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Article

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Angiotensin II plays a pathogenic role in immune-mediated renal injury in mice

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Several lines of evidence show the importance of angiotensin II (AII) in renal injuries, especially when hemodynamic abnormalities are involved. To elucidate the role of AII in immune-mediated renal injury, we studied anti–glomerular basement membrane (GBM) nephritis in AII type 1a receptor (AT1a)–deficient homozygous ($AT1a^{-/-}$) and wild-type ($AT1a^{+/+}$) mice. A transient activation of the renin–angiotensin system (RAS) was observed in both groups of mice at around day 1. A renal expression of monocyte chemoattractant protein-1 (MCP-1) was transiently induced at six hours in both groups, which was then downregulated at day 1. In the $AT1a^{+/+}$ mice, after RAS activation, the glomerular expression of MCP-1 was exacerbated at days 7 and 14. Thereafter, severe proteinuria developed, and the renal expressions of transforming growth factor-β1 (TGF-β1) and collagen type I increased, resulting in severe glomerulosclerosis and interstitial fibrosis. In contrast, glomerular expression of MCP-1, proteinuria, and tissue damage were markedly ameliorated in the $AT1a^{-/-}$ mice. Because this amelioration is likely due to the lack of AT1a, we can conclude that AII action, mediated by AT1a, plays a pathogenic role in anti-GBM nephritis, in which AII may contribute to the exacerbation of glomerular MCP-1 expression. These results suggest the involvement of AII in immune-mediated renal injuries.

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Introduction

Clinical and experimental studies indicate that angiotensin II (AII) plays a pathophysiological role in the progression of chronic renal diseases, especially when hemodynamic abnormalities are involved (1). This concept is based on the observations that angiotensin-converting enzyme inhibitors (ACEIs) and AII receptor antagonists reduced the proteinuria and tissue damage in patients with hypertension and in experimental models characterized by elevated systemic and glomerular capillary pressure (2–6). In recent clinical and experimental studies (7, 8), the inhibition of the renin–angiotensin system (RAS) with ACEIs or AII receptor antagonists has been demonstrated to ameliorate the renal diseases of normotensive patients and in normotensive experimental models. To understand the biologic significance of AII in renal diseases, it is important to view it in the broader context of the pathogenesis of renal diseases. Because most human renal diseases are of immune origin, we hypothesized that AII is also involved in the pathogenesis of immune-mediated renal diseases. In fact, ACEIs are effective for IgA nephropathy, even in normotensive patients (9). In an experimental study, ACEIs reduced the proteinuria and renal tissue damage in immune-complex glomerulonephritis (10). To explore our hypothesis, we examined the involvement of AII in the pathogenesis of immune-mediated renal disease.

Of the immune-mediated experimental renal diseases, anti–glomerular basement membrane (GBM) nephritis has been most widely studied (11). Some investigators have reported the activation of the RAS in the acute phase of anti-GBM nephritis, suggesting the role of AII (12, 13). However, the pathogenic role of AII has not been established. Therefore, we planned to examine the involvement of AII in anti-GBM nephritis. There are several methods that may be used to achieve this aim, *e.g.*, the use of inhibitors of the RAS, such as ACEIs or AII receptor antagonists. However, these agents have some problems because of nonspecific effects at high concentrations. One of the effective ways to overcome this problem is to use genetically engineered animals deficient for particular components of the RAS.

The biologic roles of AII are mediated by high-affinity membrane-bound receptors, which have recently been classified into two subtypes: AT1 and AT2. AII exerts its biologic effects mainly through AT1 receptors in the kidney (14, 15). In rodents, AT1 exists as two isoforms, AT1a and AT1b, encoded by two different genes. We recently generated an AT1a-deficient mouse strain, which is a powerful model for analyses of the effect of AII blockade (16). To elucidate the pathophysiological role of AII in immune-mediated renal injury, we induced anti-GBM nephritis in AT1a-deficient homozygous ($AT1a^{-/-}$) mice and wild-type ($AT1a^{+/+}$) mice. Using this model, we evaluated the direct effect of AII in the pathogenesis of immune-mediated renal injury.

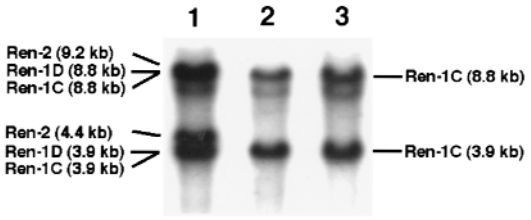

Figure 1
Genotype analysis of the renin genes. After *Eco*RI restriction enzyme digestion, the genomic DNA was analyzed by Southern blot analysis. Lane *1*, TT2 ES cells used for the generation of the AT1a$^{-/-}$ mice; Lane *2*, the AT1a$^{-/-}$ mice; Lane *3*, C57Bl/6 mice. The *Ren-2* and *Ren-1D* genes were detected as 4.4-, 9.2-kb fragments and 3.9-, 8.8-kb fragments respectively.. The *Ren-1C* gene was detected as 3.9- and 8.8-kb fragments. The AT1a$^{-/-}$ mice have the *Ren-1C* genotype identical to that of C57Bl/6 mice. *AT1a$^{-/-}$ mice,* angiotensin II type 1a receptor–deficient homozygous mice.

Methods

Animals. To obtain AT1a-deficient heterozygous (AT1a$^{+/-}$) mice that have C57Bl/6 background, a germline chimera derived from TT2 embryonic stem (ES) cells with a targeted mutation of the *AT1a* gene as described previously (16), was backcrossed for five generations with C57Bl/6 mice. The resulting AT1a$^{+/-}$ F_5 mice were then intercrossed to generate the homozygous (AT1a$^{-/-}$) mice. Concerning the genotype of the renin gene (17) in the AT1a$^{-/-}$ mice, Southern blot analysis was performed as described previously (18), and identity of the *Ren-1C* gene with that of C57Bl/6 mice was confirmed (Fig. 1). The AT1a$^{-/-}$ mice were then inbred to prepare the enough number of animals for the present study. As AT1a$^{+/+}$ mice, C57Bl/6 mice were purchased from Japan Clea Co. (Tokyo, Japan). Only male mice at the age of 10 weeks were used for the studies. For the isolation of GBM and the preparation of anti-GBM antiserum (AS), ddy mice and Japanese white rabbits, respectively, were purchased from local breeders.

