions between arachidonate cyclooxygenation or lipooxygenation pathways and the L-arginine:NO pathway may develop in the glomerular milieu in the course of glomerulonephritis. Such interactions may potentiate adverse hemodynamic and cytotoxic effects thereby amplifying the extent of renal hemodynamic impairment and glomerular cell injury. For example, NO-driven stimulation of proinflammatoty eicosanoids (TXA\textsubscript{2} and leukotrienes) would promote platelet aggregation (TXA\textsubscript{2}), leukocyte adhesion to the glomerular capillary endothelium via increased expression of adhesion molecules (TXA\textsubscript{2} and leukotrienes), and constriction of the glomerular arterioles (TXA\textsubscript{2}, LTD\textsubscript{4}). On the other hand, regulation of iNOS by eicosanoids would modulate NO generation. A diagrammatic presentation of interactions that may occur between the arachidonate cyclooxygenase and lipoxigenase pathways and the L-arginine:NO pathway is shown in Figure 2.

**RENAL HEMODYNAMIC EFFECTS OF EICOSANOIDs AND NITRIC OXIDE IN GLOMERULONEPHRITIS**

Eicosanoids and nitric oxide synthesized and released in the glomerular milieu in the course of glomerulonephritis may have opposing or additive effects on the glomerular microvasculature. NO, PGE\textsubscript{2} and PGI\textsubscript{2} preserve GFR and renal blood flow (RBF) in experimental models of glomerulonephritis via a dilatory effect on the renal vasculature. This was shown in studies assessing the impact of cyclooxygenase (COX) or nitric oxide synthase (NOS) inhibition on changes in GFR and RBF. Cyclooxygenase (COX) inhibition using non-steroidal anti-inflammatory drugs (NSAIDs) reduces glomerular synthesis of PGE\textsubscript{2} and PGI\textsubscript{2} and markedly decreases GFR and RBF in anti-GM antibody-induced glomerulonephritis in the rat [36]. In the same model, NOS inhibition using systemic administration of the L-arginine analog S\textsuperscript{α}-monomethyl-L-arginine results in reduced glomerular plasma flow rate, a drop in the glomerular capillary ultrafiltration coefficient, K\textsubscript{f}, and an increase in efferent arteriolar constriction resulting in intraglomerular capillary hypertension and exacerbation of proteinuria [37]. These adverse effects may occur due to an unopposed effect of vasoconstrictors by NO as a result of inhibition of renal NO synthesis. Established vasoconstrictors include the eicosanoids thromboxane A\textsubscript{2} and LTD\textsubscript{4}. In anti-GM or anti-Thy 1 antibody mediated glomerulonephritis, these two eicosanoids mediate the decrease in GFR by increasing the afferent arteriolar resistance and decreasing the glomerular capillary ultrafiltration coefficient [38, 39]. The changes in GFR that occur following anti-GM antibody-induced glomerulonephritis are shown in Figure 1. The marked decrement in GFR immediately following...
onset of injury (1 hr) is mediated by TxA2 and LTD4 [38, 39]. Glomerular filtration rate subsequently recovers despite sustained increments in the vasoconstrictor TxA2, indicating that the vasoconstrictor effect of this eicosanoid is opposed. Potential opposing factors are vasodilatory prostaglandins (PGE2 and PGI2) the glomerular synthesis of which is also enhanced at this stage of the immune injury [10], and NO the generation of which peaks at the point of recovery in GFR (Fig. 1) [12]. In other forms of glomerular immune injury, such as the one induced by anti-Thy 1 antibody, enhanced glomerular synthesis of TxA2 also occurs but is not accompanied by increased generation of the vasodilatory prostaglandins PGE2 and PGI2 [13]. Yet, GFR still recovers despite marked increases in glomerular TxA2 levels. This observation questions the role of vasodilatory prostaglandins in opposing the vasoconstrictor effect of TxA2 on the glomerular microvasculature following immune injury and points to NO as the likely vasodilator that may oppose the effect of TxA2. However, direct evidence for such opposing interaction between TxA2 and NO at the level of the glomerular microvasculature is lacking.

