Osteopontin expression in progressive renal injury in remnant kidney: Role of angiotensin II

XUE Q. YU, LEONARD L. WU, XIAO R. HUANG, NIANSHENG YANG, RICHARD E. GILBERT, MARK E. COOPER, RICHARD J. JOHNSON, KAR N. LAI, and HUI Y. LAN

Department of Nephrology, The First Hospital, Sun Yat-Sen University of Medical Sciences, Guangzhou, People’s Republic of China; Departments of Medicine, The University of Hong Kong, Hong Kong; Department of Medicine, Austin and Repatriation Medical Center, Victoria, Australia; and Division of Nephrology, University of Washington School of Medicine, Seattle, Washington, USA

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Background. Osteopontin (OPN) is a macrophage chemoattractive molecule and has been shown to play a role in glomerular and tubulointerstitial injury in several kidney disease models.

Methods. The present study examined whether OPN expression is involved in the progression of renal disease following subtotal nephrectomy (STNx) in rats and whether angiotensin II (Ang II) mediates the up-regulation of renal OPN expression and macrophage accumulation in this model by administering valsartan, an Ang II type I (AT1) receptor antagonist, or ramipril, an angiotensin-converting enzyme (ACE) inhibitor.

Results. In normal and sham-operated rat kidneys, OPN was expressed in a few tubules (<5%) and was absent in glomeruli. Following STNx (weeks 2 to 16), there was substantial up-regulation of OPN mRNA and protein expression in glomeruli [2 to 12 cells/glomerular cross section (gcs)] and tubular epithelial cells (20 to 75% OPN+). The up-regulation of OPN expression was associated with macrophage accumulation within the kidney, severe proteinuria, loss of renal function, and severe histologic damage, including tubulitis and tubulointerstitial fibrosis (all P < 0.001). Treatment with either valsartan or ramipril completely abrogated the up-regulation of OPN mRNA and protein expression in glomeruli and tubules. The reduction in OPN expression was associated with a significant inhibition of macrophage accumulation and progressive renal injury (P < 0.001).

Conclusion. An up-regulation of OPN expression may play a role in progressive renal injury following STNx. Inhibition of OPN expression may be one of the mechanisms by which Ang II blockade attenuated renal injury after renal ablation.

Osteopontin (OPN) is a highly acidic glycoprotein containing an adhesive arginine-glycine-aspartic acid sequence and acts as a macrophage adhesion and chemotactic molecule [1–3]. A wide range of cell types has been shown to express OPN in a constitutive or inducible fashion, including osteoclasts, some epithelial cells, macrophages, T cells, smooth muscle cells, and some tumors [4–9]. The functional importance of OPN in the recruitment of macrophages has been recently documented in vivo [10, 11]. Indeed, OPN can bind avidly to macrophages and induces prominent monocyte infiltration when injected subcutaneously in mice [10]. Furthermore, macrophage accumulation induced by intradermal injection of the chemoattractant N-formyl-met-leu-phe in rats is inhibited by the administration of a neutralizing anti-OPN antibody [11].

A strong association between the up-regulation of OPN expression and macrophage infiltration has been described in a wide range of experimental models of glomerular and interstitial nephritis [12–22]. Both the degree of OPN up-regulation in the tubules and the sites of its localization have been shown to correlate with the sites and degree of macrophage accumulation and the severity of tubulointerstitial fibrosis and renal impairment [14]. Most convincing have been recent studies in which OPN expression has either been inhibited or neutralized. The administration of anti-OPN antibody to a model of rat crescentic antiglomerular basement membrane (GBM) glomerulonephritis resulted in a remarkable inhibition of both glomerular and tubulointerstitial macrophage accumulation and a reduction in disease severity [23]. In addition, the importance of OPN in mediating macrophage-rich tubulointerstitial injury has recently been delineated in OPN−/− mice in a model of unilateral obstructive nephropathy [22].

