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Original Article

Regulatory effects of inducible nitric oxide synthase on cyclooxygenase-2 and heme oxygenase-1 expression in experimental glomerulonephritis

Prasun K. Datta[1,2], Shawn Dhupar[1] and Elias A. Lianos[1]

[1]Division of Nephrology, Department of Medicine, UMDNJ-Robert Wood Johnson Medical School, New Brunswick, NJ, and [2]Laboratory of AIDS Pathogenesis and Molecular Therapeutics, Center for Neurovirology, Temple University, Philadelphia, PA, USA

Abstract

Background. We explored whether inducible nitric oxide synthase (iNOS) driven nitric oxide (NO) production regulates expression of iNOS, endothelial NOS (eNOS), Cyclooxygenase-2 (COX-2), and Hemeoxygenase-1 (HO-1) proteins in a rat model of glomerulonephritis induced by antibody raised in rabbits against rat glomerular basement membrane (anti-GBM).

Methods. Rats were injected either with non-immune serum (control), or anti-GBM serum. In a group of rats N^6-(1-iminoethyl)-L-lysine (L-NIL) was administered prior to injection of anti-GBM serum to inhibit iNOS activity. Urinary nitrite plus nitrate (NOx) excretion was assessed to determine the extent of iNOS inhibition by L-NIL. Urinary albumin excretion was assessed to determine extent of proteinuria. Urinary PGE_2 was assessed as a marker of COX activity. Glomeruli were harvested 24 h after injection of anti-GBM serum and ED1, COX-2, iNOS, eNOS and HO-1 expression was analysed by Western blot analysis.

Results. iNOS activity in glomeruli was effectively reduced in L-NIL-treated nephritic animals. In these animals, there was exacerbation of proteinuria and reduction in urinary PGE_2 levels without changes in the extent of macrophage infiltration in glomeruli. In nephritic animals, there was an increase in glomerular protein levels of COX-2, HO-1 and iNOS, but not of eNOS. While L-NIL treatment reduced glomerular HO-1, levels of COX-2 and iNOS increased; but not that of eNOS.

Conclusions. The observations indicate that in glomerulonephritis iNOS-driven NO production acts as a negative feedback regulator of iNOS itself, suppresses COX-2 levels, and maintains HO-1 levels.

Keywords: anti-GBM nephritis; COX-2; HO-1; iNOS; L-NIL

Introduction

Inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2) and heme oxygenase-1 (HO-1) have been implicated in the pathophysiology of glomerulonephritis. Studies from this and other laboratories have demonstrated the induction of iNOS, HO-1 and COX-2 in the rat model of anti-glomerular basement membrane (GBM) antibody induced nephritis [1–3]. iNOS activation results in a sustained high output production of nitric oxide (NO), which can cause oxidative injury on its own or by interacting with superoxide anion (O_2^-), to form the relatively stable pro-oxidant peroxynitrite (ONOO) [4]. We previously demonstrated that inhibition of iNOS-driven NO production in anti-GBM nephritis worsens proteinuria and reduces synthesis of glomerular prostaglandins [1]. We also demonstrated that iNOS derived NO upregulates HO-1 expression in mesangial cells [5]. These observations, taken together, indicate that iNOS-driven NO production maintains and/or activates endogenous systems such as COX and HO-1 that can defend against renal inflammatory injury. The regulatory interactions between iNOS, COX and HO-1 in glomerular inflammation have not been characterized.

The present studies assessed whether inhibition of iNOS-derived NO generation modulates expression of COX-2 and HO-1 in anti-GBM antibody mediated nephritis.

Correspondence and offprint requests to: Prasun K. Datta, PhD Laboratory of AIDS Pathogenesis & Molecular Therapeutics, Center for Neurovirology, Temple University, 1900 N. 12th Street, Philadelphia, PA 19122, USA.
Email: dattapk@temple.edu