Preparation of rabbit anti-GBM AS. The preparation of anti-GBM AS was performed as described by Nagai *et al.* (19). In brief, glomeruli were isolated by differential sieving from the mouse renal cortex and disrupted by sonication. The GBM was collected by centrifugation and emulsified with CFA (Difco Laboratories, Detroit, Michigan, USA). Anti-GBM AS was raised in Japanese white rabbits by repeated immunization with mouse GBM.

Induction of anti-GBM nephritis. Anti-GBM nephritis was induced in the AT1a$^{-/-}$ and AT1a$^{+/+}$ mice according to the method described by Nagai *et al.* (19). In brief, mice were immunized intraperitoneally with 0.5 mg of rabbit IgG (Organon Teknika Corp., West Chester, Pennsylvania, USA) per 20 g body weight emulsified with CFA. Five days after immunization, 0.3 ml of anti-GBM AS per 20 g body weight, diluted with two parts saline, was administered intravenously via the tail vein.

Experimental design. The AT1a$^{-/-}$ mice and AT1a$^{+/+}$ mice each were divided into two groups. Mice in the first group were sacrificed at 6 h and days 1, 7, 14, 42, 84, and 126 after the administration of anti-GBM AS. Total RNA was extracted from the kidneys at every time point. The extraction of total RNA from the livers and the collection of plasma samples were performed at 6 h and days 1, 7, and 14. The kidneys were processed for histological analysis at 6 h and days 7 and 126. To examine the changes in proteinuria with the passage of time, the urinary protein excretion rate was measured at days 3, 7, 14, 21, 28, 42, 70, 98, and 126 in the mice sacrificed at day 126. The mean blood pressure (MBP) of the second group mice was measured at 6 h and days 1, 2, 3, 6, 7, 8, 9, 10, and 14. The number of mice used for each experiment was as follows. First group: Of the AT1a$^{-/-}$ mice, three mice each at 6 h and day 1, four mice at day 7, five mice at days 14 and 126, eight mice at day 42, and six mice at day 84 were used for the studies. Of the AT1a$^{+/+}$ mice, three mice at 6 h, five mice at day 1, and six mice each were used at days 7, 14, 42, 84, and 126. As control mice, in which nephritis was not induced, three nontreated AT1a$^{-/-}$ and AT1a$^{+/+}$ mice each were studied at each time point. Second group: Five AT1a$^{-/-}$ and AT1a$^{+/+}$ mice each were used.

Collection of urine and determination of urinary protein excretion rate. The mice were housed in individual metabolic cages with free access to tap water and standard chow. Urine samples were collected for 24 h. The amount of protein excreted into urine was determined using a protein assay kit (Nippon Bio-Rad Laboratories, Tokyo, Japan).

Estimation of the amount of active renin in the plasma. Blood samples were withdrawn from the AT1a$^{-/-}$ and AT1a$^{+/+}$ mice under pentobarbital anesthesia. Blood was collected into ice-cold microcentrifuge tubes containing EDTA and was then immediately centrifuged to isolate the plasma fraction. The amount of active renin in the plasma was estimated by an RIA, as described previously (20).

Measurement of MBP. The MBP was measured by a programmable sphygmomanometer (BP-200; Softron, Co., Ltd. Tokyo, Japan) using the tail-cuff method as described previously (21) under the conscious condition.

Estimation of circulating anti–rabbit IgG antibody. The circulating anti–rabbit IgG level was estimated by ELISA. Ninety-six–well ELISA plates (Nippon InterMed, Tokyo, Japan) coated with rabbit IgG (Organon Teknika Corp.) were incubated with test plasma that was diluted to 1:1,000. After being washed extensively with PBS containing 0.05% Tween-20, the plates were incubated with horseradish peroxidase–conjugated rat anti–mouse IgG (Jackson Immunoresearch Laboratories, West Grove, Pennsylvania, USA) diluted to 1:5,000. A kinetic analysis of absorbance at 650 nm was performed using 3,3′, 5,5′-tetramethylbenzidine (Nacalai Tesque, Kyoto, Japan) as a substrate. The level of anti–rabbit IgG was estimated by comparing the initial velocity of the increase in absorbance at 650 nm.

Preparation of total RNA and Northern blot analysis. Total RNA was prepared using a total RNA separator kit (CLONTECH Laboratories Inc., Palo Alto, California, USA). For the Northern blot analysis, 20 μg of total RNA was denatured with formamide, fractionated by electrophoresis through 1% agarose gels, and transferred onto nylon membranes (Amersham International, Buckinghamshire, England). The blots were hybridized with [^{32}P]dCTP–labeled specific probes. The gene expression was estimated by counting the radioactivities of ^{32}P-labeled specific probes hybridized to mRNA using an image analyzer (Instant Imager Electronic Autoradiography; Packard Instrument Co., Meriden, Connecticut, USA). To adjust the difference of sample loading, the membranes were rehybridized with [^{32}P]dCTP–labeled probes for 28S rRNA. The changes in the mRNA levels were determined by correcting for the radioactivity of 28S rRNA for each sample. The probes for AT1a (16), angiotensinogen (22), and MCP-1 (23) were described previously. To analyze the β-galactosidase (lacZ) and collagen type I expression, a 667-bp *Sal*I fragment of the *Escherichia coli* lacZ cDNA and human α2(I) cDNA (American Type Culture Collection, Rockville, Maryland, USA), respectively, were used as probes. To obtain the cDNA probe for mouse transforming growth factor-β1 (TGF-β1), a reverse transcription-PCR was performed using total RNA prepared from cultured mouse mesangial cells. The fidelity of the cDNA fragment was confirmed by a DNA sequence analysis. The probe for 28S rRNA was a generous gift from N. Kashihara.