Angiotensin II (Ang II) has been shown to play a role in macrophage-mediated tubulointerstitial injury in experimental animal models, including subtotal nephrectomy (STNx) in rats, an established model of Ang II-dependent progressive renal injury [12–14, 24, 25]. Ang II
has also been shown to stimulate OPN expression in tubules in vitro [26] and to induce OPN expression in tubules in vivo, the latter followed by macrophage infiltration and the development of tubulointerstitial disease [13]. Furthermore, in the unilateral ureteral obstruction model, the administration of angiotensin-converting enzyme (ACE) inhibitors was shown to reduce both OPN expression and the macrophage infiltration, suggesting a potential causal link [27]. In contrast, in the streptozotocin model of diabetes, the administration of ACE inhibitors actually increased OPN expression in tubules via a bradykinin-dependent effect, and the OPN expression in this model did not correlate with macrophage infiltration or injury [28]. Given these contrasting results, we sought to determine whether OPN expression is increased in the remnant kidney model and whether its expression is associated with macrophage infiltration and renal injury. Furthermore, we examined the role of Ang II in the regulation of OPN expression in this model by administering either an ACE inhibitor or an angiotensin type I (AT1) receptor antagonist.

**METHODS**

**Experimental design**

The present study consisted of two experimental protocols that have been described previously [24, 29]. The first experiment contained 50 male Sprague-Dawley rats (weighing 200 to 250 g). Rats were obtained from the Animal House, Repatriation Campus (A & R Medical Center, Heidelberg, Australia). Twenty-five animals underwent STNx consisting of right subcapsular nephrectomy and infarction of approximately two thirds of the left kidney by selective ligation of two of the three extra-renal branches of the left renal artery. At the same time, 25 rats underwent a sham surgical procedure (control group) consisting of laparotomy and tactile manipulation of left kidney before wound closure. All animals were housed in a temperature-controlled (22°C) room with ad libitum access to commercial standard rat chow (Norco, Lismore, N.S.W., Australia) and water for the duration of the study. Groups of five animals in both STNx and sham groups were killed at 2, 4, 8, 12, or 16 weeks after surgery. In addition, one group of five normal rats was used as the normal control.

The second experiment consisted of 32 male Sprague-Dawley rats weighing 200 to 250 g. Twenty-four animals underwent STNx as described previously in this article. After the surgery, the rats were randomly assigned into three groups: (1) STNx alone (N = 8), (2) STNx with an ACE inhibitor, ramipril (STNxR; 3 mg/L drinking water, N = 8; Hoechst AG, Frankfurt, Germany), and (3) STNxV with an AT1 receptor antagonist, valsartan (STNxV; 30 mg/kg/day by daily gavage, N = 8; Ciba-Geigy, Basel, Switzerland). In addition, a group of eight rats was used as a sham surgery control group. Animals were sacrificed 12 weeks after surgery.

The experiments were approved by the Animal Welfare and Ethics Committee of the Austin and Repatriation Medical Center.

**Blood pressure and renal function**

Systolic blood pressure was monitored weekly in conscious rats using an occlusive tail-cuff plethysmograph attached to a pneumatic pulse transducer (Narco Biosystem, Houston, TX, USA). Prior to sacrifice, rats were housed in metabolic cages for 24 hours for urine collection. Total urinary protein excretion was measured using the Coomassie Brilliant Blue Method. At the time of sacrifice, serum was collected for measurement of urea and creatinine concentrations.

**Histopathology**

Renal tissues were fixed in 10% neutral-buffered formalin and were embedded in paraffin. Four-micron paraffin sections were stained with hematoxylin and periodic acid-Schiff (PAS) or with Masson’s trichrome. Glomerulosclerosis was assessed on PAS-stained sections using a slightly modified version of the semiquantitative scoring method [24, 29]. The extent of tubulointerstitial injury was evaluated on trichrome-stained sections to assess the relative proportion of tubular dilation, tubular atrophy, and interstitial fibrosis, using a standard point-counting technique [24, 29]. In addition, tubulitis was also used as an index of severe tubular injury. This was assessed by the presence of more than two ED1+ macrophages infiltrating within the tubules defined by the ED1 monoclonal antibody (mAb) labeling on PAS-counterstained tissue sections. Data were expressed as a percentage of tubules exhibiting tubular lesions.

