Expression of endothelial and inducible nitric oxide synthase in human glomerulonephritis

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Expression of endothelial and inducible nitric oxide synthase in human glomerulonephritis. The presence of nitric oxide (NO) in the kidney has been implicated in the pathogenesis of human glomerulonephritis. However, the exact type of glomerular cells that express NO synthase (NOS) and the NO isoform involved in the local production of NO has not been identified in the human diseased kidney. We examined the expression of three isoforms of NOS, inducible NOS (iNOS), endothelial NOS (eNOS), and brain NOS (bNOS) in the renal tissue of patients with IgA nephropathy (IgAN, N = 10), lupus nephritis (LN, N = 5), membranous nephropathy (MN, N = 5) and minimal change nephrotic syndrome (MCNS, N = 5). Sections were immunostained and the correlation between the expression of each NOS and the degree of glomerular injury in that section was also examined. Normal portions of surgically resected kidneys served as controls. eNOS was present in glomerular endothelial cells and endothelium of cortical vessels in the control and diseased kidneys. iNOS was localized in mesangial cells, glomerular epithelial cells and infiltrating cells in the diseased glomeruli, whereas immunostaining for iNOS was hardly detected in control kidneys. In addition, the expression pattern of eNOS in each glomerulus was the reverse of that of iNOS. In IgAN and LN, the extent of staining for eNOS correlated negatively with the degree of glomerular injury, while the extent of staining for iNOS correlated positively with the degree of glomerular injury in the same tissues. bNOS was not detected in normal or nephritic glomeruli. Our results indicate the presence of a NO pathway in human glomeruli.

Nitric oxide (NO) has broad biological properties as an intercellular messenger molecule acting as a vasodilator and neurotransmitter, and is also involved in inflammation, tissue injury and cell defense [1, 2]. The synthesis of NO is catalyzed by nitric oxide synthase (NOS) [1] and three isoforms of NOS, endothelial NOS (eNOS), brain NOS (bNOS) and inducible NOS (iNOS), have been identified [3, 4]. The constitutive forms of NOS, such as bNOS and eNOS, are present in endothelial cells and neurons, while iNOS is present in different types of cells, including macrophages, vascular endothelial cells, vascular smooth muscle cells, epithelial cells, neutrophils and fibroblasts [1]. eNOS and bNOS generate small quantities of NO, which is important in signal transduction including the regulation of vascular tone [2, 5], whereas iNOS, which can be induced by cytokines and endotoxin, produce large quantities of NO with cytotoxic/cytostatic effects [2, 6].

With regard to the role of NO in the kidney, previous reviews [2, 7] showed that in the physiological state, NO is mainly derived from constitutive NOS, such as eNOS and bNOS, and that NO contributes to the regulation of the glomerular microcirculation and the inhibition of platelet aggregation and adhesion. In contrast, in pathological conditions NO is generated by isolated nephritic glomeruli, as shown in four different rat models of immune complex-mediated glomerulonephritis [8–11]. However, the role of NO in renal diseases is still controversial. Inhibition of NO production in vivo in experimental glomerulonephritis produces inconsistent results. For example, in anti-Thy-1 model and MRL-lpr/lpr mice, inhibition of NO production by administration of the NOS inhibitor NG monomethyl-L-arginine reduced glomerular injury [12], while the same treatment worsened the degree of proteinuria in nephrotoxic nephritis [13].

Several studies have examined the constitutive NOS expression in kidney [14–16]. Lamas et al [14] showed that eNOS mRNA is synthesized by cultured glomerular endothelial cells. More recently, Goto et al [15] showed that glomerular expression of eNOS mRNA in anti-Thy-1 glomerulonephritis increased biphasically and that bNOS mRNA was not detected in the same model; however, the localization of eNOS mRNA was not determined in that study. In normal human kidney, using immunohistochemistry and in situ hybridization, Bachmann, Bosse and Mundel [16] identified the expression of eNOS in glomerular endothelial cells and afferent/afferent arterioles, while bNOS was expressed in the efferent arterioles, macula densa and Bowman’s capsule. However, the expression of eNOS and bNOS has not been reported in human diseased kidney.