Materials and methods

Anti-GBM nephritis group

The studies were performed on male Lewis rats (175–200 g) obtained from Charles River Laboratories. The studies have been approved by the Institutional Use and Care of Animal Committee and conducted in accordance with standards of the NIH Guide to the Care and Use of Laboratory Animals, NIH Publication No. 85-23, 1985. Rats were immunized intraperitoneally with 1 mg of rabbit IgG (Pel-Freez Biologicals, Rogers, AR) emulsified in complete Freund's adjuvant (Sigma Chemical Co., St. Louis, MO). Seven days after this immunization, rats ($n=4$) were injected in the tail vein with two doses (0.3 ml/100 g body weight given 24 h apart) of rabbit immune serum raised against rat particulate GBM. Controls received two doses of non-immune rabbit serum ($n=4$). This protocol results in heavy proteinuria and accelerated onset of nephritis characterized by glomerular infiltration by macrophages and, at later stages, crescent formation and scarring.

iNOS inhibition group

The selective iNOS inhibitor N^6-(1-iminoethyl)-L-lysine (L-NIL) (Alexis Biochemicals, San Diego, CA) was used. This inhibitor has an IC_{50} of 3.3 μmol/l for macrophage iNOS compared to an IC_{50} of 92 μmol/l for rat brain constitutive NOS [6]. L-NIL (15 mg/kg of body weight) was injected in the tail vein of rats ($n=8$) 30 min prior to each injection of the anti-rat GBM immune serum to prevent the early iNOS activation known to occur in this model [1–3].

Estimation of urine protein (albumin), creatinine, Nitrite and PGE_2 excretion

Before sacrifice of animals, timed urine collections (24 h) were performed using metabolic cages placed on an automated refrigerated collection rack that freezes urine down to −20°C while it is being collected. This prevents bacterial growth and provides stability of urinary proteins and nitrite. Urinary albumin was measured using nephrat rat albumin ELISA kit (Exocell Inc. Philadelphia, PA). Urinary creatinine concentrations were determined using a creatinine assay kit (Sigma Chemical Co., St. Louis, MO). To measure urine NOx (nitrate and nitrite), urine samples were diluted in water and NO_3 was converted to NO_2 using vanadium chloride. NO_2^- was detected in samples using the Griess reagent (Promega, Madison, WI). Results were compared against a standard curve of $NaNO_3$ treated in an identical manner as the samples. Results were expressed as ratios of urine albumin to urine creatinine and of urine NOx to urine creatinine. Urinary PGE_2 levels were determined using a Prostaglandin E_2 EIA kit (Cayman Chemical Co. Ann Arbor, MI). Results are expressed as ratios of urine PGE_2 to urine creatinine.

Preparation of glomerular protein lysates

Animals were nephrectomized 24 h after the second injection of anti-rat GBM serum or of non-immune rabbit serum (NIS). This time point was chosen on the basis of our previous observations that demonstrated a peak glomerular expression and enzyme activity of iNOS as well as a marked increase in glomerular expression of HO-1 [3] and activity of COX [1]. Glomeruli were isolated by differential sieving of minced cortex. This procedure typically yields glomerular preparations of 95–98% purity. The isolated glomeruli were resuspended in lysis buffer (50 mM HEPES, pH 7.5, 150 mM NaCl, 1.5 mM $MgCl_2$, 1 mM ethyleneglycol bis (β-aminoethyl ether)-*N,N′*-tetra-acetic acid, 10% glycerol, 1% Triton X-100, 200 μM sodium orthovanadate) in the presence of the protease inhibitors aprotinin and leupeptin (1 μg/ml) and 1 mM phenylmethylsulfonyl fluoride. Lysates were centrifuged at 14 000 rpm for 30 min and aliquots were stored frozen at −70°C. Protein concentrations were estimated using the Bio-Rad protein assay reagent (Bio-Rad Laboratories, Hercules, CA).

Western blot analyses of ED1, iNOS, HO-1, COX-2 and eNOS protein levels in glomeruli