Histological analysis. For the immunohistochemistry of rabbit IgG, mouse IgG, and collagen type I, the kidneys were fixed in 4% buffered formaldehyde and embedded in paraffin, and 4-μm-thick cryostat sections were prepared. The immunos-

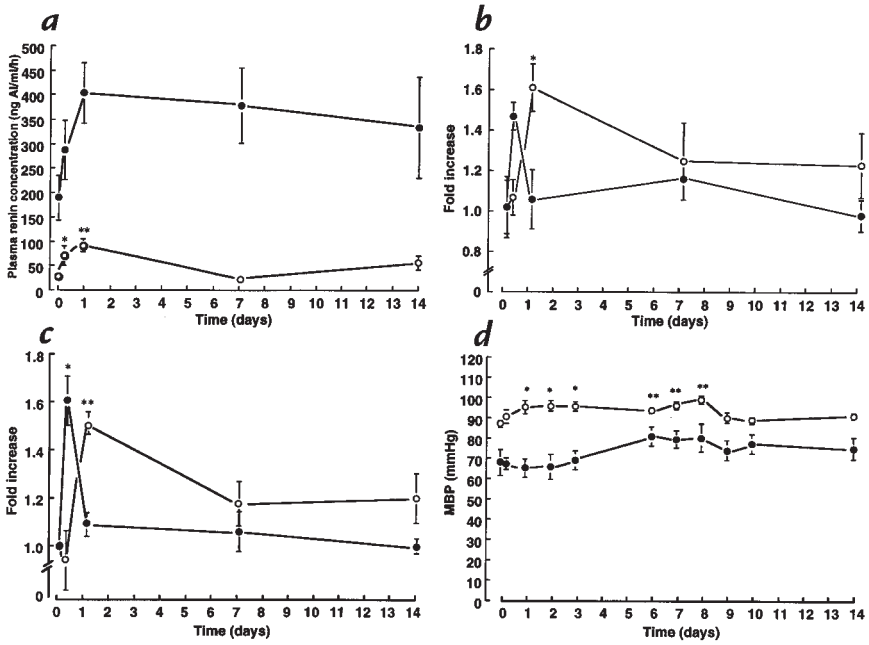

Figure 2
Activation of the RAS after administration of anti-GBM AS. Mice were sacrificed at 6 h and days 1, 7, and 14 after the administration of anti-GBM AS. (***a***) The amount of active renin in the plasma was estimated by RIA as described in the Methods. (***b***) The expression of the angiotensinogen mRNA in the liver was examined by Northern blot analysis. Open circles represent the AT1a$^{+/+}$ mice, and closed circles represent the AT1a$^{-/-}$ mice. (***c***) Renal expressions of AT1a mRNA in the AT1a$^{+/+}$ mice and lacZ mRNA in the AT1a$^{-/-}$ mice. Open circles and closed circles represent the gene expressions of AT1a and lacZ, respectively. Results at each time point in *b* and *c* were given as the fold increase compared with those observed before the induction of nephritis (day 0). (***d***) Changes in the MBP. Open circles represent the AT1a$^{+/+}$ mice, and closed circles represent the AT1a$^{-/-}$ mice. Data are mean ± SE. Statistical significance was evaluated by comparing the data at each time point with those obtained before the administration of anti-GBM AS (day 0). $*P < 0.05$, $**P < 0.01$. *AI, angiotension I; AS,* antiserum; *AT1a,* angiotensin II type 1a receptor; *AT1a$^{+/+}$ mice,* AT1a wild-type mice; *GBM,* glomerular basement membrane; *MBP,* mean blood pressure; *RAS,* renin–angiotensin system.

taining of rabbit IgG and mouse IgG was performed using rabbit IgG and mouse IgG detection kits (Vector Laboratories, Burlingame, California, USA), respectively. For the immunohistochemistry of the third component of the complement system (C3), MCP-1 and TGF-β1, the kidneys were snap frozen in liquid nitrogen, and 8-μm-thick cryostat sections were prepared and then fixed in acetone. The sections were incubated with the following antibodies: goat anti–human collagen type I cross-reactive with mouse collagen type I (Southern Biotechnology Associates, Birmingham, Alabama, USA), goat anti–mouse C3c (Nordic Immunological Laboratories, Tilburg, the Netherlands), and goat anti–mouse MCP-1 (Santa Cruz Biotechnology Inc., Santa Cruz, California, USA). For the immunostaining of TGF-β1, rabbit polyclonal antibody to human TGF-β1 cross-reactive with mouse TGF-β1 (Santa Cruz Biotechnology) was conjugated with biotin using NHS-Biotin (Pierce Chemical Co., Rockford, Illinois, USA). After being incubated with biotin-conjugated second antibody, except for TGF-β1, the tissue sections were processed using a streptoavidin-biotin immunoperoxidase kit (Vector Laboratories), and peroxidase enzyme were detected using 3,3′-diaminobenzidine tetrahydrochloride (Wako Pure Chemical Industries, Osaka, Japan) as a substrate.

For semiquantification of renal tissue damage, the sections at day 126 were stained using the Azan-Mallory method. Areas occupied by extracellular matrix was calculated using a photoimaging system (Imaging Research Inc., Ontario, Canada) by an operator unaware of the origin of each slide. The glomerular and interstitial scores were determined following the modified criteria originally described by Floege *et al.* (24) and Shih *et al.* (25), respectively. Glomerular score: 0 = normal or <1%, 1 = 1%–<25%, 2 = 25%–50%, 3 = 50%–75%, 4 = >75% of the glomerulus; interstitial score: 0 = normal, 1 = <10%, 2 = 10%–25%, 3 = 25%–75%, 4 = >75% of the cortex.

Statistical analysis. Results are expressed as means ± SEM. Statistical significance was determined using Student's *t* test. $P < 0.05$ was considered significant.

Results

Activation of the RAS in the acute phase of renal injury. To evaluate the activation of the RAS after the induction of nephritis, we examined the changes in the amount of active renin in the plasma. Six hours after the administration of anti-GBM AS, the amount of active renin was increased in both the AT1a$^{+/+}$ and AT1a$^{-/-}$ mice. The maximum increase was observed at day 1, with 3.3- and 2.1-fold increases in the AT1a$^{+/+}$ (27.6 ± 5.2 ng AI/ml/h at day 0 vs. 91.9 ± 13.7 ng AI/ml/h at day 1) and AT1a$^{-/-}$ mice (189.3 ± 51.5 ng AI/ml/h at day 0 vs. 403.9 ± 62.6 ng AI/ml/h at day 1), respectively (Fig. 2*a*).