With regard to iNOS expression, Mohaupt et al [17] reported that iNOS mRNA was produced by cultured mesangial cells stimulated by tumor necrosis factor-α (TNF-α) and interferon-γ.
(IFN-γ), and that microdissected glomeruli of normal and lipopolysaccharide-treated rats also synthesized the mRNA. Although it was suggested that iNOS is synthesized in mesangial cells in vivo, two experimental models of glomerulonephritis showed that infiltrating leukocytes, not mesangial cells, expressed iNOS [15, 18]. Using immunohistochemistry, Jansen et al [18] showed that the majority of iNOS expressing cells in glomeruli of rats with immune complex glomerulonephritis were macrophages. In contrast, in an anti-Thy1 model, most of the cells positive for iNOS in the glomeruli possessed a marker for polymorphonuclear leukocytes [15].

These findings suggest the involvement of NO in the development and progression of human glomerulonephritis. However, whether glomerular resident cells express the NOS isoform or which isoform of NOS is involved in the local production of NO have not been clarified in human diseased kidney. In the present study, we examined the expression of the above three NOS isoforms in biopsy specimens obtained from patients with various forms of glomerulonephritis using immunohistochemistry, and also analyzed the correlation between the expression of each NOS and the degree of glomerular injury.

METHODS

Kidney tissues

We examined a total of 25 renal biopsy specimens from 25 patients with renal diseases, including 15 patients with proliferative [10 IgA nephropathy (IgAN) and 5 lupus nephritis-type IV (LN)] and 10 nonproliferative [5 membranous nephropathy (MN) and 5 minimal change nephrotic syndrome (MCNS)] glomerulonephritis. Five tissue specimens from uninvolved areas of adenocarcinomatous kidneys served as normal controls. All patients gave informed consent to renal biopsy and the present study. The diagnosis was established based on clinical, immunofluorescence, light and electron microscopic findings. The clinical data are summarized in Table 1. The following laboratory parameters were determined: proteinuria, hematuria and serum creatinine. The severity of hematuria was graded as: grade 0, representing 0 to 5 erythrocytes per high-power field; grade 1, 5 to 30 erythrocytes per high-power field; grade 2, >30 erythrocytes per high-power field; and grade 3, representing macroscopic hematuria. All patients were normotensive and none received steroids or immunosuppressive drugs prior to renal biopsy. Immunofluorescence, light and electron microscopic examination were performed routinely to establish the histological diagnosis. For immunohistochemistry, a portion of the cortical fragment was embedded in O.C.T. compound (Miles Inc., Elkhart, IN, USA) and quickly frozen in ethanol bath with dry ice and stored –80°C until use.

<table>
<thead>
<tr>
<th>Case</th>
<th>N</th>
<th>Age (years)</th>
<th>Proteinuria (g/day)</th>
<th>Hematuria index</th>
<th>Serum creatinine (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgAN</td>
<td>10</td>
<td>38 ± 10</td>
<td>1.0 ± 0.9</td>
<td>1.7 ± 1.2</td>
<td>1.1 ± 0.3</td>
</tr>
<tr>
<td>LN</td>
<td>5</td>
<td>30 ± 10</td>
<td>2.5 ± 1.3</td>
<td>2.0 ± 1.1</td>
<td>1.1 ± 0.3</td>
</tr>
<tr>
<td>MCNS</td>
<td>5</td>
<td>23 ± 6</td>
<td>7.2 ± 6.5</td>
<td>0.4 ± 1.0</td>
<td>0.8 ± 0.4</td>
</tr>
<tr>
<td>MN</td>
<td>5</td>
<td>45 ± 17</td>
<td>1.7 ± 1.0</td>
<td>0.7 ± 0.7</td>
<td>0.8 ± 0.1</td>
</tr>
</tbody>
</table>

Data are mean ± sd. Abbreviations are: IgAN, IgA nephropathy; LN, lupus nephritis-type IV; MCNS, minimal change nephrotic syndrome; MN, membranous nephropathy.