Glomerular protein lysates (50 μg) were mixed with sodium dodecyl sulfate (SDS) sample buffer (62.5 mM Tris-HCl, pH 7.4, 2% SDS, 10% glycerol, 2.5% β-mercaptoethanol and dye), boiled for 5 min, and separated by SDS-polyacrylamide gel electrophoresis using 4–20% Tris-glycine gel (Bio-Rad Laboratories, Hercules, CA). The separated proteins were transferred electrophoretically to Hybond-PVDF membranes (Amersham, Arlington Heights, IL) at 25 V at 4°C as described previously [5]. The membranes were blocked for 1 h in TBS-T (10 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.05% Tween 20) containing 5% non-fat milk. Membranes were then incubated overnight at 4°C with monoclonal antibody against the rat monocyte/macrophage marker ED1 (1:1000 dilution, Serotec, Raleigh, NC), or rabbit polyclonal antibody against rat HO-1 (1:1000 dilution, StressGen Biotechnologies, Vancouver, British Columbia, Canada), or polyclonal antibody against iNOS or eNOS (1:1000 dilution, Upstate Biotechnology, Charlottesville, VA), or monoclonal antibody against β-actin (1:5000 dilution, Sigma Chemical Co., St. Louis, MO). The membranes were subsequently washed thoroughly in TBS-T and incubated with anti-mouse or anti-rabbit horseradish peroxidase-conjugated antibody (1:7500 dilution) for 2 h. Blots were developed with ECL reagent from Amersham Biotech (Piscataway, NJ), followed by detection, capture and quantification of signal using a Kodak Image Station 400 CF (NEN, Boston, MA).

Statistical analysis

Data are expressed as mean ± SEM. Comparisons used *t*-test statistics for unpaired observations. $P<0.05$ was considered significant.

Results

Inhibition of iNOS activity exacerbates proteinuria

The efficacy of iNOS inhibition by L-NIL treatment was assessed by estimating the ratio of urinary NOx (U_{Nox})/urinary creatinine (Uc). In L-NIL+anti-GBM-treated animals there was a significant reduction (2.7-fold) in urinary NOx excretion ($p<0.05$). Values (U_{NOx}/Uc) were: 356.62 ± 6 in anti-GBM-treated

Table 1. Renal excretion of nitrate and nitrite (U_{NOx}), albumin (U_{alb}) and prostaglandin E_2 (U_{PG}) in animals treated with anti-GBM serum and L-NIL+anti-GBM serum

	Anti-GBM-treated	L-NIL+Anti-GBM-treated
U_{NOx}/U_c (μM/mg)	356.62 ± 6	131.12 ± 11[a]
U_{alb}/U_c (mg/mg)	0.51 ± 0.12	2.04 ± 0.4[a]
U_{PG}/U_c (pg/mg)	1127.5 ± 98.0	246.6 ± 29.1[a]

Data are expressed as mean ± SEM. U_{NOx}, urinary nitrate+nitrite; U_c, urinary creatinine; U_{alb}, urinary albumin; U_{PG}, urinary PGE_2. [a]$P < 0.05$ anti-GBM serum plus L-NIL *vs* Anti-GBM serum, $n = 4$–8 animals/group.

animals compared to 131.12 ± 11 in L-NIL + anti-GBM-treated animals. In L-NIL + anti-GBM-treated animals, urinary albumin to creatinine (Ualb/Uc) ratios determined 24 h following onset of nephritis increased by 4-fold ($P < 0.05$). Values (Ualb/Uc; mg/mg) were: 0.51 ± 0.12 in anti-GBM-treated animals, and 2.04 ± 0.4 in L-NIL + anti-GBM-treated animals. In L-NIL + anti-GBM-treated animals, urinary PGE_2 to creatinine (U_{PG}/Uc) ratios 24 h following onset of nephritis decreased by 4-fold ($P < 0.05$). Values (U_{PG}/Uc; pg/mg) were 1127.5 ± 98.0 in anti-GBM-treated animals compared to 246.6 ± 29.1 in L-NIL + anti-GBM-treated animals. The results are summarized in Table 1.

Inhibition of iNOS activity does not alter infiltration of glomeruli by activated macrophages

The anti-GBM antibody model of injury used in our studies is characterized by prominent macrophage infiltration in glomeruli [3]. We therefore assessed whether L-NIL treatment increased macrophage infiltration thereby accounting for the exacerbation of proteinuria. To assess the extent of macrophage infiltration in glomeruli, changes in levels of the rat monocyte/macrophage marker, ED1 were determined. ED1 levels were assessed using Western blot analysis in glomerular protein lysates obtained from NIS-treated, anti-GBM-treated, and L-NIL+anti-GBM-treated animals. The blot in Figure 1A (upper panel) demonstrates that there was no change in the levels of ED1 in anti-GBM-treated animals (lanes 3 and 4) compared to L-NIL+anti-GBM-treated animals (lanes 5, 6 and 7). ED1 was barely detectable in glomerular lysates from non-nephritic animals (lanes 1 and 2). Figure 1A (lower panel) shows the levels of β-actin in the same glomerular lysates. No alteration in levels of β-actin was observed. A densitometric analysis of ED1 factored by β-actin levels is shown in Figure 1B.