We also analyzed the gene expression of angiotensinogen in the liver. In the AT1a$^{+/+}$ mice, the expression of the angiotensinogen mRNA was augmented at day 1, corresponding to the increase of the active renin in the plasma. In the AT1a$^{-/-}$ mice, the augmentation of angiotensinogen gene expression was observed at six hours (Fig. 2*b*).

The expression of AII receptor is a very important factor in evaluating the RAS activation. Because AT1a is one of the prominent receptors that mediate a biologic action

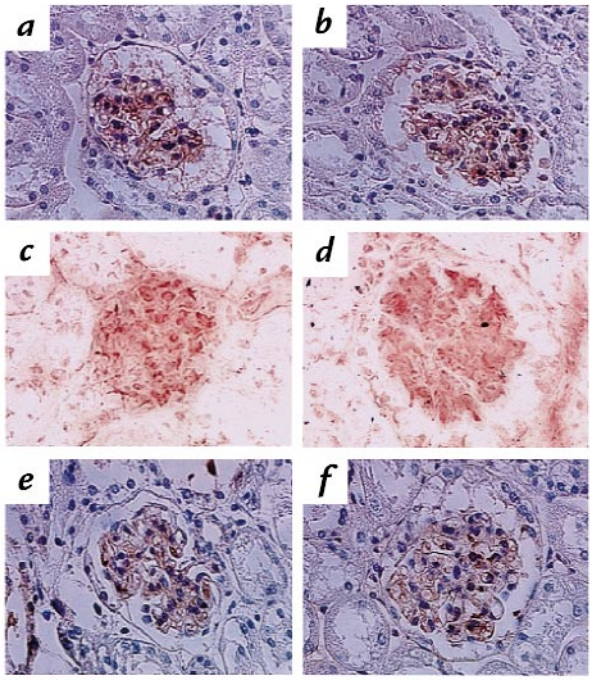

Figure 3
Immunohistochemical findings in the kidney after the administration of anti-GBM AS in the AT1a$^{+/+}$ (***a***, ***c,*** and ***e***) and AT1a$^{-/-}$ mice (***b***, ***d,*** and ***f***). Linear binding of rabbit IgG to the GBM at 6 h (*a* and *b*). Fine granular deposits of C3 along the capillary wall, with additional deposition in the mesangium at 6 h (*c* and *d*). Linear binding of mouse IgG, identical to rabbit IgG, in the glomeruli at 7 days (*e* and *f*). ×400.

of AII in the kidney, the gene expression of AT1a was examined in the AT1a$^{+/+}$ mice. In the AT1a$^{-/-}$ mice, the *AT1a* gene is replaced with the *lacZ* gene adjacent to the AT1a promoter, as we reported previously (16). Therefore, the AT1a promoter activity can be estimated by the examination of *lacZ* expression in the AT1a$^{-/-}$ mice. After the anti-GBM AS administration, the gene expression of AT1a was suppressed transiently (but not significantly) and then elevated by 1.5-fold at day 1. Thereafter, the expression was decreased. In the AT1a$^{-/-}$ mice, the expression of *lacZ* was also augmented transiently and peaked at six hours with a 1.6-fold increase (Fig. 2*c*).

To confirm the lack of AT1a-mediated action of AII in the AT1a$^{-/-}$ mice, changes in the MBP were examined during the first 14 days after the administration of anti-GBM AS. In the AT1a$^{+/+}$ mice, a slight but significant elevation of the MBP was observed in two phases. In the first phase, the MBP showed an upward trend at six hours and was significantly elevated between days 1 and 3 (from 87 ± 2 mmHg before the anti-GBM AS administration to 96 ± 3 mmHg at day 2). In the second phase, an elevation of the MBP (up to 99 ± 2 mmHg) was observed between days 6 and 8. Subsequently, the MBP declined to values similar to those observed before the administration of anti-GBM AS (89 ± 2 mmHg at day 10). The MBP was normotensive throughout the rest of the study period (data not shown). In the AT1a$^{-/-}$ mice, the MBP was not elevated in the first phase, but a tendency of the MBP elevation was observed in the second phase (Fig. 2*d*). In the control experiments in which normal rabbit serum was administered, the MBP showed an upward trend between days 6 and 8 corresponding to the second phase (data not shown), indicating that the elevation of the MBP in the second phase was nonspecific. Because the RAS was activated at around day 1, the elevation of the MBP observed in the AT1a$^{+/+}$ mice in the first phase was thought to be caused by the activation of the RAS. Thus, the lack of the MBP elevation in the first phase confirmed that the AT1a-mediated action of AII was abolished in the AT1a$^{-/-}$ mice.

Examination of inflammatory and immunological responses. To compare the acute-phase injuries, we examined the inflammatory and immunological responses provoked in the AT1a$^{+/+}$ and AT1a$^{-/-}$ mice at six hours and day 7. Similar bindings of rabbit IgG (Fig. 3, *a* and *b*) and C3 (Fig. 3, *c* and *d*) on the glomeruli was observed at six hours in both groups of mice, suggesting that comparable inflammatory responses were provoked. We also performed an enzyme-linked immunosorbent assay (ELISA) to examine the production of circulating autologous antibody (mouse IgG) to rabbit IgG. An increase in the autologous antibody production was observed at day 1 (data not shown), and a further increase was observed at day 7. There was no significant difference in the amount of autologous antibody between the AT1a$^{+/+}$ and AT1a$^{-/-}$ mice at day 7 (214.4 ± 7.26 mOD/min in the AT1a$^{+/+}$ mice vs. 258.4 ± 16.61 mOD/min in the AT1a$^{-/-}$ mice). The immunoperoxidase staining revealed the similar binding of mouse IgG to rabbit IgG fixed in the glomeruli, which confirmed the formation of an *in situ* immune complex in both groups (Fig. 3, *e* and *f*).

