Renal Interstitial Fibrosis Is Reduced in Angiotensin II Type 1a Receptor-Deficient Mice

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Abstract. Unilateral ureteral obstruction (UUO) results in tubulointerstitial fibrosis of the affected kidney by stimulating the renin-angiotensin system. This study established a UUO model in angiotensin type 1a receptor (AT1a) deficient (mutant) mice to elucidate the role of angiotensin II through AT1a on the fibrosis of the obstructed kidney (OBK). The relative volume of the tubulointerstitium was measured by an image analyzer; deposition of collagen types III and IV and monocyte/macrophage infiltration were histologically examined using specific antibodies. Also determined were the mRNA levels of transforming growth factor-β by Northern blot analysis. Nuclear factor-κB activity was assessed by gel shift assay. UUO in wild mice resulted in a marked expansion of relative volume of the tubulointerstitium, together with increased deposition of collagen types III and IV and number of infiltrated monocytes/macrophages in the interstitium, relative to sham-operated mice. In comparison, these changes were significantly lower in mutant mice with UUO. The mRNA level of transforming growth factor-β was significantly higher in the OBK of wild mice with UUO compared with sham-operated mice. In contrast, the increase in mRNA level in the OBK of mutant mice was significantly less than in wild mice. Finally, UUO resulted in activation of nuclear factor-κB in wild mice but was inhibited in the OBK of mutant mice. The results provide direct evidence that angiotensin II acting via the AT1a plays a pivotal role in the development of tubulointerstitial fibrosis in UUO.

It is widely recognized that progressive renal disease is accompanied by tubulointerstitial changes characterized by tubular atrophy, increased number of interstitial fibroblasts, phenotypic change of interstitial cells, accumulation of matrix proteins, and interstitial infiltrate of mononuclear cells. Deterioration of renal function is determined to a large extent by the degree of tubulointerstitial changes rather than by the extent of histologic changes in the glomeruli in many forms of glomerulonephritis. However, the pathogenic mechanisms of tubulointerstitial changes have not yet been elucidated fully. Evidence from many studies suggests that common pathogenic mechanisms exist in the pathogenesis of tubulointerstitial changes (1).

Unilateral ureteral obstruction (UUO) is a well-established model of progressive tubulointerstitial fibrosis.
To elucidate the functional importance of AT1a-mediated signal transduction pathways in the pathophysiology of renal diseases, especially tubulointerstitial injury, we established AT1a-deficient (mutant) mice with a targeted replacement of AT1a loci by the lacZ gene (14,15). Using these mice, we determined in the present study the role of the Ang II–AT1a system in the pathophysiology of tubulointerstitial injury after experimentally induced UUO. Our results indicate that the action of Ang II via AT1a plays an important role in the development of tubulointerstitial fibrosis with UUO.

Materials and Methods

Experimental Protocol

Female C57BL/6 (wild) mice at the age of 10 wk, weighing 20 to 22 g, and female mutant mice at the age of 10 wk, weighing 25 to 35 g, were used in these experiments. With the mice under pentobarbital anesthesia, the left ureter was ligated with 4-0 silk at two locations and cut between the ligatures to prevent retrograde urinary tract infection in both wild (n = 14) and mutant mice (n = 14). Mice that were operated on were killed under pentobarbital anesthesia 2 (n = 5), 5 (n = 5), and 10 (n = 4) d after UUO. Sham operation was performed in both wild (n = 4) and mutant mice (n = 4); mice had their ureters manipulated but not ligated. Five d after sham operation, mice were killed to obtain control kidneys. The experimental protocol was approved by the Ethics Review Committees for Animal Experimentation of the participating institutions.

Morphometric Evaluation of Interstitial Fibrosis of Renal Cortex

Obstructed kidneys (OBK) were removed, sliced axially into 3-mm-thick sections, fixed in 10% buffered formalin, and embedded in paraffin. Paraffin sections (4-μm thick) were stained with Azan-Mallory stain. Blue-stained interstitial fibrotic areas were assessed by using an image analyzer (MCID image analyzer, Fujifilm, Tokyo, Japan). Five consecutive fields were randomly selected in renal cortex and evaluated at ×400 on a 10 × 10 grid-imprinted reticule. All points not counted within tubular cells, lumen, glomerulus, or vascular space were considered interstitial. This fraction represented the relative interstitial volume. Results were expressed as percentage of the measured area, which represented the interstitial space and was determined as the relative volume (Vv) of the interstitium.