**Immunohistochemistry**

Immunohistochemical staining for OPN protein and macrophage accumulation was performed on formalin-fixed, paraffin-embedded sections using a microwave antigen retrieval method [5, 30]. Sections were dewaxed and treated with 10 minutes of microwave oven heating in 400 mL of 0.01 mol/L sodium citrate, pH 6.0, at 2450 MHz and 800 W. After preincubation with 10% fetal calf serum (FCS) and 10% normal goat serum in phosphate-buffered saline (PBS), the sections were drained and then labeled with mouse mAbs to rat OPN (MPIIIB10; obtained from the Developmental Studies Hybridoma Bank, Iowa City, IA, USA) or rat macrophages (ED1) for 60 minutes, washed (three times) in PBS, and endogenous peroxidase inactivated by incubation in 0.3% H2O2 in methanol. Sections then were washed in PBS, incubated with peroxidase-conjugated goat anti-mouse IgG, washed in PBS, incubated with
mouse peroxidase antiperoxidase complexes, and developed with 3,3-diaminobenzidine to produce a brown color.

Double immunostaining was used to detect OPN and macrophages within the same section. Once staining with the ED1 mAb was completed, as described previously in this article, sections were given a second round of microwave oven heating to block antibody cross-reactivity and enhance detection of OPN. Following precubation, as described previously in this article, sections were incubated with the MPIIIB10 mAb and then incubated sequentially with alkaline phosphatase-conjugated goat anti-mouse IgG and mouse alkaline phosphatase antialkaline phosphatase complexes and developed with Fast Blue BB Salt (Ajax Chemicals, Melbourne, Australia). No staining was seen in negative control sections using the 73.5 IgG1 (anti-human CD45R) irrelevant isotype control mAb. All peroxidase and alkaline phosphatase-conjugated antibodies and complexes were purchased from Dakopatts (Glostrup, Denmark).

Probes

A 1 kb cRNA probe was generated from the 2B7 cDNA clone of rat smooth muscle OPN [8]. Sense and antisense cRNA probes were labeled with digoxigenin (DIG)-UTP using a T7 RNA polymerase kit (Boehringer Mannheim GmbH, Mannheim, Germany). In addition, a 358 bp fragment of rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA was amplified by reverse transcription-polymerase chain reaction (RT-PCR) and labeled with DIG-DUTP using the High-Prime random priming kit (Boehringer Mannheim GmbH). Probes were precipitated, and incorporation of DIG was determined by dot blotting.

Northern blot analysis

Northern blotting was performed as previously described [5, 23]. Briefly, total cellular RNA was extracted using the RNAzol reagent (GIBCO BRL, Gaithersburg, MD, USA), and 20 µg samples were denatured with glyoxal and dimethylsulfoxide, size fractionated on 1.2% agarose gels, and capillary blotted onto positively charged nylon membranes (Boehringer Mannheim). Membranes were hybridized overnight at 68°C or 42°C with DIG-labeled cRNA or cDNA probes, respectively, in DIG Easy Hyb solution (Boehringer Mannheim). Following hybridization, membranes were washed finally in 0.1 X standard saline citrate (SSC)/0.1% sodium dodecyl sulfate (SDS) at 68°C or 0.2 X SSC/0.1% SDS at 42°C. Bound probes were detected using sheep anti-DIG antibody (Fab) conjugated with alkaline phosphatase and development with CPD-star enhanced chemiluminescence (Boehringer Mannheim). Chemiluminescence emissions were captured on Kodak XAR film, and densitometry analysis was performed using the public domain NIH ImagePC program (developed at the U.S. National Institutes of Health and available on the Internet at http://rsb.info.nih.gov/nih-image/).