Renal expression of MCP-1. To analyze further the early inflammatory response, the renal expression of MCP-1 was examined by Northern blot analysis. Six hours and 1, 7, and 14 days after the anti-GBM AS administration, total RNA was prepared from the whole kidneys, and the expression of mRNA for MCP-1 was examined. In the nontreated AT1a$^{+/+}$ and AT1a$^{-/-}$ mice, the MCP-1 mRNA was not detectable. Six hours after the anti-GBM AS administration, the gene expression of MCP-1 was induced in both the AT1a$^{+/+}$ and AT1a$^{-/-}$ mice, which was then downregulated at day 1. The expression was induced again at day 7, and further induction was observed at day 14 in the AT1a$^{+/+}$ mice (Fig. 4, *a* and *b*). This is in good agreement with the findings of Tang *et al.* (26), who reported the similar biphasic induction of MCP-1 expression in rat anti-GBM glomerulonephritis. Our immunohistological analysis confirmed the glomerular localization of MCP-1 protein (Fig. 4*c*, *left*). In addition, electron microscopy revealed the presence of infiltrated macrophages in the glomeruli of the AT1a$^{+/+}$ mice at day 7 (data not shown). In contrast, only a slight induction of MCP-1 gene expression was observed at days 7 and 14 in the AT1a$^{-/-}$ mice (Fig. 4, *a* and *b*). The immunohistochemical analysis confirmed that the glomerular expression of MCP-1 protein was reduced in the AT1a$^{-/-}$ mice at day 7 (Fig. 4*c*, *right*).

Urinary protein excretion. The excretion of urinary protein was observed by day 3 in the AT1a$^{+/+}$ mice, peaked at day 14 (3.61 ± 0.32 mg/24 h/g body weight), and per-

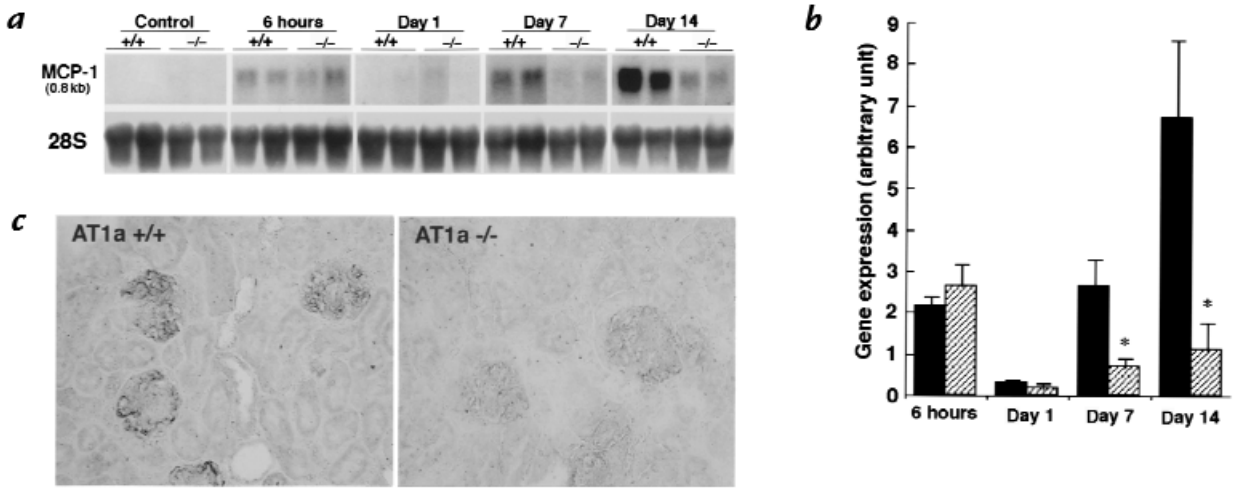

Figure 4

Expression of MCP-1. (***a***) Gene expression of MCP-1 during the first 14 days after the anti-GBM AS administration was examined by Northern blot analysis. Total RNA from mice at day 0 (control), 6 h, and days 1, 7, and 14 was separated on an agarose gel and probed for MCP-1. The blots were stripped and rehybridized with a cDNA probe for the 28S rRNA. Two representative results of each animal group were shown. (***b***) The gene expression of MCP-1 was estimated as described in Methods. The closed bars represent the AT1a$^{+/+}$ mice, and the hatched bars represent the AT1a$^{-/-}$ mice. Data are mean ± SE in arbitrary units. *$P < 0.05$ compared with the AT1a$^{+/+}$ mice. (***c***) Immunoperoxidase staining for MCP-1 at day 7 after anti-GBM AS administration. In the AT1a$^{+/+}$ mice, MCP-1 expression was propagated in the glomeruli. In contrast, MCP-1 expression was reduced in the AT1a$^{-/-}$ mice. ×200. *MCP-1,* monocyte chemoattractant protein-1.

sisted throughout the period of study. In contrast, a marked reduction of proteinuria (peaked at day 7 with 0.57 ± 0.37 mg/24 h/g body weight) was observed in the AT1a$^{-/-}$ mice (Fig. 5).

Expressions of TGF-β1 and collagen type I. To analyze the development of renal injury, we examined the gene expressions of TGF-β1 and collagen type I by Northern blot analysis. An immunohistochemical analysis was also performed at day 126 to examine the localization of these proteins. In the AT1a$^{+/+}$ mice, the gene expression of TGF-β1 was increased by 3.5-fold at day 14; after declining at day 42, it increased again and continued to increase until day 126, when a 3.0-fold increase was observed. In contrast, the expression of TGF-β1 in the AT1a$^{-/-}$ mice was increased slightly throughout the period of study, with 1.9- and 1.3-fold increases observed at six hours and day 126, respectively (Fig. 6*a* and Fig. 6*b*, *top*). The immunohistochemical analysis revealed that the expression of TGF-β1 was propagated in the glomeruli of the AT1a$^{+/+}$ mice (Fig. 6*c*, *top left*) but was markedly reduced in the AT1a$^{-/-}$ mice (Fig. 6*c*, *top right*).

The gene expression of collagen type I was examined by Northern blot analysis using a specific probe for α2(I). In the AT1a$^{+/+}$ mice, the increase in α2(I) expression showed an upward trend at day 7. A significant increase in α2(I) expression was observed at day 14. Similar to the TGF-β1 results, the α2(I) expression decreased at day 42, then elevated again, and gradually increased until day 126. The magnitude of the increase in α2(I) gene expression in the AT1a$^{+/+}$ mice was 7.7- and 6.4-fold at days 14 and 126, respectively. In contrast, the α2(I) expression in the AT1a$^{-/-}$ mice was increased by threefold at day 1, but no further increase was observed (Fig. 6*a* and Fig. 6*b, bottom*). The localization of collagen type I was confirmed by immunohistochemical analysis. In the AT1a$^{+/+}$ mice, the expression of collagen type I was propagated in the interstitium (Fig. 6*c*, *bottom left*). In the AT1a$^{-/-}$ mice, the collagen type I expression was markedly reduced (Fig. 6*c*, *bottom right*).