Indirect immunoperoxidase staining was performed using monoclonal antibody against eNOS, iNOS and bNOS (N30020, N32020 and N31020, respectively) purchased from Transduction Laboratories (Lexington, KY, USA). A monoclonal antibody against CD68 (M718; Dako, Glostrup, Denmark) was also used to identify any infiltrating monocytes. Using the avidin biotin complex method with kits from Vector Laboratories (PK-6102; Burlingame, CA, USA), staining for NOS and CD68 was performed as previously described [19]. Briefly, cryostat sections (4 µm) were fixed with 4% paraformaldehyde in phosphate buffered saline (PBS) and washed in PBS. The sections were incubated for 30 minutes with 3% H2O2 to eliminate endogenous peroxidase activity. After rinsing in PBS, the sections were treated with blocking serum and incubated with the primary antibody. In the next step, the sections were washed with PBS and incubated with the biotinlated secondary antibodies. After washing, the sections were incubated for 30 minutes with the Vectastain ABC reagent. Color was developed by reacting with 3,3′-diaminobenzidine/tetrahydrochloride and H2O2.

To determine the contribution of macrophages to the population of cells positive for iNOS in glomeruli, double immunolabeling for iNOS and CD68, a surface marker of macrophages, was performed according to the methods of Berkes et al [20]. After staining iNOS using the protocol described above, the tissue sections were washed in PBS and then treated with blocking serum and incubated with anti-CD68 antibody for 60 minutes at room temperature. Biotinylated secondary antibody and Vectastain ABC reagent applied sequentially as mentioned above, and second chemogen, TrueBlue Substrate (71-00-64; KPL, Gaithersburg, MD, USA) was applied, resulting in the staining of positive cells in blue color.

Histological examination and evaluation of the expression of NOS isoforms in each biopsy were performed by two observers independently without prior knowledge of the clinical and laboratory data. For semiquantitative histological grading of the glomeruli, after the color development of immunohistochemistry, the sections were stained by periodic acid-Schiff reaction (PAS), which allowed the identification of histological changes such as mesangial expansion and the exact location of those cells positive for NOS isoforms relative to the glomerular basement membrane and mesangial area. Since the degree of glomerular injury is heterogeneous among glomeruli during the progression of glomerulonephritis [21], it is possible that the level of expression of NOS isoforms may relate to the degree of injury in each glomerulus. Hence, we evaluated the degree of glomerular injury as well as expression of each NOS isoform in each glomerulus rather than averaging the injury in each section. Five to seven glomeruli, each with an equatorial plane cross-section, were analyzed per biopsy. The glomerular changes were graded into four classes ranging from 0 to 3+ (minimal, mild, moderate and severe) according to the proportion of the involved region in the sectioned areas of each glomerulus, based on the method described by Okada et al [22] with a minor modification. Grade 0 represented injury involving of less than 10% of the glomerulus, grade 1 between 10% and 30%, grade 2 between 30 and 60% and grade 3 represented involvement exceeding 60%.

After determining the degree of glomerular injury, in the same
Fig. 1. Immunohistochemistry for endothelial nitric oxide synthase (eNOS) in normal kidney (A) and in renal sections of patients with IgA nephropathy (IgAN; B-D), lupus nephritis (LN, E-F), minimal change nephrotic syndrome (MCNS; G), and membranous nephropathy (MN; H). To identify the precise localization of eNOS positive cells in the glomeruli, the renal sections are counterstained by PAS (×200). (A) In normal kidney, a number of glomerular endothelial cells are positively stained for eNOS (arrows). (B) Endothelial expression of eNOS in the glomerulus (arrows) and in peritubular capillaries (arrowheads) in renal tissue of IgAN with mild lesion. (C) Note absence of positive cells for eNOS in the area of mesangial expansion (the area surrounded by arrows) in glomeruli of a representative patient with IgAN. In contrast, staining for eNOS is still observed in mildly injured area (the area surrounded by arrowheads). (D) eNOS is hardly detected in the sclerotic glomeruli of patient with IgAN. (E) In the renal tissue of LN with moderate lesion, glomerular endothelial cells in the area of mesangial expansion are not stained for eNOS, whereas in mildly injured areas, cells positive for eNOS are observed. (F) Only a few cells positive for eNOS are observed in severely injured glomeruli of patients with LN. (G and H) The expression patterns of eNOS in the renal tissue of MCNS (G) and MN (H) are similar to that in control (A).
glomeruli, semiquantitation of the expression of each NOS isoform in kidney tissues from patients with various types of glomerulonephritis was performed according to the methods of Roy-Chaudhury et al [23]. Briefly, a scoring scale from 0 to 3 was used as follows: 0 = no specific staining, 0.5 = possibly positive, 1+ = weakly positive, 2+ = moderately positive, 3+ = strongly positive. Scoring was generally influenced by the extent rather than the intensity of staining.