In situ hybridization

In situ hybridization was performed on 4 µm paraffin sections of formalin-fixed tissue using a microwave-based protocol as described in detail elsewhere [5, 23, 31]. After dewaxing, sections were treated with a microwave oven for 2 x 5 minutes as described previously in this article, incubated with 0.2 mol/L HCl for 15 minutes, followed by 1% Triton X-100 for 15 minutes, and then digested for 20 minutes with 10 µg/mL Proteinase-K at 37°C (Boehringer Mannheim). Sections then were washed in 2 x SSC, prehybridized, and then hybridized with 0.3 ng/µL DIG-labeled sense or antisense OPN cRNA probe overnight at 37°C in a hybridization buffer containing 50% deionized formamide, 4 x SSC, 2 x Denhardt's solution, 1 mg/mL salmon sperm DNA, and 1 mg/mL yeast tRNA. Sections were washed finally in 0.1 x SSC at 37°C, and the hybridized probe was detected using sheep anti-DIG antibody (Fab) conjugated with alkaline phosphatase and color development with NBT/ X-phosphate (Boehringer Mannheim). Sections were mounted in an aqueous medium. No signal was seen with the sense riboprobe labeled to the same specific activity.

Quantification of in situ hybridization and immunohistochemistry staining

Positively stained cells were quantitated in tissue sections as previously described [5, 23]. Briefly, the number of cells labeled with the OPN cRNA probe or the different mAb was counted in at least 50 glomeruli cross-sections per animal. Interstitial ED1+ macrophages were counted in at least 20 consecutive high-power fields moving from the outer cortex to the inner cortex by means of a 0.02 mm2 graticule fitted in the eyepiece of microscope. The number of tubules positive for OPN mRNA and protein was scored from at least 1000 cortical tubules. In addition, a point-counting technique was used to quantitate the number of interstitial ED1+ cells surrounding OPN+ or OPN− tubules. The number of ED1+ macrophages that are immediately surrounding and/or infiltrating either OPN+ or OPN− tubules was counted using at least 1000 cortical tubules and expressed as ED1+ cells/tubules per animal. Finally, OPN+ or OPN− cortical tubules were assessed for tubulitis by scoring the presence of ED1+ macrophages between and/or within the tubules in at least 1000 cortical tubules per animal. Data are expressed as the mean ± SEM for groups of six animals.

All morphological analysis and cell counting were performed on blinded slides, and the areas evaluated were in the renal cortex at a site distant from the surgical infarct.
Statistical analyses

One-way analysis of variance (ANOVA) from the Complete Statistical Analysis program (CSS, Statsoft, Tulsa, OK, USA) was used to analyze differences in clinical data and quantitative immunohistochemistry scoring. The Pearson single-correlation coefficient was used to compare OPN protein expression with macrophage and T-cell accumulation, proteinuria, and renal impairment. The relationship between OPN expression and glomerular and tubulointerstitial damage was compared using the Spearman’s rank correlation coefficient.

RESULTS

Histology, renal function, and blood pressure

Following STNx, rats developed elevated systolic blood pressures, heavy proteinuria, renal insufficiency, progressive glomerulosclerosis, and tubulointerstitial disease as described previously [24, 29]. All of these parameters remained within the normal range in the sham-operated control group for the duration of the experiment [24, 29].

OPN expression in normal and sham-operated rat kidneys

In normal rat kidney, in situ hybridization and immunostaining identified the constitutive expression of OPN mRNA and protein in some distal tubules and in thick ascending limbs of the loop of Henle, accounting for less than 5% (3.3 ± 1.8%) of all cortical tubules (Figs. 1a, 2a, and 3B). There were no OPN-positive cells within the glomerulus (Figs. 1a, 2a, and 3A). Similarly, there was no increase in the number of glomerular or tubular OPN mRNA and protein-positive cells in sham-operated kidneys throughout the 16-week period of observation (Figs. 2b and 3A, B). There was also no increase in ED1+ macrophages within glomeruli and tubulointerstitium in all sham-operated kidneys (Figs. 2b and 3C, D).