Immunohistochemical Studies

Indirect immunofluorescence was performed as described previously (16). Briefly, surgically removed kidney specimens were immediately snap-frozen and unfixed cryostat sections (4-μm thick) were prepared. The sections were washed in phosphate buffered saline (PBS) three times, 5 min each, and then incubated with the primary antibody in PBS for 1 h at room temperature. Rabbit polyclonal antibodies against type III collagens (LSL Corp., Tokyo, Japan; dilution 1:60) and type IV collagen (LSL Corp.; dilution 1:30) were used as primary antibodies. Each section was washed three times in PBS and incubated with the secondary antibody in PBS for 30 min. Fluorescence isothiocyanate-conjugated goat anti-rabbit IgG (Zymed Laboratories, Inc., San Francisco, CA; dilution 1:80) was used as the secondary antibody. After the section was washed with PBS three times, it was mounted with fluoromount-G. Deposition of type III and type IV collagens in the tubulointerstitium of renal cortex was assessed semiquantitatively by fluorescence microscopy as described previously (17). A matrix deposition score ranging from 0 to 3 was determined on the basis of the intensity and distribution of type III and type IV collagens in the tubulointerstitium: 0, no change; 1, mild change; 2, moderate change; 3, severe change. The matrix score was determined in each section selected at random and more than 40 fields were examined under ×200 magnification.

Identification and Quantitation of Infiltrating Monocytes/Macrophages

Infiltration of monocytes/macrophages was examined by immunohistochemistry as described previously (18). Briefly, formalin-fixed, paraffin-embedded sections were deparaffinized, and endogenous peroxidase was inactivated with 0.3% hydrogen peroxide. Sections were then washed in PBS three times, 5 min each, preincubated in a blocking solution (10% goat serum in PBS) for 30 min, washed in PBS three times, and then incubated for 1 h with F4/80 (Serotec, Oxford, England; dilution 1:100) as the primary antibody against monocytes/macrophages. Control sections using wild OBK sections were treated similarly but without addition of the primary antibody. Each section was washed three times in PBS and then incubated with the second antibody for 30 min. Biotinylated rabbit anti-goat IgG (Nichirei Corp., Tokyo, Japan; dilution 1:1000) was used as the second antibody. After the sections were washed with PBS three times, they were placed in streptavidin labeled with peroxidase (Nichirei Corp.). They were placed in diaminobenzidine/hydrogen peroxide solution, counterstained with hematoxylin, dehydrated, and enclosed in synthetic resin. Monocyte/macrophage infiltration was determined by counting the number of F4/80 positively stained cells in the renal cortex. The number of F4/80-positive cells was determined in 20 randomly selected nonoverlapping ×200 fields in each section of the individual mouse renal cortex. The average number of F4/80-positive cells from three separate animals was calculated and averaged.

RNA Extraction and Northern Hybridization Analysis

Total RNA from the cortex was extracted by the acid guanidium thiocyanate phenol chloroform extraction method, as described previously (19). RNA concentration and purity were determined spectrophotometrically. A total of 20 μg of RNA was run on 0.8% agarose 6% formaldehyde gels. RNA integrity was verified by examination of 28S and 18S ribosomal bands of ethidium bromide–stained material under ultraviolet light. The RNA was then transferred onto nylon membranes (Hybond-N; Amersham Life Science, Buckinghamshire, England) by capillary action and fixed by ultraviolet irradiation. Membranes were hybridized with a cDNA probe coding for mouse TGF-β labeled with [α-32P]dATP by random primed DNA synthesis. Membranes were hybridized in a solution containing 1% bovine serum albumin, 7% sodium dodecyl sulfate, 0.5 M NaH2PO4, and 1 mM ethylenediaminetetraacetate (EDTA). The filter was then exposed to x-ray film at −80°C for 1 to 7 d. The membrane was rehybridized with a cDNA probe coding for glyceraldehyde phosphate dehydrogenase (GAPDH) labeled with [α-32P]dATP. The relative intensity of autoradiograms was determined by scanning densitometry. Results were expressed as the ratio of optical density of TGF-β to GAPDH relative to baseline control kidneys that were arbitrarily assigned a value of 1.