Inhibition of iNOS activity increases glomerular COX-2 expression

Earlier studies from this laboratory [1] demonstrated that in anti-GBM nephritis glomerular cyclooxygenase activity is iNOS dependent. In these studies it was demonstrated that inhibition of iNOS-derived NO by

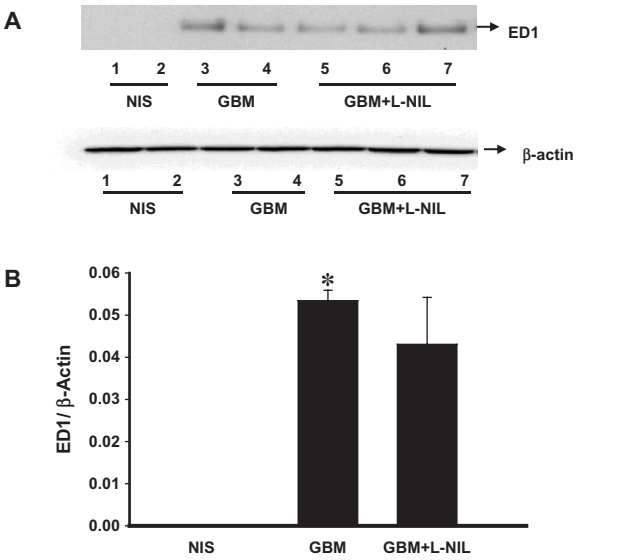

Fig. 1. Changes in levels of the macrophage marker ED1 and β-actin protein in glomeruli of rats with anti-GBM nephritis. (**A**) Representative Western blot of ED1 protein (upper panel) and β-actin (lower panel) in glomeruli obtained from control, NIS-treated rats (lanes 1 and 2), anti-GBM-treated rats (lanes 3 and 4), and L-NIL+anti-GBM-treated rats (lanes 5–7). (**B**) Densitometric analysis of ED1/β-actin protein ratios. There is increased ED1/β-actin protein ratio in anti-GBM-treated rats *vs* NIS-treated rats (*$p < 0.05$). No significant difference is observed in ED1/β-actin protein ratios in anti-GBM-treated rats *vs* L-NIL+anti-GBM-treated rats. Values are means ± SEM.

L-NIL reduced glomerular synthesis of the arachidonic acid cyclooxygenation products PGE_2 and PGI_2 without an effect on thromboxane A_2. These observations raise the question of whether inhibition of iNOS activity downregulates glomerular COX-2 expression. The Western blot shown in Figure 2A demonstrates that glomerular levels of COX–2 protein were increased in anti-GBM-treated animals (lanes 3 and 4) compared to NIS-treated controls (lanes 1 and 2). Glomerular levels of COX–2 in L-NIL + anti-GBM-treated animals (lanes 5, 6 and 7) were further increased compared to levels in animals with anti-GBM nephritis not treated with this iNOS inhibitor (lanes 3 and 4). A densitometric analysis of COX-2 factored by β-actin levels is shown in Figure 2B.

Inhibition of iNOS activity increases glomerular iNOS without an effect on eNOS expression

To determine whether the decrease in urinary nitrite was associated with changes in glomerular iNOS or eNOS protein levels in animals treated with L-NIL, we performed Western blot analysis of iNOS and eNOS proteins in glomerular lysates. The representative blot shown in Figure 3A, demonstrates that there was no change in glomerular eNOS levels in anti-GBM-treated animals (lanes 3 and 4) compared to NIS-treated controls (lanes 1 and 2). Moreover, in L-NIL+anti-GBM-treated animals (lanes 5, 6 and 7), glomerular eNOS levels were not different from those in