Examination of chronic renal tissue damage. To examine chronic tissue damage, we stained the kidney sections at day 126 using the Azan-Mallory method. As expected from the results of TGF-β1 and collagen type I expressions, an excessive production of extracellular matrix protein was observed in the glomeruli and interstitium, and inflammatory cells were infiltrated into the interstitium, which resulted in severe glomerulosclerosis and tubulointerstitial fibrosis in the AT1a$^{+/+}$ mice (Fig. 7*a*, *top*). In the AT1a$^{-/-}$ mice, these histological changes were markedly reduced (Fig. 7*a*, *bottom*). Semiquantitative analysis of renal tissue damage revealed that both the glomerular and interstitial injuries were ameliorated in the AT1a$^{-/-}$ mice. The glomerular and interstitial scores in the AT1a$^{-/-}$ mice decreased by 87% and 51%, respectively, compared with those in the AT1a$^{+/+}$ mice (glomerular score: 1.81 ± 0.14 in the AT1a$^{+/+}$ mice vs. 0.23 ± 0.10 in the AT1a$^{-/-}$ mice; interstitial score: 2.55 ± 0.18 in the AT1a$^{+/+}$ mice vs. 1.24 ± 0.19 in the AT1a$^{-/-}$ mice) (Fig. 7*b*). The degree of decrease in the interstitial score in the AT1a$^{-/-}$ mice was somewhat smaller than that of the glomerular score, which is likely due to the occasionally observed perivascular fibrosis in the AT1a$^{-/-}$ mice. This appearance of perivascular fibrosis is consistent with the observation that perivascular injuries were appeared in the AT1a$^{-/-}$ mice with C57Bl/6 genetic background (27).

Discussion

In the present study, we examined the involvement of AII in renal injuries induced by the administration of anti-GBM AS. For the first set of experiments, we examined the RAS activation after the administration of anti-GBM AS. Immediately after the administration of anti-GBM

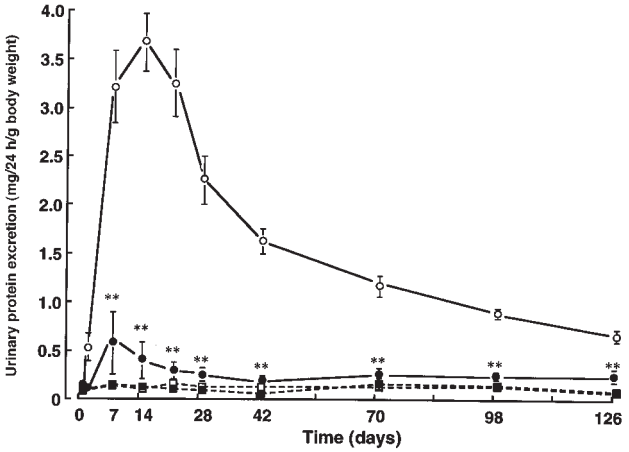

Figure 5

Changes in urinary protein excretion after the administration of anti-GBM AS. Urinary protein excretion was measured in the mice sacrificed at day 126. The open circles and closed circles represent the $AT1a^{+/+}$ and $AT1a^{-/-}$ mice, respectively. The open squares with a dotted line and the closed squares with a dotted line represent the physiological protein excretion of the $AT1a^{+/+}$ and $AT1a^{-/-}$ mice, respectively. Data are mean ± SE. Statistical significance was evaluated by comparing the data of the $AT1a^{-/-}$ mice with those of the $AT1a^{+/+}$ mice at each time point. $^{**}P < 0.01$.

AS, the amount of active renin in the plasma was increased in both the $AT1a^{+/+}$ and $AT1a^{-/-}$ mice. The gene expression of angiotensinogen in the liver was also elevated transiently in both groups of mice. Boyce and Holdsworth (28) demonstrated that in an isolated perfused kidney system, the deposition of anti-GBM antibody on glomeruli increased the intrarenal renin release. In addition, it has been reported that the gene expression of angiotensinogen in the liver was elevated by inflammatory mediators such as interleukin-1 and tumor necrosis factor-α (29). Therefore, it is suggested that in both the $AT1a^{+/+}$ and $AT1a^{-/-}$ mice, the binding of anti-GBM antibody on glomeruli produced the direct increase of intrarenal renin release and the elevation of angiotensinogen gene expression in the liver through the increase of inflammatory mediator levels in the circulation. The Northern blot analysis revealed the transient elevations of AT1a and lacZ gene expressions. Timmermans *et al.* (12) demonstrated that in rat anti-GBM nephritis, the change in AII receptor density took place in response to changes in the circulating AII level, with a ligand/receptor relationship in a feedback fashion. The same mechanism is thought to be operative in our experiment. However, in the $AT1a^{-/-}$ mice lacking such a ligand/receptor relationship, the induction of *lacZ* expression was observed, suggesting the direct elevation of AT1a gene expression independent of a receptor/ligand relationship. Thus, the elevation of AII receptor gene expression may amplify the effect of the RAS activation. The results of the MBP measurements confirmed that the AT1a-mediated action of AII was abolished in the $AT1a^{-/-}$ mice.

The observed differences between the $AT1a^{+/+}$ and $AT1a^{-/-}$ mice might be due to the differences in blood pressure between both groups of mice. Therefore we conducted control experiments using the $AT1a^{+/+}$ mice, whose blood pressure was kept lower. The $AT1a^{+/+}$ mice received daily intraperitoneal injections of 3.9 mg/kg hydralazine throughout the experiment. The values of MBP were as follows: 51.56 ± 2.42, 57.09 ± 1.30, and 78.21 ± 2.04 mmHg at days 0, 1, and 6, respectively. These values were lower than those of the $AT1a^{-/-}$ mice at every time point. The hypotensive $AT1a^{+/+}$ mice showed similar urinary protein excretion to that of the normotensive $AT1a^{+/+}$ mice (2.96 ± 0.22 and 3.12 ± 0.37 mg/24 h/g body weight in the hypotensive $AT1a^{+/+}$ mice and normotensive $AT1a^{+/+}$ mice at day 7, respectively). In addition, immunohistochemistry revealed that the glomerular expression of MCP-1 at day 7 in the hypotensive $AT1a^{+/+}$ mice was also similar to that of the normotensive $AT1a^{+/+}$ mice (data not shown). These results indicate that even the $AT1a^{+/+}$ mice with blood pressure lower than that of the $AT1a^{-/-}$ mice could develop initial injuries similar to those of the normotensive $AT1a^{+/+}$ mice. Thus, the marked amelioration of renal injuries that resulted from the lack of AT1a suggests the crucial role of AII in anti-GBM nephritis, at least in part, independent of the blood pressure effects.