EICOSANOIDS AND NITRIC OXIDE AS MEDIATORS OF CYTOTOXICITY IN GLOMERULONEPHRITIS

A sustained synthesis of arachidonate cyclooxygenation products (PGs and TxA2) and a sustained generation of NO occur in the course of glomerular immune injury (Fig. 1). This is likely due to activation of the inducible form of cyclooxygenase (COX-II) and of the inducible isoform of nitric oxide synthase (iNOS) [16, 17].

Cyclooxygenase (COX) catalyzes the committed step in prostaglandin biosynthesis from arachidonic acid, specifically the oxygenation of arachidonic acid to PGG2 and reduction of PGG2 to PGH2. It has recently become apparent that there are two different isoforms of COX, referred to as COX-I and COX-II, and encoded by separate genes [40]. Both COX-I and COX-II probably catalyze reactions that are mechanistically identical and the two enzymes exhibit the same $K_m$ values for their substrate, arachidonic acid. The biological basis for the existence of two distinct cyclooxygenases is not well understood; COX-I is constitutively expressed in most tissues and is believed to mediate “housekeeping” functions. Surprisingly, however, COX-I deficient mice are generally healthy and develop normally. Their kidneys show minimal abnormalities, mainly scarce foci of immature tubules [41]. In contrast, COX-2 deficient mice die early (by eight weeks). Pathologically, their tissues are normal except for kidney tissue, the maturation of which stops early after only a small number of nephrons have developed. The majority of glomeruli and tubules are immature while the residual nephrons develop glomerular sclerosis, interstitial inflammation and progression to end stage disease [42]. Different patterns of expression of COX-I and COX-II have been identified in the kidney, that is, COX-I is mainly expressed in the collecting tubule while COX-II in the macula densa [43].

The most dramatic difference between the two COX isoenzymes relates to regulation of their expression. While expression of COX-I is maintained at constant levels, that of COX-II can be dramatically increased during inflammation [44], an observation that has led to the proposal that activation of COX-II results in generation of proinflammatory prostaglandins. The induction of COX-II by proinflammatory cytokines and during inflammatory responses in cells that mediate inflammation, that is, the macrophage, and the reduced expression of this enzyme by anti-inflammatory agents such as corticosteroids, provides circumstantial evidence to support this role.

It was originally presumed that COX-I is the site of action of non-steroidal anti-inflammatory drugs (NSAIDS). However, the discovery of the COX-II isoenzyme and its association with inflammation has suggested that the therapeutic target of NSAIDS functioning in their anti-inflammatory capacity is COX-II. This observation has advanced the attractive concept that use of non-steroidal anti-inflammatory agents that can selectively...
inhibit COX-II would result in a beneficial anti-inflammatory effect without the adverse consequences of COX-I inhibition, such as peptic ulcer formation and renal functional impairment (decreased RBF and GFR). This attractive concept has not yet been tested in inflammatory processes involving the kidney, such as glomerulonephritis. Preliminary evidence does indicate that in experimental glomerulonephritis there is up-regulation of glomerular COX-II expression [17]. Whether this promotes glomerular inflammatory injury is unknown. For example, in mice lacking COX-II, the severity of inflammatory responses to tetradecanoyl phorbol acetate to or arachidonic acid is not diminished in a model of acetone-induced ear inflammation [42].

As mentioned earlier, NO can up-regulate the activity of both COX-I and COX-II. It is, therefore, quite possible that in the course of glomerular immune injury the sustained high-output generation of NO originating from activation of iNOS can “drive” synthesis of proinflammatory prostaglandins and TxA2 by simulating COX-II. This could amplify the inflammatory injury to intrinsic glomerular cells. Whether sustained generation of proinflammatory eicosanoids in the glomerular milieu in the course of glomerulonephritis is injurious to glomerular cells is debatable. Solid evidence that proinflammatory eicosanoids including PGs, TxA2, and the leukotrienes can directly effect cell injury is lacking. Indeed, in other inflammatory conditions such as osteoarthritis, inhibition of cyclooxygenase using NSAIDS reduces synthesis of PGs and TxA2 and alleviates symptoms. Yet, the destruction of joints by the inflammatory process is not prevented. In contrast, iNOS-derived NO can directly effect cell injury. This can occur either directly by NO itself or by oxidation products of NO. Examples of direct toxic effects mediated by NO itself include: (a) inhibition of cytochrome P450 [45]; (b) reversible inactivation of ribonucleotide reductase, which is a critical enzyme for synthesis of DNA precursors [46]; and (c) irreversible inhibition of cytochrome C oxidase, which may promote leakage of superoxide from the mitochondrial electron transport chain [47].