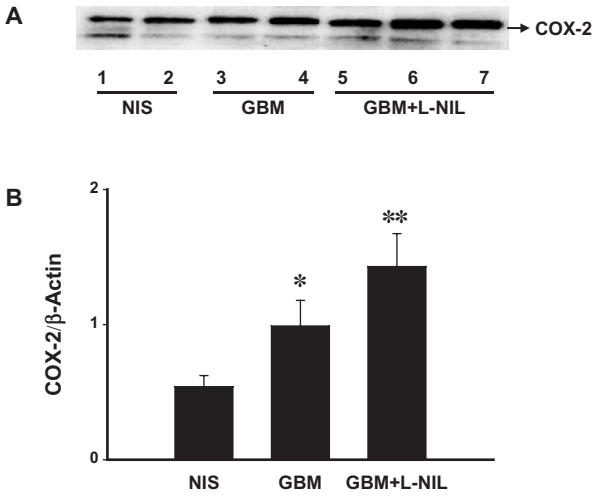

Fig. 2. Changes in COX-2 protein levels in glomeruli of rats with anti-GBM nephritis. (**A**) Representative Western blot of COX-2 protein in glomeruli obtained from control, NIS-treated rats (lanes 1 and 2), anti-GBM-treated rats (lanes 3 and 4), and L-NIL+ anti-GBM-treated rats (lanes 5–7). (**B**) Densitometric analysis of COX-2/β-actin protein ratios. There is an increased COX-2/β-actin protein ratio in anti-GBM-treated rats *vs* NIS-treated rats ($*P < 0.05$). A significant increase is also observed in COX-2/β-actin protein ratios in anti-GBM-treated rats *vs* L-NIL+anti-GBM-treated rats ($**P < 0.05$). Values are means ± SEM.

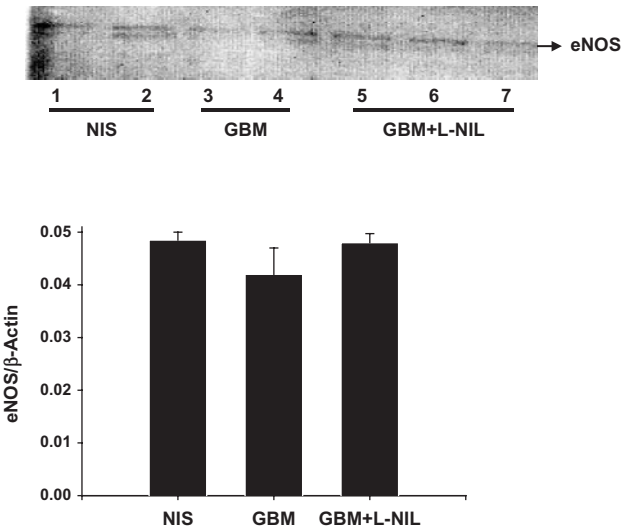

Fig. 3. Changes in eNOS protein levels in glomeruli of rats with anti-GBM nephritis. (**A**) Representative Western blot of eNOS protein in glomeruli obtained from control, NIS-treated rats (lanes 1 and 2), anti-GBM-treated rats (lanes 3 and 4), and L-NIL+anti-GBM-treated rats (lanes 5–7). (**B**) Densitometric analysis of iNOS/β-actin protein ratios. There is no change in the eNOS/β-actin protein ratios in anti-GBM-treated rats compared to L-NIL+anti-GBM-treated rats or to NIS-treated rats. Values are means ± SEM.

anti-GBM-treated animals . A densitometric analysis of eNOS factored by β-actin levels is shown in Figure 3B. In contrast, as shown in Figure 4A, while glomerular iNOS was undetectable in glomeruli of NIS-treated (lanes 1 and 2), it was clearly detectable in anti-GBM-treated animals (lanes 3 and 4). There was a marked