Detection of Renal AT1a and AT1b mRNA Expression

First, cDNA strand was synthesized from total RNA (1 μg) by Moloney-murine leukemia virus reverse transcriptase (Amersham Pharmacia Biotech, Little Chalfont, England) with random hexamers as a primer. AT1a, AT1b, and GAPDH cDNA were amplified by
AmpliTaq DNA polymerase (Applied Biosystems, Foster City, CA). PCR was performed using 35 cycles for AT1a and GAPDH, each cycle consisting of 30 s at 94°C, 45 s at 60°C, and 60 s at 72°C. For AT1b, 35 cycles were used, each cycle consisting of 60 s at 94°C, 90 s at 55°C, and 90 s at 72°C. PCR products were separated on 0.8% agarose gel and stained with ethidium bromide.

**Preparation of Nuclear Extracts**

Nuclear extracts were prepared from the kidney cortex according to the procedure of Asanuma et al. (20). In brief, the kidney cortex was minced and suspended in 1 ml of TBS buffer (25 mM Tris-HCl [pH 7.9], 130 mM NaCl, and 5 mM KCl) and homogenized for 20 strokes in a glass homogenizer. The homogenates were centrifuged at 7000 × g for 30 s, and the pellets were resuspended with 1 ml of TBS buffer and vortexed for 10 s followed by centrifugation of the homogenates at 5000 × g for 30 s. The pellets were suspended in 1 ml of buffer A (10 mM HEPES [pH 7.9], 10 mM KCl, 0.1 mM EDTA, 0.1 mM ethyleneglycol-bis(β-aminoethyl ether)-N,N′-tetraacetic acid, 1 mM phenylmethylsulfonyl fluoride, and 1 mM dithiothreitol [DTT]) and chilled on ice for 20 min. In the next step, 100 μl of 10% Nonidet P-40 was added and vigorously vortexed for 15 s. The nuclear fraction was precipitated by centrifugation at 12,000 × g for 7 min and suspended in 100 μl of buffer B (20 mM HEPES [pH 7.9], 400 mM NaCl, 1 mM EDTA, 1 mM ethyleneglycol-bis(β-aminoethyl ether)-N,N′-tetraacetic acid, 1 mM DTT, and 1 mM phenylmethylsulfonyl fluoride). The mixture was left on ice for 2 h with frequent agitation. Nuclear extracts were prepared by centrifugation at 12,000 × g for 7 min and stored at −80°C. Protein concentration was determined by the protein assay reagent (Bio-Rad Laboratories, Hercules, CA).

**Gel Shift Assay**

To detect the DNA binding activity, gel shift assay was performed as described previously (21). Consensus sequences of nuclear factor-κB (NF-κB) oligodeoxynucleotide (ODN) were as follows: 5′-AGTTGAGGGGACTTTCCCAGGC-3′. The ODN was labeled by T4 polynucleotide kinase (Promega, Madison, WI). Binding reactions were performed with 15 μg of nuclear extracts in a binding buffer consisting of 10 mM Tris-HCl (pH 7.5), 50 mM NaCl, 0.5 mM EDTA, 0.5 mM DTT, 1 mM MgCl2, 4% glycerol, and 50 ng/ml poly(dI-dC). Each 30-μl reaction mixture was incubated for 10 min at room temperature, and then approximately 200,000 cpm of [γ-32P]-ATP-labeled ODN probe was added to the reaction mixture and incubated for an additional 20 min at room temperature. Samples were then electrophoresed on a 4% polyacrylamide gel containing 45 mM Tris (pH 8.0), 45 mM sodium acetate, 2.5% glycerol, and 1 mM EDTA. Electrophoresis was carried out for 2 h. After electrophoresis, the gel was dried and autoradiographed and the relative intensity of autoradiograms was determined by scanning densitometry. To assess the specificity of the reaction, we performed competition assay with 50- and 10-fold excess of unlabeled consensus sequences of NF-κB. The unlabeled probes were added to the binding reaction 10 min before the reaction of the labeled probe. When supershift assays were carried out, polyclonal antibodies against p50 or p65 were added to the extracts 30 min before the labeled probe. Each experiment was performed three times.