Specificity of anti-inducible nitric oxide synthase antibody

To determine the specificity of anti-iNOS antibody, various control studies were performed. First, an absorption test using mouse iNOS (60862; Cayman Chemical Company, Ann Arbor, MI, USA) was performed. Briefly, 125 μl of PBS containing 40 μg/ml of monoclonal antibody was incubated with 125 μl of PBS containing 0 μg, 5 μg, 10 μg of mouse iNOS for one hour at 4°C and processed for indirect immunohistochemistry as described above. Second, irrelevant monoclonal antibodies with the same IgG subclass of the first antibodies, nonspecific mouse IgG1 (Dako X931) and IgG2 (Dako X0943), were used instead of the specific antibody for negative controls in immunohistochemistry. Third, omission of primary antibody in the peroxidase system served as additional control. Finally, the specificity of immunostaining for iNOS was also verified in a few samples using a different anti-iNOS antibody, a rabbit polyclonal antibody to iNOS (SA-200; BIOMOL, Plymouth Meeting, PA, USA).

Furthermore, in order to demonstrate synthesis of iNOS by glomerular cells, in situ hybridization for iNOS mRNA was performed using some renal sections and 25-mer digoxigenin-labeled oligonucleotide, which corresponded to the base number 3525-3549 of human iNOS cDNA [24], as described elsewhere [18]. The selected sequences showed no significant similarity with known sequences deposited in the gene data bank (GenBank Rel. 85, December 1996). In brief, a fresh frozen renal tissue, fixed with 4% paraformaldehyde, was deproteinated using HCl and protease K. After hybridization with the probe, immunohistochemistry was performed to visualize DIG-labeled probe using mouse monoclonal anti-DIG antibody (Boehringer 1333 062), followed...
Fig. 4. Immunohistochemistry for iNOS and CD68 on serial sections from a representative patient with IgAN (A and B; ×200), and double immunostaining method demonstrating iNOS positive cells (blown color) and CD68 positive cells (blue color) on the same section from a representative patient with LN (C and D; ×200). Although monocytes are occasionally observed in glomeruli (A, arrowheads), the staining pattern for iNOS (B, arrows) is different from that of CD68. (C) Glomerular cells negative for CD68 stained iNOS (arrows), iNOS positive cell (arrowhead) in the glomeruli also stained with anti-CD68 antibody. Macrophages stained blue but negative for iNOS were also observed (open arrows). (D) In some glomeruli, no macrophages were present, while immunoreactive iNOS is present in the glomeruli (arrowheads).

Table 2. Glomerular expression of eNOS and iNOS in normal and diseased kidney

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>eNOS score/glomerulus</th>
<th>iNOS score/glomerulus</th>
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<tbody>
<tr>
<td>IgAN</td>
<td></td>
<td></td>
</tr>
<tr>
<td>minimal-mild lesion</td>
<td>1.59 ± 0.44</td>
<td>0.96 ± 0.39</td>
</tr>
<tr>
<td>moderate-severe lesion</td>
<td>0.42 ± 0.33</td>
<td>1.23 ± 0.57</td>
</tr>
<tr>
<td>LN</td>
<td></td>
<td></td>
</tr>
<tr>
<td>minimal-mild lesion</td>
<td>2.24 ± 0.56</td>
<td>0.90 ± 0.62</td>
</tr>
<tr>
<td>moderate-severe lesion</td>
<td>0.47 ± 0.33</td>
<td>1.56 ± 0.73</td>
</tr>
<tr>
<td>MCNS</td>
<td>1.81 ± 0.61</td>
<td>0.10 ± 0.20</td>
</tr>
<tr>
<td>MN</td>
<td>1.57 ± 0.50</td>
<td>0.08 ± 0.18</td>
</tr>
<tr>
<td>Control kidney</td>
<td>1.13 ± 0.34</td>
<td>0.07 ± 0.18</td>
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</table>