Up-regulation of OPN expression, macrophage accumulation, and renal injury following STNx

In situ hybridization and immunostaining showed a significant increase in the number of OPN+ glomerular and tubular cells at week 2 following STNx (Figs. 1b, 2c, and 3A, B). An important observation was that the de novo OPN expression by proximal tubules at week 2 preceded the development of significant macrophage infiltration at week 4 (Fig. 3), demonstrating that up-regulation of renal OPN temporally precedes the macrophage accumulation following renal ablation. Notably, OPN mRNA and protein expression by cortical tubules continued to increase throughout the disease course, eventually being positive in up to 80% of tubules (Figs. 1d and 3B). The OPN expression was paralleled by progressive macrophage accumulation and tubulointerstitial damage over weeks 4 to 16 (Figs. 1c, d; 2c, d; and 3B, D). In situ hybridization and double immunohistochemistry staining showed that the marked up-regulation of tubular OPN mRNA and protein expression occurred in areas of focal tubulointerstitial macrophage infiltration, resulting in the development of macrophage-rich tubulitis and tubulointerstitial fibrosis (Figs. 1c, d and 2c, d). In contrast, there was minimal macrophage accumulation in the areas where tubular OPN expression was absent (Fig. 2c). Quantitative analysis confirmed that most infiltrating peritubular macrophages accumulated around OPN+ tubules (Fig. 4A) and that the incidence of tubulitis was much greater in OPN+ tubules as compared with OPN− tubules (Fig. 4B).

Correlation of OPN expression with macrophage accumulation and progressive renal injury

As shown in Table 1, the number of glomerular and tubular OPN+ cells correlated significantly with the number of ED1+ macrophages within the tubulointerstitium, the presence of tubulitis and tubulointerstitial fibrosis, and proteinuria and the loss of renal function over the 16-week time course following renal ablation.

Inhibition of OPN expression, macrophage accumulation, and progressive renal injury by Ang II blockade

Double immunostaining, in situ hybridization, and Northern blotting demonstrated a marked up-regulation of OPN mRNA and protein expression within the kidney in control animals 12 weeks following STNx (Figs. 1d, 2d, 5, and 6). This was particularly striking within the damaged tubulointerstitium. The up-regulation of tubular OPN mRNA and protein expression was associated with focal infiltration of ED1+ macrophages and the development of severe tubulointerstitial damage such as tubu-
lar hypertrophy, macrophage-rich tubulitis, and interstitial fibrosis (Figs. 1d, 2d, 5, 6, and 7). In addition, the up-regulation of OPN expression by glomerular parietal epithelial cells was found to be associated with early glomerular crescent formation (Fig. 1d). In contrast, in rats administered the AT1 receptor antagonist valsartan or the ACE inhibitor ramipril, the glomerular and tubular up-regulation of OPN expression was nearly abrogated (Figs. 1e, f, 2e, f, 5, and 6). The decrease in OPN expression was associated with a significant reduction of macrophage accumulation within the glomeruli [6.6 ± 1.9 cells/glomerular cross-section (gcs) in STNx vs. 3.0 ± 0.3 cells/gcs in STNxV and 1.9 ± 0.5 cells/gcs in STNxR, P < 0.05 or < 0.01, respectively].
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and in the cortical tubulointerstitium (394 ± 168 cells/mm² vs. 179 ± 33 cells/mm² in STNxV and 136 ± 143 cells/mm² in STNxR, P < 0.01, respectively). This was associated with a substantial inhibition of tubulitis (Fig. 7). In addition, valsartan or ramipril treatment was also associated with a reduction of proteinuria (156 ± 36 in STNx vs. 65 ± 17 in STNxV and 58 ± 20 mg/24 h in STNxR, P < 0.01, respectively), a less decline in creatinine clearance (0.6 ± 0.2 in STNx vs. 1.8 ± 0.3 in STNxV and 1.6 ± 0.4 mL/min in STNxR, P < 0.05) and less glomerulosclerosis (index: 0.9 in STNx vs. 0.2 in STNxV and STNxR, P < 0.01) and tubulointerstitial fibrosis (index: 5.5 in STNx vs. 0.9 in STNxV and 0.8 in STNxR, P < 0.01), as described previously [29, 32].