**Statistical Analyses**

Data, shown as mean ± SD, were analyzed by the unpaired t test using the Macintosh StatView program (Hulinks, Tokyo, Japan). One-way ANOVA was used to determine the significance of the relative volume of the renal interstitium, F4/80 cell infiltration, type III collagen or type IV collagen matrix score, and results of Northern blots and gel shift assay. P < 0.05 denoted the presence of a statistically significant difference.

**Results**

**Expression of Renal AT1a and AT1b mRNA**

The expression of AT1a and AT1b mRNA was detected by reverse transcription-PCR (RT-PCR). AT1a mRNA was expressed in wild mice but was absent in mutant mice (Figure 1). Renal AT1b mRNA expression was not detected in either wild or mutant mice.

**Morphology of the Renal Cortex**

Figure 2 demonstrated sections of the renal cortex stained with Azan-Mallory stain. In sham-operated mutant mice, hypertrophy of juxtaglomerular apparatus and focal hypertrophy of intrarenal artery was evident compared with sham-operated wild mice (Figure 2, B and H), although the tubules and interstitium were normal (Figure 2, A and B). Five d after UUO in wild mice, fibrous material and monocyte infiltration increased in the interstitial space. Furthermore, thickening of the tubular basement membrane and widening of the interstitial space of the renal cortex were noted (Figure 2, C and E). In contrast, the interstitial space in the mutant mice did not show a significant increase (Figure 2D). The prevalence of atrophic tubules as a result of UUO was prominent (Figure 2F), and there were no changes in the glomeruli of either the OBK or the contralateral kidney at 5 d (Figure 2, G and H). There was no particular change in renal function (serum creatinine level: sham 0.22 ± 0.05 to UUO after 5 d 0.25 ± 0.06 mg/dl).

**Figure 1.** Angiotensin type 1a receptor (AT1a) and AT1b mRNA of the unilateral ureteral obstruction (UUO) model in wild or homozygous mutant mice. Reverse transcription-PCR of the cortex mRNA from wild or homozygous mutant mice after sham operation, 2 and 5 d after UUO. Total RNA from adrenal gland were used for positive control.
The volume of the interstitial fibrotic area relative to the cortical tubulointerstitial area was expressed as the volume fraction (Vv) (Figure 3). The interstitial volume of the OBK was significantly increased at 2 and 5 days after UUO in wild mice. In the mutant mice, Vv of OBK at 2 days was significantly less than in wild mice (11.2 ± 1.5% versus 16.8 ± 2.2%; P < 0.01). At 5 days, the difference between the two groups was smaller but still significant (26.2 ± 8.3% versus 37.8 ± 4.9%; P < 0.05; Figure 3), but at 10 days after UUO, the interstitial volume of the OBK in mutant mice was the same as in wild mice (40.7 ± 8.1% versus 41.1 ± 7.9%). No significant difference was observed in Vv of the contralateral kidney between the two groups (data not shown).

**Immunohistochemical Analysis**

Type III collagen was clear stained and confined to the renal interstitial space in control wild mice. In OBK of wild mice at 5 days, interstitial type III collagen was markedly increased in the interstitial space compared with sham-operated kidneys (SOK; data not shown). In contrast, type III collagen was not increased in mutant mice; there was little staining of type III collagen in the interstitial space. In sham-operated kidney, there was no difference between wild and mutant mice (Figure 4, A and B). Increased deposition of type IV collagen at 5 days was observed in wild mice. In mutant mice, there was a significantly less deposition of type IV collagen in OBK at 5 days compared with that in wild mice (Figure 4, C and D). The matrix scores for interstitial types III and IV collagen expression are summarized in Table 1. Two days after UUO, the matrix deposition score of type III collagen was significantly higher in wild mice than in SOK of wild mice. However, no such increase was observed in mutant mice. At 5 days after UUO, however, the difference between wild and mutant mice became smaller but was still significant (P < 0.05). At 10 days after UUO, there was no significant difference between the two groups. For matrix deposition score of type IV collagen, deposition was significantly higher 2 days after UUO in the wild mice compared with SOK (Table 1; P < 0.01). In contrast, increased deposition measured at the same day in mutant mice was not significant relative to SOK of mutant mice. Changes at days 5 and 10 in both groups were similar to those of type III collagen (Table 1).
Monocyte/Macrophage Infiltration in the Interstitium