Data are means ± SD.

a The glomerular injury was graded from 0 to 3+ according to the proportion of involved regions in sectioned surface areas of each glomerulus according to the method described by Okada et al [22] with a minor modification. Grade 0 represented involvement of less than 10%, grade 1 between 10% and 30%, grade 2 between 30% and 60%, and grade 3 represented involvement exceeding 60%.

b In each glomerulus, the degree of eNOS and iNOS staining were scored according to the methods of Roy-Chaudhury et al [23]. Five to seven glomeruli in each section were analyzed. Differences between groups were tested for statistical significance using one-way analysis of variance (ANOVA) with Scheffe’s F test.

by horseradish peroxidase-conjugated antibodies. Color was developed by reacting with H2O2 and 3,3'-diaminobenzidine/tetrahydrochloride. To evaluate the specificity of the technique, various control experiments including pre-treatment of RNase, a study with a sense probe and a competitive study were performed as described previously [25, 26].

Statistical analysis

Data were expressed as mean ± SD. Differences between different groups were tested for statistical significance using one-way analysis of variance (ANOVA) with Scheffe’s F test. Correlation between the degree of glomerular injury and expression of each NOS isoform per glomerular cross-section was analyzed using the Spearman’s rank correlation test as described previously [27].

RESULTS

The results of staining by immunohistochemistry for eNOS are shown in Figure 1 and summarized in Table 2. In the control tissue, eNOS was present in glomerular endothelial cells (Fig. 1A), endothelial cells of afferent and efferent arterioles, and cortical vessels. The expression pattern of eNOS in the renal tissue of IgAN with mild lesion (Fig. 1B), MCNS (Fig. 1G) and MN (Fig. 1H) was similar to the control (Fig. 1A). The degree of the expression of eNOS in IgAN with minimal to mild lesion, LN with minimal to mild lesion, MCNS and MN was higher than control (Table 2; P = 0.157, < 0.01, < 0.01, = 0.142, respectively). In contrast, in glomeruli with moderate lesion in IgAN (Fig. 1C) and LN (Fig. 1E), eNOS expression was low in glomerular endothelial cells within the area of mesangial expansion, while staining of eNOS was still observed in areas with mild injury. In the sclerotic glomeruli of patients with IgAN and LN, eNOS was hardly detected (Fig. 1D, F). In IgAN and LN, the extent of staining for eNOS correlated significantly and negatively with the degree of glomerular injury (Table 3).

The results of immunohistochemical staining for iNOS are shown in Figure 2 and Table 2. While iNOS expression was hardly detected in glomeruli of MCNS, MN (Fig. 2G) and control tissue (Fig. 2A), cells positive for iNOS were clearly detected in the glomeruli of IgAN (Fig. 2B, C) and LN (Fig. 2D-F). In IgAN and LN, iNOS was localized in the mesangial area (Fig. 2 C, E, F) and glomerular epithelial cells (Fig. 2 E, F). We also demonstrated the synthesis of iNOS mRNA in glomerular cells using in situ hybridization. As shown in Figure 3, iNOS mRNA signals were detected in glomeruli of IgAN and LN. The degree of iNOS expression in the glomeruli of IgAN and LN was significantly higher than that in MCNS, MN and control kidney (Table 2). In IgAN and LN, the extent of staining for iNOS correlated positively with the degree of glomerular injury (Table 3).