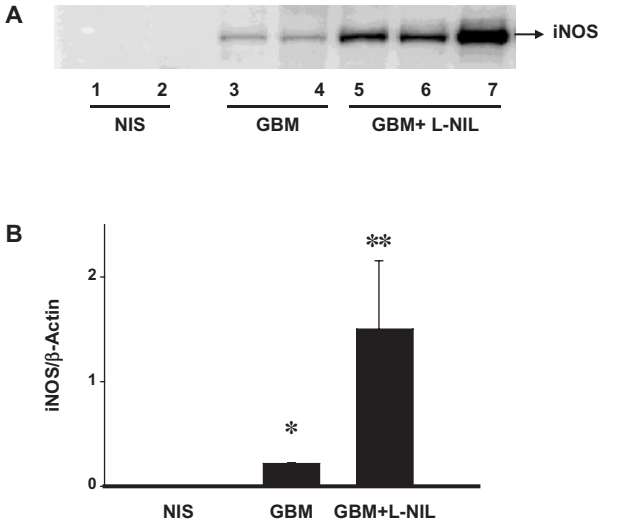

Fig. 4. Changes in iNOS protein levels in glomeruli of rats with anti-GBM nephritis. **(A).** Representative Western blot of iNOS protein in glomeruli obtained from control, NIS-treated rats (lanes 1 and 2), anti-GBM-treated rats (lanes 3 and 4), and L-NIL+anti-GBM-treated rats (lanes 5–7). **(B).** Densitometric analysis of iNOS/β-actin protein ratios. There is a significant increase in iNOS/β-actin protein ratio in anti-GBM-treated rats *vs* NIS-treated rats ($*P < 0.05$). A significant increase is also observed in L-NIL+anti-GBM-treated rats compared to anti-GBM-treated rats ($**P < 0.05$). Values are means ± SEM.

increase in glomerular iNOS levels in L-NIL+anti-GBM-treated animals (lanes 5, 6, 7). A densitometric analysis of iNOS factored by β-actin levels is shown in Figure 4B.

Inhibition of iNOS activity reduces glomerular HO-1 expression

HO-1 expression is modulated by iNOS-derived NO both *in vitro* and *in vivo* [5,7]. As shown in the Western blot in Figure 5A, HO-1 was undetectable in glomerular lysates of NIS-treated animals (lanes 1 and 2), while there was a marked increase in HO-1 levels in anti-GBM-treated animals (lanes 3 and 4). In L-NIL + anti-GBM-treated animals, glomerular HO-1 levels decreased (lanes 5 and 6). A densitometric analysis of changes in glomerular HO-1 normalized to β-actin levels is shown in Figure 5B.

Discussion

In this study, we investigated the effects of selective iNOS inhibition on glomerular COX-2, HO-1, iNOS and eNOS expression. Our observations indicate that iNOS-driven NO production acts as negative feedback regulator of iNOS itself, suppresses COX-2 protein levels and maintains HO-1 levels. To elucidate the role of NO in glomerulonephritis, earlier studies utilized non-selective inhibitors of NOS such as N^G-monomethyl-L-arginine (L-NMMA) or N^G-nitro-L-arginine methyl

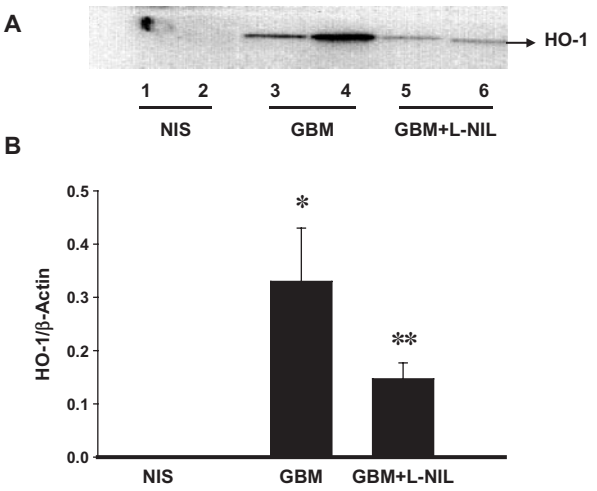

Fig. 5. Changes in HO-1 protein levels in glomeruli of rats with anti-GBM nephritis. **(A).** Representative Western blot of HO-1 protein in glomeruli obtained from control, NIS-treated rats (lanes 1 and 2), anti-GBM-treated rats (lanes 3 and 4), and L-NIL+anti-GBM- treated rats (lane 5–6). **(B).** Densitometric analysis of HO-1/β-actin protein ratios. There is an increased HO-1/β-actin protein ratio in anti-GBM-treated rats *vs* NIS-treated rats ($*P < 0.001$). A significant decrease is observed in HO-1/β-actin protein ratios in anti-GBM-treated rats *vs* L-NIL+anti-GBM-treated rats compared to anti-GBM-treated rats ($**P < 0.05$). Values are means ± SEM.