Mononuclear cell infiltrates that were positive for F4/80 were observed in the cortex after UUO in both groups (Figure 5). In wild mice at 5 d, there was an infiltration of the renal cortex by F4/80-positive monocytes/macrophages (Figure 5C). In contrast, there was no significant infiltrate of F4/80-positive cells in the renal cortex of mutant mice after UUO (Figure 5D). There was no staining of F4/80 in the negative control section (Figure 5E). Results of quantitative analysis of monocytes/macrophages in the interstitium are shown in Figure 6. The number of monocytes/macrophages in the interstitium increased significantly 2 d after UUO in the wild mice, compared with SOK, with a further increase at 5 d. In mutant mice, although the number of these cells also increased at days 2 and 5, the counts were significantly less at 2 and 5 d compared with the wild mice (52 and 44% of wild mice, respectively).

Expression of TGF-β mRNA

Figure 7 demonstrates the relative level of expression of TGF-β mRNA at 2 and 5 d of UUO by Northern blot analysis. The amount of TGF-β mRNA in the OBK in wild mice was larger after UUO than in SOK. At 5 d of UUO, the mRNA level was 3.41 ± 0.41-fold compared with sham-operated mice (Table 1). However, in mutant mice, the increase in TGF-β mRNA levels was blunted. The increase in TGF-β mRNA level in OBK above that in SOK was significantly less at 2 d (39%, P < 0.01) and 5 d (43%, P < 0.05).

NF-κB Activation

In wild mice, UUO increased NF-κB activity after 2 and 5 d relative to that in SOK (Figure 8). It increased 3.2-fold at 2 d and 5.5-fold at 5 d. In contrast, in mutant mice, NF-κB activation was not increased at 2 d. Even at 5 d of UUO, the degree of NF-κB activation was significantly suppressed compared with that in SOK (Figures 9 and 10).

Table 1. Immunohistochemical analysis and TGF-β mRNA expression in the kidney of wild and mutant mice with experimentally-induced unilateral ureteral obstruction

<table>
<thead>
<tr>
<th>Wild Mice</th>
<th>Mutant Mice</th>
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<tr>
<td>Sham Operated</td>
<td>2 D after UUO</td>
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<tr>
<td>2 D after UUO</td>
<td>10 D after UUO</td>
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<tr>
<td>Type III collagen matrix score b</td>
<td>0.19 ± 0.18</td>
</tr>
<tr>
<td>Type IV collagen matrix score b</td>
<td>0.43 ± 0.50</td>
</tr>
<tr>
<td>Relative density of TGF-β mRNA c</td>
<td>1.00 ± 0.09</td>
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<td>a</td>
<td>b</td>
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a TGF-β, transforming growth factor-β; UUO, unilateral ureteral obstruction; GAPDH, glyceraldehyde phosphate dehydrogenase; ND, not done.
b Values represent the mean ± SD of the matrix score (average of 20 independent fields) of each of three separate obstructed kidneys.
c Values represent the mean ± SD of the ratio of optical density for TGF-β mRNA to that of GAPDH mRNA.

P < 0.01 compared with sham-operated mice of the same group.
P < 0.05 compared with wild mice at the same days after UUO.