A key mechanism of injury attributed to production of nitric oxide is its reaction with superoxide (O$_2^-$) to form peroxynitrite (ONOO$^-$). Peroxynitrite is not a free radical and its formation is irreversible. In infiltrative forms of glomerulonephritis, both NO and superoxide can be released in the glomerular milieu from infiltrating leukocytes. Therefore, formation of peroxynitrite is likely to occur. However, the concentration of superoxide is generally kept low by its scavenger superoxide dismutase the intracellular concentration of which is quite high (estimated 4 to 10 μM in brain and liver). Thus, very high concentrations (μM levels) of NO must be attained in order for NO to effectively compete with superoxide dismutase and react with superoxide to form peroxynitrite. This may only occur following activation of the inducible NOS, which, as reviewed above, results in high-output generation of NO. Whether peroxynitrite is indeed generated in the glomerular milieu in the course of glomerulonephritis is unknown. One indirect approach to detect its formation is to search for an in vivo “fingerprint” of interaction between peroxynitrite and protein tyrosine residues. Detection of nitrotyrosine-containing proteins has recently become possible using antibodies against these proteins. Using these antibodies, it was demonstrated that extensive nitration of protein tyrosine residues occurs in human atherosclerotic lesions [49].

Once formed as a result of the reaction between NO and superoxide, peroxynitrite can be particularly toxic. Thus, protonation of peroxynitrite results in its isomerization to peroxynitrous acid. This can subsequently yield hydroxyl radical and nitrogen dioxide. In addition to generating these two toxic radicals, peroxynitrous acid can itself oxidize iron/sulfur centers, zinc fingers and protein thiols. Examples of peroxynitrite-induced cytotoxicity include: (a) inactivation of α1-anti-protease inhibitor [50], which is a protease inhibitor that plays an important protective role in the lung against neutrophile-derived proteases; (b) damage of amiloride-sodium sensitive channels [51]; (c) mitochondrial injury the extent of which is more severe than that induced by NO [52]; and (d) oxidation of low density lipoproteins to a form that facilitates their uptake by activated macrophages [53].

**IS INHIBITION OF NITRIC OXIDE A REASONABLE GOAL IN GLOMERULONEPHRITIS?**

As reviewed above, generation of NO in the course of glomerulonephritis is important in preserving GFR. Yet, NO is most likely derived from activation of iNOS [14, 15]. Therefore, its generation can be sustained and of high output and can cause cell injury by the mechanisms reviewed above. A critical dilemma thus emerges: should generation of NO (particularly of iNOS-derived NO) be left unopposed to preserve GFR or should it be inhibited in order to protect glomerular cells from oxidative injury at the risk of causing renal vasoconstriction and intraglomerular or systemic hypertension? An additional risk of inhibiting glomerular generation of NO in infiltrative forms of glomerulonephritis is that this maneuver may enhance platelet and leukocyte adherence to the glomerular capillary endothelium thereby promoting platelet and leukocyte-induced injury. A number of recent studies support both the argument of leaving NO generation unopposed and that of inhibiting NO generation. In anti-GBM antibody-induced glomerulonephritis, inhibition of NO synthesis using systemic administration of nonselective NOS inhibitors or systemic NOS substrate (L-arginine) depletion, results in intraglomerular hypertension [37] and worsening of proteinuria [37, 54]. Contrasting these findings are those in murine lupus nephritis in which long-term pharmacologic NOS inhibition improved proteinuria and renal histopathology [55].