ester. These NOS inhibitors ameliorated the severity (histopathology, proteinuria) of anti-Thy1.1 antibody-mediated mesangial nephritis but worsened that of thrombotic microangiopathy and of anti-GBM antibody induced nephritis (see review [8]). Since, glomerular iNOS is specifically and prominently upregulated in anti-GBM nephritis, we used the selective iNOS inhibitor L-NIL. L-NIL, an acetamide-containing analog of arginine, is an iNOS dimerization inhibitor. It displays a 28-fold higher selectivity for iNOS compared to nNOS and is 49-fold more selective for iNOS *vs* eNOS [6]. L-NIL has no other pharmacologic effects on enzymes known to be activated in nephritic glomeruli i.e. the arachidonic acid cyclooxygenase or lipoxygenase, and has superior bioavailability *in vivo* in comparison to L-NMMA [9]. It has been extensively used to study the effects of iNOS inhibition [8].

The present study confirms observations that inhibition of iNOS activity worsens proteinuria in renal immune injury (glomerulonephritis and interstitial [1]. Since iNOS inhibition was initiated before induction of anti-GBM nephritis, one potential concern is that L-NIL could modify the extent of glomerular infiltration by macrophages and the degree of macrophage-mediated injury exacerbating proteinuria. Our observation that there was no alteration in the protein levels of monocyte/macrophage marker ED1 (Figure 1) argues against this possibility.

In glomerulonephritis, iNOS may become a major catalyst of NO production. For example, in a model of experimental crescentic glomerulonephritis glomerular levels of constitutive forms of NOS such as eNOS decrease in the course of the disease while those of iNOS are sustained [10]. Furthermore, in IgA nephropathy and lupus nephritis, eNOS was shown to correlate negatively with the degree of glomerular injury [11]. In the present studies, iNOS levels increased within 24 h of onset of anti-GBM antibody-induced injury in contrast to those of eNOS which did not change (Figures 3 and 4). Therefore, the exacerbation of proteinuria in nephritic animals treated with the iNOS inhibitor, L-NIL, observed in the present studies and in previous reports raises the question of whether iNOS-driven NO production attenuates glomerular injury by maintaining endogenous renoprotective systems. Two such systems are the cyclooxygenase and the heme oxygenase enzymes.

In the nephritic glomerulus, there is increased synthesis of the vasodilatory and cytoprotective prostaglandins PGE_2 and PGI_2 as a result of increased cyclooxygenase expression and activity. In various forms of renal injury including glomerulonephritis, these prostaglandins become critically important in preserving renal function and in limiting the extent of inflammation. Recent evidence indicates that in renal injury both the constitutive and the inducible forms of cyclooxygenase (referred to as COX-1 and COX-2, respectively) become important in preserving renal function as inhibition of either isoform can intensify existing haemodynamic compromise or the extent of underlying injury [12]. NO increases enzyme activity of COX-1 and COX-2 [13]. To this end, we previously demonstrated that, in the model of anti-GBM antibody induced nephritis, glomerular COX activity (assessed by measuring production of PGE_2) is iNOS-dependent [1]. Therefore, the decrease in urinary PGE_2 excretion in rats with anti-GBM nephritis treated with L-NIL in the present studies is most likely due to a reduction in COX-2 enzyme activity as COX-2 protein levels in glomeruli of nephritic animals treated with this iNOS inhibitor actually increased (Figure 2).

HO catalyses the NADPH, O_2 and cytochrome P450-dependent oxidation of heme to carbon monoxide (CO), iron and biliverdin, which is reduced to bilirubin by biliverdin reductase [14]. Two catalytically active isoenzymes of HO have been characterized, HO-1 and HO-2. HO-1 is induced by a host of stimuli that have in common the ability to produce oxidative stress. In contrast, HO-2 is a constitutive form and, to date, only the adrenal glucocorticoids have been identified as the inducers of its gene [14]. While HO-2 is constitutively expressed in the rat kidney and its activity may modulate physiological functions under basal conditions, expression of HO-1 is relatively low and its contribution to renal HO activity becomes apparent only under pathophysiological conditions causing its induction [15]. Most evidence indicates that the overall effect of HO-1 induction is anti-inflammatory [16]. Mechanisms by which induction of HO-1 attenuates inflammation include: (a) generation of biliverdin and bilirubin which can scavenge reactive oxygen species and inhibit lipid peroxidation, (b) release of CO, which can suppress production of cytokines such as platelet-derived growth factor and