DISCUSSION

This study documents that OPN mRNA and protein are markedly up-regulated following STNx. The up-regulation of glomerular and tubular OPN expression was strongly associated with macrophage accumulation and progressive renal injury and correlated with the development of tubulitis, tubulointerstitial fibrosis, proteinuria, and renal function impairment. The observation that both treatment with an AT1 receptor antagonist or an ACE inhibitor inhibited the OPN expression suggests that Ang II either directly or indirectly regulates OPN expression in this model. Furthermore, the close association between the inhibition of OPN expression and the reduction in macrophage infiltration and tubulointerstitial injury suggest that one of the mechanisms by which Ang II blockade is effective may be via inhibition of OPN expression.

It is now well known that OPN is a potent chemotactic and adhesive molecule for monocyte/macrophages [1–3]. An association between up-regulation of tubular OPN expression and focal macrophage infiltration has been described in a number of experimental models of renal injury, suggesting a pathological role for OPN in progressive glomerular and tubulointerstitial injury [5, 12–23]. Interestingly, in models in which tubulointerstitial injury is mild or transient, OPN expression is also transient [12]. Fewer studies have been performed of chronic models of renal injury, but some sustained OPN expression that correlates with chronic macrophage accumulation has been shown [18]. The functional role of OPN in tubulointerstitial injury has been further confirmed by a recent study of obstructive nephropathy induced in OPN null mutant mice (OPN−/−). Indeed, the OPN−/− mice exhibited a significant reduction in early interstitial macrophage influx and fibrosis [22]. The current study is of some interest as the increase in OPN was progressive and eventually was observed in over 80% of cortical tubules. Furthermore, as noted in other models, the tubular OPN expression correlated with both the sites and degree of macrophage accumulation and the development of tubulitis and interstitial fibrosis [5, 14, 23]. In contrast to our previous finding in a rat model of anti-GBM glomerulonephritis in which glomerular OPN expression and macrophage accumulation are prominent [5, 23], only a small, but significant number of glomerular OPN+ cells and macrophages were found. This may be due to the less severe glomerular injury seen in this disease model compared with anti-GBM crescentic glomerulonephritis. Importantly, the documentation that OPN expression preceded the macrophage accumulation in the current model provides strong suggestive evidence that OPN may be at least partially responsible for the subsequent macrophage infiltration. Similar observations were noted in a model of tubulointerstitial injury induced by Ang II infusion [13].

The mechanism by which OPN may promote macrophage accumulation within the kidney is likely through its adhesive and chemotactic functions. OPN can bind
Table 1. Correlation of osteopontin (OPN) expression with macrophage accumulation, histology and renal function

<table>
<thead>
<tr>
<th>OPN expression</th>
<th>Glomerular ED1⁺ cells</th>
<th>Interstitial ED1⁺ cells</th>
<th>Glomerulosclerosis</th>
<th>Tubulointerstitial fibrosis</th>
<th>Tubulitis</th>
<th>Proteinuria</th>
<th>Serum urea</th>
<th>Serum creatinine</th>
</tr>
</thead>
</table>
| Glomerulus     | 0.472
| Tubules        | —                     | 0.457              | —                | —             | 0.259
|                | 0.153                |                      | —                | 0.780         | 0.441
|                |                       |                      | —                | 0.535         | 0.507