Careful experiments are needed to clarify whether inhibition of NO will have a net beneficial or detrimental effect in glomerulonephritis. The design of such experiments should include attempts to determine whether NO generation in immunologically injured glomeruli reaches biologically relevant levels. One should not underestimate the efficiency with which nitric oxide can be removed and neutralized immediately after its generation at the site of inflammatory injury, and the fact that nitric oxide itself is far less reactive and toxic at biologically relevant concentrations than in *in vitro* experiments suggest. Most studies in the literature in general and in the nephrology literature in particular, have not measured the biologically relevant concentrations of NO in glomeruli and may have erroneously assumed that NO is being continuously generated because of its short half-life. However,
NO is an uncharged molecule that can diffuse through most cells and tissues with little consumption or direct reaction [56]. A very efficient removal mechanism of NO is generated at the site of inflammation and is provided by red blood cells which rapidly destroy NO following its avid binding to red blood cell oxyhemoglobin. Thus, red blood cells can rapidly reduce intracellular concentrations of NO in NO-producing cells or extracellular concentrations of NO at a site of inflammatory injury. Glomeruli are typically hyperemic in glomerulonephritis. Therefore, a quite efficient removal of NO generated within the glomerular milieu can be expected. Whether NO concentrations in nephritic glomeruli reaches levels sufficient to cause cell injury via formation of peroxynitrite is unknown. Most studies have measured ex vivo production of NO in nephritic glomeruli by assessing concentration of nitrite/nitrate. In these studies, prolonged (up to 48 hr) incubations are usually required in the presence of the NO substrate, L-arginine [12–14]. This approach may not provide an estimate of the biologically relevant concentrations of NO attained within nephritic glomeruli. For example, NO concentrations of about 5 nm cause vasodilation by activating guanylate cyclase and can, therefore, be assumed as “homeostatic.” In contrast, μM concentrations of NO are required to compete with superoxide dismutase and react with superoxide to generate peroxynitrite. Are such concentrations of NO achieved in nephritic glomeruli? Newer direct NO detection methods using chemiluminescence detectors may provide accurate estimates of “glomerular” NO concentrations.

The demonstration that expression of iNOS is enhanced in glomerulonephritis raises the obvious question of whether selective inhibition of this NOS isoform while leaving the constitutive isoform intact would have a beneficial anti-inflammatory effect. Increasingly selective iNOS inhibitors are now becoming available and may serve as powerful tools to definitively address the role of iNOS-derived NO in the pathobiology of glomerulonephritis. Such inhibitors have shown promising results in other forms of inflammation, specifically in adjuvant-induced arthritis in rats and in carrageenan-induced subcutaneous inflammation. In these models, it was shown that iNOS inhibition attenuates nitrite accumulation, leukocyte infiltration and the histopathological severity of the lesions [57, 58]. Moreover, these beneficial effects were not associated with systemic hypertension as a result of NO synthesis inhibition. The efficacy of iNOS inhibition in ameliorating the extent of glomerular injury and proteinuria in glomerulonephritis is unknown. Selective inhibition of iNOS would still reduce NO generation thereby promoting renal vasoconstriction, platelet aggregation and leukocyte adherence to the glomerular capillary endothelium. This could offset the potential benefit of selective iNOS inhibition. Indeed, in a rat model of rapidly progressive crescentic nephritis, iNOS inhibition using a relatively selective inhibitor worsened proteinuria [65]. Although increasingly selective iNOS inhibitors are being developed, they are not entirely without an inhibitory effect on constitutive NOS and this may be sufficient to cause renal vasoconstriction. The recent development of transgenic mice lacking the iNOS gene [59] may provide a definitive tool to assess the role of iNOS in the biology of glomerulonephritis.