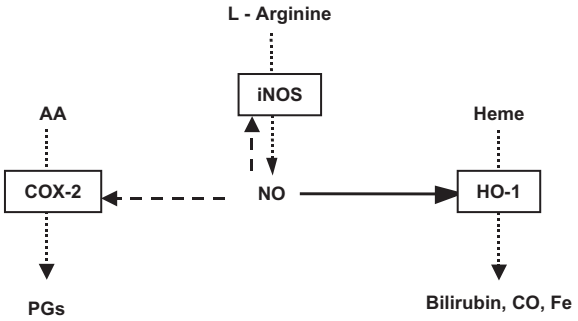

Fig. 6. Scheme of proposed regulatory effect of iNOS catalysed NO production on iNOS, COX-2 and HO-1 expression in anti-GBM nephritis. Immune injury results in up-regulation of glomerular COX-2, iNOS and HO-1 expression and metabolism of arachidonic acid (AA), L-arginine and heme to generate respective end products. iNOS catalysed NO production is a negative regulator (---►) of iNOS and COX-2 expression and a positive regulator (——►) of HO-1 expression.

endothelin, (c) upregulation of ferritin which sequesters iron thus preventing iron-dependent oxidative stress and (d) blockade of leukocyte activation by complement and other factors.

Our observations indicate that iNOS-driven NO production maintains glomerular HO-1 protein levels (Figure 5). This can be explained on the basis of observations demonstrating that NO derived either from NO donors or from cytokine-induced iNOS increases HO-1 mRNA and protein levels in cultured mesangial cells [5]. The mechanism by which NO upregulates HO-1 is being investigated.

The marked increase in glomerular iNOS protein levels in nephritic animals treated with the iNOS inhibitor, L-NIL, points to autoregulation of iNOS by NO; specifically, iNOS-driven NO production downregulates iNOS. We have previously demonstrated this phenomenon in mesangial cells [5]. Regulation of iNOS expression is governed predominantly by the transcription factor NF-κB [17], whose induction can be under a negative feedback regulation by NO, either directly or via up-regulation/stabilization of NF-κB inhibitory protein IκBα [18]

The exacerbation in proteinuria in nephritic animals treated with the iNOS inhibitor, L-NIL, could also be due to a reduced generation of NO allowing accumulation and/or an unopposed effect of superoxide. In the macrophage-dependent model of anti-GBM nephritis employed in the present studies, there is an increased glomerular production of both NO and superoxide (O_2^-), the infiltrating macrophages being a major source of these radicals. NO reacts with O_2^- at diffusion-controlled rates, the reaction constant being $4.3–6.7 \times 10^9\,m^{-1}\,s^{-1}$ [19]. This reaction constitutes an important 'sink' for O_2^- because it is twice as fast as the maximum velocity of superoxide dismutase [19]. These observations have led investigators to propose that NO could attenuate O_2^- mediated oxidative injury by scavenging superoxide. In the NO/O_2^- reaction, fluxes of NO determine the rate constant while fluxes of O_2^- determine the amount of the reaction product, peroxynitrite ($ONOO^-$) [20]. Therefore, in nephritic rats treated with the iNOS inhibitor, L-NIL, fluxes of O_2^- would predominate. Thus, the exacerbation of proteinuria observed in L-NIL-treated nephritic animals could be mediated by an excess of O_2^-.

In summary, our observations identify regulatory interactions between iNOS, COX-2 and HO-1 in anti-GBM antibody-induced nephritis. iNOS-driven NO production autoregulates iNOS expression, maintains HO-1 expression and has a suppressive effect on COX-2 expression [Figure 6]. The exacerbation of proteinuria following iNOS inhibition could be due to loss of renoprotective effects of NO including maintenance of prostaglandin synthesis and of HO-1 levels.

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Conflict of interest statement. None declared.

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