Data are from 25 animals with STNx analyzed using the Pearson single correlation coefficient.
* P < 0.05, * P < 0.01, * P < 0.001

Fig. 5. Northern blot demonstrates the inhibition of OPN mRNA expression in rat remnant kidneys by valsartan or ramipril. Northern blot analysis shows that OPN mRNA is markedly up-regulated at week 12 after STNx, while treatment with valsartan (STNxV) and ramipril (STNxR) abrogated OPN mRNA up-regulation. OPN mRNA is normalized for GAPDH mRNA (OPN/GAPDH ratio). Shown are the mean ± SEM for five animals. **P < 0.01 compared with sham-operated animals.

Fig. 6. In situ hybridization and immunostaining demonstrate the inhibition of glomerular and tubular OPN mRNA and protein expression in the rat remnant kidney by valsartan and ramipril. Shown are OPN mRNA (A and B) and OPN protein (C and D) expression in glomeruli and tubules in sham-operated (●), untreated disease control (STNx, ■), valsartan-treated (STNxV, □), and ramipril-treated (STNxR, ○) animals. Data are expressed as mean ± SEM for eight animals. ***P < 0.001 compared with sham-operated animals.

To a number of ligands including the αvβ3 integrin and CD44, an adhesion molecule that is expressed by infiltrating leukocytes and markedly up-regulated by intrinsic kidney cells during the disease [2, 5, 23]. The administration of a neutralizing anti-OPN antibody to rat anti-GBM glomerulonephritis causes marked inhibition of renal OPN and its ligand CD44 expression, which suggests that the OPN–CD44 interaction may promote leukocyte adhesion in the development of both glomerular and tubulointerstitial injury [23]. However, not all isoforms
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Kaneto et al have also reported that in a unilateral model of obstruction, the administration of an ACE inhibitor could prevent OPN protein but not mRNA expression, and this was also associated with a reduction in macrophage infiltration and injury [27]. AT1 receptor blocker therapy also reduces OPN expression and macrophage accumulation in a model of cyclosporine-induced nephropathy [14]. In contrast, Fischer et al reported that ACE inhibitors increase OPN expression in the kidneys of rats with streptozotocin-induced diabetes, an effect that was mediated by bradykinin generated by the ACE inhibitor [28]. However, the observation in the current study that both ACE inhibitor and AT1 receptor antagonist therapy were protective strongly implicates a role for Ang II in the up-regulation of OPN in this model.

The mechanism by which Ang II stimulates OPN expression in tubules in this model could be direct or indirect. Ang II may act directly to stimulate tubular expression of OPN [26]. However, Ang II might also induce OPN expression indirectly, such as by inducing glomerular hypertension and proteinuria, the latter of which has been shown to induce tubular OPN expression in a model of protein overload nephropathy [16]. Ang II is also able to induce transforming growth factor-β (TGF-β) expression in vitro [37], which in turn, up-regulates OPN expression in cultured normal rat tubular epithelial cells [38]. In this disease model, we have shown that blocking Ang II activity significantly inhibited the up-regulation of renal OPN expression in the present study.

An amplification system for OPN expression is also possible. We have recently shown that IL-1 can act directly to up-regulate OPN expression in renal epithelial cells in vivo and in vitro [39]. Activated macrophages are a major source of IL-1; therefore, local macrophage accumulation could further induce tubular OPN expression with the additional recruitment of more monocyte-macrophages. The observation of continuously increased up-regulation of tubular OPN expression through out the entire disease course may possibly be accounted for by this inflammatory amplification response.

In summary, this study demonstrates that Ang II is a key stimulator to up-regulate renal OPN expression following STNx. Inhibition of OPN expression may be one of the mechanisms by which Ang II blockade suppresses macrophage-mediated renal injury in the remnant kidney.

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