The results of immunostaining for iNOS and CD68 in the serial sections are shown in Figure 4 A and B. Although monocytes were occasionally observed in the glomeruli, the staining pattern for...
iNOS was different from that for CD68. Double immunostaining for iNOS and CD68 showed that glomerular cells negative for CD68 were stained iNOS (Fig. 4C). In some glomeruli without macrophage infiltration, immunoreactive iNOS was still detected in the glomeruli (Fig. 4D). These results indicated that most of iNOS-positive cells were glomerular resident cells and that a small population of iNOS-positive cells was macrophages.

Comparison of the staining pattern of eNOS and iNOS on serial sections showed that the staining pattern of eNOS was the reverse of that of iNOS. The expression of iNOS was observed in the areas where eNOS was not expressed (Fig. 5) and vice versa. Expression of bNOS was not detected in normal and nephritic glomeruli.

The specificity of the anti-iNOS antibody was confirmed in several experiments. First, preincubation of the antibody with mouse iNOS abolished the reactivity of monoclonal antibody (data not shown). Second, irrelevant monoclonal antibody of the same subclass, nonspecific mouse IgG1 and IgG2 instead of the primary antiserum, used as negative control, yielded no staining (Fig. 2H). In addition, negative control with omission of the primary antibody was also consistently negative (data not shown). Moreover, samples immunostained with the polyclonal antibody showed a staining pattern similar to that of the monoclonal anti-iNOS antibody (data not shown).

None of the clinical parameters investigated in the present study, that is, age, proteinuria, hematuria index and the level of serum creatinine, correlated with the expression of eNOS and iNOS.

**DISCUSSION**

The major finding of the present study was the detection of specific immunostaining for eNOS and iNOS in the glomeruli of human kidney. Cells positive for eNOS included glomerular endothelial cells and the endothelium of afferent/effluent arterioles and cortical vessels in normal and diseased glomeruli. iNOS was expressed in mesangial cells, glomerular epithelial cells and infiltrating cells in IgAN and LN. Interestingly, at each glomerulus, a reciprocal expression pattern of eNOS and iNOS was observed. Furthermore, in IgAN and LN, the expression of eNOS correlated negatively with the degree of glomerular injury, whereas the expression of iNOS correlated positively with the degree of glomerular injury. To our knowledge, this is the first presentation of the cellular distribution of eNOS and iNOS in glomeruli of human renal biopsy specimens of patients with various types of glomerulonephritis.

Our results of eNOS expression in normal kidney are in agreement with those described in a previous immunohistochemical study [16], and also established the expression of eNOS in human nephritic glomeruli. Our findings are also in agreement with the results of several in vitro studies [14, 28] demonstrating eNOS expression in glomerular endothelial cells but not in mesangial cells by Northern blot analysis. Thus, the present results together with those of previous studies suggest that glomerular endothelial cells participate in the NO pathway in normal and diseased glomeruli through the synthesis of eNOS.

In terms of iNOS expression, it is important to search for glomerular infiltrating monocytes as a possible source of NO, since the macrophages are known to produce iNOS in certain experimental model [18] and in vitro [17, 29]. In fact, Jansen et al [18] reported that iNOS predominantly localized in macrophages in glomeruli of rat models with immune complex glomerulonephritis. The difference in the findings of iNOS expression between rats and human macrophages suggests that iNOS expression may differ between species. This hypothesis is supported by Weinberg et al [30], who reported that rodent macrophages appear to have higher iNOS expression than human macrophages. In a human study, Kashem and coworkers [31] reported that most of the iNOS expressing cells appeared to be interstitial infiltrating cells, although some weak focal staining for iNOS was found in the glomerular cells in the renal tissue samples from patients with IgAN. The reason for the discrepancy between the present results and those of Kashem et al is unclear, but may be due to differences in the methodologies employed, such as fixation of tissue sections and the sensitivity of used avidin biotin complex method. To differentiate infiltrating monocytes from iNOS-expressing cells, we performed two experiments. The first was immunohistochemistry for iNOS on one section and for the surface marker of monocytes (CD68) on serial sections. The other experiment was the double staining for iNOS and surface marker of macrophage. Based on the results of these experiments, it is clear that while some macrophages express iNOS, the majority of cells that contained iNOS were intrinsic glomerular cells, such as mesangial cells and glomerular epithelial cells. Our results are in agreement with some previous studies. Nicolson et al [32] showed that the synthesis of iNOS in cultured human mesangial cells stimulated by cytokines. Wilkes and colleagues [33] demonstrated that iNOS could be induced by cytokine stimulation in cultured glomerular epithelial cells. In another study, Jansen et al [18] showed weak focal immunostaining of iNOS in glomerular epithelial cells in rat immune complex glomerulonephritis. Furthermore, to confirm the iNOS production by the glomerular resident cells, two different antibodies were used and in situ hybridization for iNOS mRNA was performed. Thus, our results indicated that iNOS is induced in mesangial cells, and glomerular epithelial cells and some infiltrating cells in the glomeruli of IgAN and LN.