Little is known about activation of inducible cyclooxygenase or nitric oxide synthase in human forms of glomerulonephritis. This is particularly important insofar as activation of iNOS is concerned as this form of iNOS may not be induced in human macrophages infiltrating immunologically injured glomeruli. Indeed, the circumstances required for the release of iNOS-derived NO in human macrophages are controversial. That human monocytes/macrophages contain and can express the iNOS gene is well established. The accumulation of iNOS mRNA and protein has been documented in interferon-gamma (IFN-γ) and TNF-stimulated human monocytes, and in monocytes cultured with HIV-1 [60, 61]. However, the actual production of NO by human monocytes/macrophages differs from that of rodent macrophages. While treatment of rodent macrophages with established iNOS inductants such as interferon gamma (IFN-γ), tumor necrosis factor (TNF) or lipopolysaccharide (LPS) results in rapid and abundant accumulation of NO, the amount of NO metabolites released by human macrophages is modest when compared with production in rodent cells. Also, induction of iNOS requires a period of stimulation of several days [62]. This has lead to the proposal that the human macrophage iNOS is a relatively low-output system. However, notable exceptions to this slow induction/slow output system also exist. Human monocytes produce large amounts of NO2 when cultured with M. avium [62]. Unstimulated human peripheral blood monocytes produce relatively high levels of NO2 (26 mmol/106 cells/day) during the first day of culture and production increases linearly or abruptly after six days in culture [63]. Finally, cross-linking of the CD69 cell surface receptor in human monocytes results in rapid production of NO2 [64]. Collectively, these observations point to limitations of the animal (in particular rodent) models of inflammation in studying activation of the L-arginine:NO system. They also suggest a more sophisticated and tightly regulated system for iNOS-derived NO in human as compared to rodent forms of macrophage-dependent forms of inflammation.

CONCLUDING REMARKS

In glomerulonephritis the arachidonic acid pathway to cyclooxygenation and lipoxygenation eicosanoids and the L-arginine to NO pathway are coactivated. Regulatory interactions between the two pathways may occur as a result of NO binding to iron containing centers of cyclooxygenase and lipoxygenases. Evidence that NO can up-regulate cyclooxygenase activity is firm while evidence that NO may also regulate activity of lipoxygenases is emerging. In experimental forms of glomerulonephritis, inducible isoforms of cyclooxygenase and nitric oxide synthase are expressed. This may lead to sustained generation of proinflammatory prostaglandins and TxA2, and in high output generation of NO. Whereas evidence that eicosanoids have a direct cytotoxic effect is lacking, the cytotoxic effect of NO is well established particularly when NO reacts with superoxide to form the strong and relatively stable oxidant peroxynitrite. Whether the concentrations of NO or peroxynitrite attained in the glomerular milieu in the course of glomerulonephritis are sufficient to cause cytotoxicity by this mechanism is unknown. Detection of nitrotyrosine-containing proteins in glomeruli using immunohistochemical methods may provide a means of assessing the extent of peroxynitrite-induced cell injury. Without such demonstration, the argument that iNOS-derived NO causes cell injury in the course of glomerulonephritis remains unfounded. Obtaining such evidence is important in order to justify use of selective iNOS inhibitors to reduce generation of iNOS-derived NO in glomerulonephritis. A serious adverse effect of NO inhibition is renal vasoconstriction with intraglomerular and/or systemic hypertension. Therefore,
beneficial effects expected as a result of iNOS inhibition should be carefully weighed against such adverse sequelae. Oxy-hemoglobin in red blood cells provides a very efficient system of removing NO following its release. On the other hand, superoxide dismutase provides an efficient system to remove O₂⁻. These two systems prevent reaction of O₂⁻ with NO to form the potent oxidant ONOO⁻. This, and the ability of NO to diffuse through cells and tissues with little consumption or direct reaction, raises skepticism and urges carefully conducted studies to confirm the cytotoxic potential of NO in glomerulonephritis. This is particularly true in humans because human macrophages infiltrating nephritic glomeruli may be unable to up-regulate activity of the inducible NOS.

References

18. CAREY T, CRUET E, MUNGER K, SERHAN CN, BADR KF: Cyclooxygenase II (COX-II) expression and lipoxin (LX) biosynthesis in passive (NTS) and accelerated (aNTS) nephrotoxic serum nephritis in the rat: Modulation by aspirin (ASA) therapy. (abstract) J Am Soc Nephrol 6:753, 1995