Several investigators suggested that the genetic background had a profound effect on the severity of renal injury (30, 31). We generated AT1a-deficient mice using the TT2 ES cells that have a mixed genetic background of C57Bl/6 and CBA (16). Also, we studied C57Bl/6 mice as the $AT1a^{+/+}$ mice. However, the $AT1a^{-/-}$ mice used in the present study were derived from the intercrossings of the $AT1a^{+/-}$ F_5 mice, as described in Methods. Concerning the genotype of the renin gene in the $AT1a^{-/-}$ mice, identity of the *Ren-1C* gene with that of C57Bl/6 mice was confirmed (Fig. 1). Therefore, we considered that the $AT1a^{-/-}$ mice used in the present study have a genetic background similar to that of C57Bl/6. In addition, with respect to the phenotype of $AT1a^{-/-}$ mice, Oliverio *et al.* (27) reported that perivascular injuries were apparent in the mice with a genetic background of C57Bl/6. We also observed injuries in the nontreated $AT1a^{-/-}$ mice similar to those noticed in their study, which confirmed that the $AT1a^{-/-}$ mice used in the present study have a genetic background of C57Bl/6. Moreover, the $AT1a^{-/-}$ mice, generated by intercrossings of the AT1a ± F_7 mice, showed a reduction of proteinuria similar to that shown in the present study (2.91 ± 0.41 and 0.38 ± 0.23 mg/24 h/g body weight in the $AT1a^{+/+}$ and $AT1a^{-/-}$ mice at day 7, respectively). To confirm further that the observed differences between the $AT1a^{+/+}$ and $AT1a^{-/-}$ mice were not due to the effect of genetic background, we performed a control experiment using the mice with mixed background of C57Bl/6 and CBA. We induced anti-GBM nephritis in C57Bl/6 × CBA F1 mice, which showed similar urinary protein excretion (2.68 ± 0.98 and 2.88 ± 0.85 mg/24 h/g body weight at day 7 in C57Bl/6 × CBA F1 mice and C57Bl/6 mice, respectively) and glomerular protein expression of MCP-1 (data not shown) to those of C57Bl/6 mice. These results suggest that even though the $AT1a^{+/+}$ mice have mixed genetic background of C57Bl/6 and CBA, their susceptibility to anti-GBM nephritis is similar to that of the $AT1a^{+/+}$ mice with C57Bl/6 background. From these facts, it is implausible that the remarkable reduction of renal injuries in the $AT1a^{-/-}$ mice is due to the effect of the difference in the genetic background.

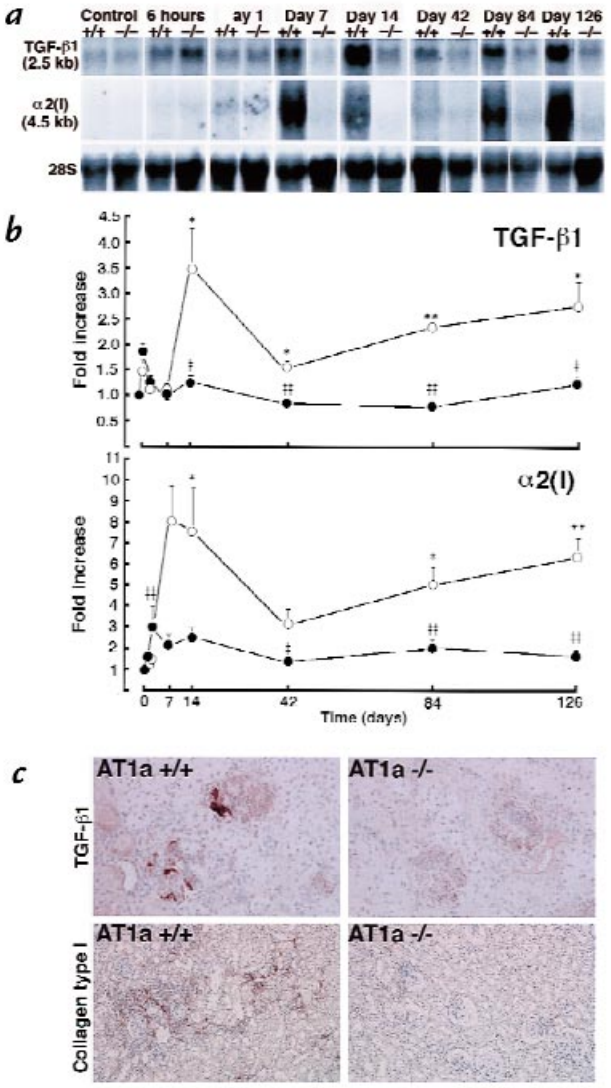

Figure 6

Expressions of TGF-β1 and collagen type I. (***a***) Northern blot analysis was performed using specific probes for TGF-β1 and α2(I). The blots were stripped and rehybridized with a cDNA probe for the 28S rRNA. A representative result of each animal group was shown. (***b***) The gene expressions of TGF-β1 (*top*) and collagen type I (*bottom*) were estimated as described in Methods. The results are presented as the fold increase compared with the values obtained before the administration of anti-GBM AS (day 0). The open circles represent the $AT1a^{+/+}$ mice, and the closed circles represent the $AT1a^{-/-}$ mice. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with day 0; $^{\ddagger}P < 0.05$, $^{\ddagger\ddagger}P < 0.01$ compared with the $AT1a^{+/+}$ mice at each time point. (***c***) Immunoperoxidase staining for TGF-β1 (*top left and top right*) and collagen type I (*bottom left and bottom right*) at day 126 after the anti-GBM AS administration. In the $AT1a^{+/+}$ mice, the expression of TGF-β1 was propagated in the glomeruli, (*top left*)and the expression of collagen type I was propagated in the interstitium(*bottom left*). In the $AT1a^{-/-}$ mice, reduced staining of TGF-β1 (*top right*) and collagen type I (*bottom right*)was observed. TGF-β1, ×200; collagen type I, ×100. *TGF-β1,* transforming growth factor-β1.