Regarding the mechanism of iNOS production in the diseased glomeruli, it is possible that certain cytokines are involved in the induction of iNOS in the diseased glomeruli. In fact, the expression of IL-1β and TNF-α in the glomeruli of IgAN and LN has already been reported [34, 35]. Moreover, in pathological states, under the influence of various cytokines such as IL-1β and TNF-α, NO is synthesized mainly by iNOS [2, 36]. The regulatory mechanism of iNOS and eNOS should be clarified in further studies.

In the present study, a negative correlation was observed between eNOS expression and the degree of glomerular injury. The decreased expression of eNOS in IgAN and LN with moderate to severe lesion suggests that this alteration may contribute to the progression of these renal diseases. Although the down-regulatory mechanism of eNOS production in damaged glomeruli was not clarified in this study, it is probable that glomerular endothelial cells of injured glomeruli may reduce the expression of eNOS during the progression of renal diseases. It is important to determine whether reduced eNOS expression with enhanced iNOS expression is the cause or result of renal injury in IgAN and LN.

Although the exact role of NO in normal and diseased glomeruli is not fully understood, our results showed a reciprocal staining pattern of eNOS and iNOS in each glomerulus. This finding, when considered in conjunction with the different relationships between each NOS and the degree of glomerular injury, allows us to
speculate that the induction of iNOS may compensate the reduced production of eNOS or that eNOS and iNOS may have different roles in the diseased glomeruli. Since the half-life of NO is very short, the biological effects of NO depend on both the concentration of NO at the site of action as well as the location of production [2]. In fact, the amount of NO generated by eNOS is small, in nmol quantities, whereas iNOS synthesizes NO in large (mmol) quantities [2]. NO, which constitutively derived from eNOS in the glomeruli, regulates glomerular microcirculation under normal physiological conditions by modifying the tone of the afferent arteriole and mesangial cells, and at the same time maintains the antithrombogenic properties of the endothelium [2, 37]. The fact that the expression of eNOS decreased with the progression of glomerular injury suggests that these physiological effects of NO, produced by eNOS, diminish in severely damaged glomeruli, leading to disturbances in microcirculation. In contrast, high levels of NO, derived from iNOS, are cytotoxic or cytostatic at least in vitro [36], and hamper cell function by reacting with iron in numerous vital enzymes of the mitochondrial electron transport chain and the citric acid cycle [2, 36]. In addition, NO may also react with superoxide anion generated locally to form the reactive peroxynitrite anion, which causes further tissue injury and lipid peroxidation [2, 37]. On the other hand, NO may have a beneficial effect in the nephritic glomeruli. Trachtman et al [38] reported that NO inhibits the production of extracellular matrix protein by mesangial cells. Increased production of extracellular matrix protein in glomeruli, a process known to occur during the progression of several forms of glomerulonephritis [39], may be inhibited by locally generated NO. Based on these observations, together with the present results, we suggest that NO derived from eNOS and iNOS may be involved in the progression of glomerulonephritis via complex pathways. Further studies are warranted to understand the role of NO in the inflammatory process in human glomerulonephritis.

In conclusion, our results indicate the presence of NO pathway in the human diseased kidney. Our results also suggest that eNOS and iNOS may participate in the process of glomerular injury through different mechanisms.

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