Figure 4. Immunofluorescent micrographs of OBK stained for type IV collagen. Wild sham-operated kidney (A), mutant sham-operated kidney (B), wild OBK at 5 d after UUO (C), and mutant OBK at 5 d after UUO (D). Magnification, ×200.
Discussion

Previous studies have shown that UUO in rats resulted in tubulointerstitial fibrosis (3,5,17,22) and that such pathologic changes could be reduced by ACE inhibitors or AT1 receptor antagonists (10,17). Furthermore, reduced angiotensinogen expression attenuated renal interstitial fibrosis as a result of UUO in mice (23). These results suggest that in this model, RAS plays an important role in the pathogenesis of interstitial fibrosis. The physiologic effects of Ang II within the kidney are mediated primarily by the AT1 receptor subtype (24). The AT1 in rat and mouse has been further subclassified by molecular cloning into AT1a and AT1b subtypes (13,25,26), which show 94% homology at the amino acid level and are pharmacologically indistinguishable from each other (13). A complete block of the AT1a receptor is present in AT1a-deficient mice from birth; thus, this mouse is useful for investigating the specific function of AT1a. Using this model, we examined in this study the role of the Ang II–AT1a system in the development of interstitial fibrosis. First, we demonstrated a lack of expression of AT1b in this mouse by RT-PCR and thus confirmed that in this model, Ang II does not act through AT1b.

Figure 5. F4/80 staining of the tubulointerstitium after UUO in wild or homozygous mutant mice. Wild-sham operated kidney (A), mutant-sham operated kidney (B), wild OBK at 5 days after UUO (C) and mutant OBK at 5 days after UUO (D). Negative control staining using wild OBK section are shown in E. Original magnification: ×400.

Figure 6. Number of infiltrating F4/80-positive cells in the tubulointerstitium of the UUO model in wild or homozygous mutant mice. Data are mean ± SD. □, wild mice; ■, mutant mice. The number of F4/80-positive cells per ×200 field of kidney cortex of four separate OBK was counted as described in the Materials and Methods section. *, P < 0.01 compared with wild, day 2; **, P < 0.05 compared with wild, day 5.

Figure 7. Transforming growth factor-β (TGF-β) mRNA of UUO model in wild or homozygous mutant mice. Northern blots of the cortex and inner medulla RNA from wild or homozygous mutant mice after sham operation, 2 and 5 d after UUO. A total of 20 μg of RNA was loaded in each lane.

Morphologic examination of the kidney of homozygous mutant mice showed hypertrophy of juxtaglomerular apparatus and focal hypertrophy of intrarenal artery. Angiotensinogen-deficient mice and ACE-deficient mice typically develop abnormal kidney with atrophy of renal papillae and renal vascular thickening (27,28). Unlike these mice, homozygous mutant mice used in our study did not show any histologic abnormalities in the tubulointerstitium of the renal cortex.

Our results showed that the volume fraction (Vv) of the cortical interstitium in the OBK in mutant mice was significantly less than in wild mice. After 2 d of UUO, wild mice showed a 3.5-fold increase in interstitial volume, but mutant mice showed only a 1.2-fold increase. However, after 5 d of UUO, the difference in Vv between two groups had become
smaller. After 10 d, there were no differences between wild mice and mutant mice. These differences in the early and late pathologic responses to UUO might be due to different mechanisms inducing these changes. In this regard, previous studies have demonstrated that both vitamin E (29), an antioxidant, and arginine (30), which increases nitric oxide (NO) production, improved renal tubulointerstitial fibrosis. Reduced production of intrarenal NO and increased production of reactive oxygen species have been implicated in the tubulointerstitial fibrosis process, which is characteristic in the late phase in the UUO model. Furthermore, because of the lack of Ang II–AT1a system in our mutant mice, certain compensatory mechanisms, such as endothelin-1 (1), might explain the difference in the late and early phases in our model. It has been demonstrated that angiotensin regulates at least 50% of the renal interstitial fibrotic response in obstructive nephropathy using mice altering the number of copies of the angiotensinogen gene (23).

Immunohistochemical studies showed less deposition of types III and IV collagen in mutant mice relative to wild mice. Type IV collagen, a major component of the basement membrane, was observed in the thickened tubular basement membrane and widened interstitial space. The amount of type IV collagen in the interstitium of mutant OBK after 2d of UUO was similar to the sham-operated mice. However, after 5 d, type IV collagen deposition increased in the interstitial space to a level similar to that in the wild mice.

TGF-β has a pleiotropic effect on matrix protein production. TGF-β stimulates gene expression of ECM components (31). Our results showed that the level of TGF-β mRNA was increased in the cortex of OBK of the wild mice compared with SOK. This finding suggests that an increased level of TGF-β in

Figure 8. Nuclear factor-kB (NF-kB) activation. Gel shift assay of NF-kB are shown. Lane 1, wild sham-operated kidney; lane 2, wild OBK after 2 d; lane 3, wild OBK after 5 d; lane 4, mutant sham-operated kidney; lane 5, mutant OBK after 2 d; lane 6, mutant OBK after 5 d.

Figure 9. Scanning densitometry of NF-kB activation. The relative intensity to sham groups is shown. Data are mean ± SD. □, wild mice; ■, mutant mice. n = 4 for each sham-operated group; n = 5 for each group at days 2 and 5. *, P < 0.005 compared with wild, day 2; **, P < 0.01 compared with wild, day 5.

Figure 10. Specificity of NF-kB DNA binding. Lane 1, nuclear extracts from wild OBK after 5 d; lane 2, pretreated with 50× excess cold NF-kB; lane 3, pretreated with 10× excess cold mutant NF-kB; lane 4, pretreated with anti-p60 antibody; lane 5, pretreated with anti-p50 antibody.
the cortex augmented the synthesis of the ECM component. In mutant mice, a UUO-induced increase in the level of TGF-β mRNA in the OBK was blunted relative to wild mice, suggesting that increased levels of Ang II stimulate gene expression of TGF-β and result in accumulation of ECM. Fern et al. (33) also showed that renal expression of TGF-β was not increased by UUO in mice that did not express the angiotensinogen gene. ECM might accumulate not only by upregulation of its synthesis but also by downregulation of its degradation. ECM proteins are degraded by various proteinases. Among these, the plasmin activator-plasmin system plays a major role. Plasminogen activator inhibitor-1 inhibits plasmin activation, which causes ECM storage. In this regard, Ang II is known to stimulate the production of TGF-β and plasminogen activator inhibitor-1 leading to a rapid matrix accumulation (32).

Infiltration of monocytes/macrophages was observed in the renal cortex of the OBK during UUO. Ishidoya et al. (10) reported that administration of ACE inhibitor but not Ang II receptor antagonist resulted in a significant reduction in the number of infiltrating monocytes/macrophages. In contrast, Lafayette et al. (33) showed that ACE and AT1 blockade resulted in similar beneficial effects on tissue injury after subtotal nephrectomy. In another study, Wu et al. (34) demonstrated that treatment with both drugs reduced the infiltration of monocytes/macrophages in the interstitium. In the present study, the number of monocytes/macrophages in the OBK of mutant mice was less than in wild mice. Several chemotactants for monocyte/macrophage infiltration have been identified, including macrophage-colony stimulating factor (18), a lipid factor (35), monocyte chemotactic protein-1 (MCP-1) (36), and osteopontin (37). MCP-1 gene transcription has been shown to be regulated by NF-κB (38). Recently, several reports have indicated the possible role of Ang II to induce NF-κB activation; ACE inhibitor prevented NF-κB activation, MCP-1 expression, and macrophage infiltration in a rabbit model of early accelerated arteriosclerosis and in a rat model of immune complex nephritis (39,40). More recent, Morrissey and Klahr (41) showed that ACE inhibitor decreased NF-κB activation in OBK. We also showed that in the UUO model, NF-κB activity in the mutant mice was lower than in wild mice. Together, these results indicate that NF-κB activation was mediated by the Ang II–AT1a system in renal cortex.

In summary, we demonstrated that the relative volume of the tubulointerstitium was significantly increased in the mouse renal cortex of the OBK at 2 or 5d after UUO compared with SOK. This expansion was due to an increase in the deposition of type III collagen and type IV collagen and monocyte/macrophage infiltration in the tubulointerstitium. We also showed that the expression of TGF-β mRNA and NF-κB activation was increased in OBK. In the mutant mouse, the increase in the interstitial volume, deposition of type III collagen and type IV collagen, the number of infiltrating monocytes/macrophages in the tubulointerstitium, expression of TGF-β mRNA, and NF-κB activation were significantly reduced compared with the wild mice. These results indicated that the Ang II–AT1a system plays an important role in the progression of tubulointerstitial fibrosis in UUO in mice.

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