Two distinct phases are identified in murine anti-GBM nephritis: the heterologous phase and the autologous phase. The former is a result of a rapid binding of injected heterologous antibodies to glomeruli. The latter is characterized by the immune response of the host to the heterologous antibodies. We found a similar binding of rabbit IgG and C3 on the glomeruli in the $AT1a^{+/+}$ and $AT1a^{-/-}$ mice at six hours, corresponding to the heterologous phase. The autologous phase appeared by day 1 with the increase of autologous antibody production (data not shown). We did not observe a significant difference in the production of autologous antibody and its binding on the glomeruli between the $AT1a^{+/+}$ and $AT1a^{-/-}$ mice at day 7. These results suggest that the marked amelioration of renal injury in the $AT1a^{-/-}$ mice was not a result of an insufficiency of normal inflammatory response or of autologous antibody production.

To analyze the changes in the expression of chemotactic factors that may be involved in the early inflammatory processes, the renal expression of mRNA for MCP-1 was examined. A comparable induction of MCP-1 gene expression in the kidney was observed at six hours in both the $AT1a^{+/+}$ and $AT1a^{-/-}$ mice. Stahl *et al*. (32) reported that the glomerular deposition of IgG immune complexes stimulated the expression of MCP-1 in antithymocyte antibody–induced glomerulonephritis. Because a similar binding of rabbit IgG on the glomeruli was observed at six hours in both groups of mice, the comparable induction of MCP-1 at this time point was thought to be caused by the binding of immune complexes on the glomerular Fc receptors. At day 1, the expression of MCP-1 was reduced, which is likely due to the focal mesangiolysis. The marked difference in the MCP-1 expression between the $AT1a^{+/+}$ and $AT1a^{-/-}$ mice was found after the RAS activation. In the $AT1a^{+/+}$ mice, the gene expression of MCP-1 was induced again at day 7, and a further induction was observed at day 14. In contrast, the induction of MCP-1 gene expression was markedly attenuated in the $AT1a^{-/-}$ mice. The immunostaining for MCP-1 at day 7 revealed that the MCP-1 expression was exacerbated in the glomeruli of the $AT1a^{+/+}$ mice and markedly attenuated in the $AT1a^{-/-}$ mice. Because the RAS activation observed at around day 1 seemed to result in the induction of the MCP-1 expression at day 7 in the $AT1a^{+/+}$ mice, it is suggested that AII was involved in the exacerbation of the glomerular MCP-1 expression. Increasing evidence suggests that MCP-1 plays an important role in the induction of anti-GBM nephritis and that it also plays a critical role in the progression of irreversible tissue damage (33, 34). Therefore, a reduced expression of MCP-1 likely accounts for the marked attenuation of proteinuria and subsequent glomerular and interstitial injuries in the $AT1a^{-/-}$ mice.

The precise mechanism by which AII exacerbated the glomerular MCP-1 expression in the $AT1a^{+/+}$ mice remains to be elucidated. Tang *et al*. (26) suggested that in rat anti-GBM nephritis, MCP-1 is produced by intrinsic glomerular cells and infiltrating monocytes/macrophages in paracrine and autocrine fashions. Recently, it was reported (35) that AII stimulates the expression of MCP-1 in mesangial cells through NF-κB activation. These facts suggest that AII is involved in the induction of MCP-1 expression in glomerular cells, macrophages, or both. Alternatively, because the chemotactic activity of AII on monocytes was reported (36), it is possible that AII stimulated the infiltration of macrophages into glomeruli and this resulted in the propagation of MCP-1 expression.

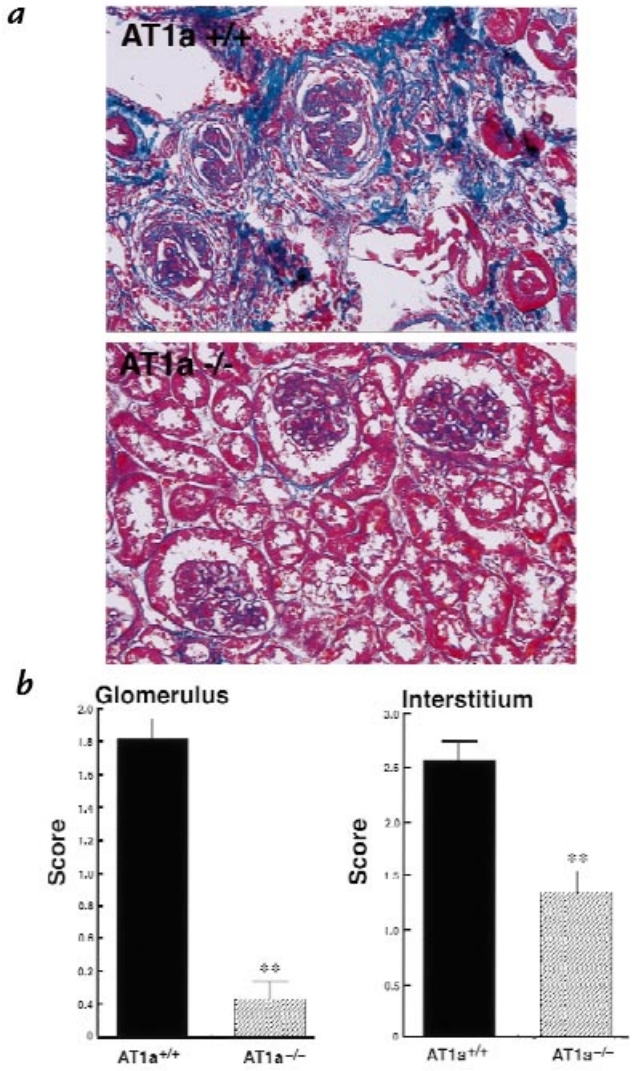

Figure 7
Histological examination of the chronic renal injuries. (***a***) Light microscopy of the kidney at day 126 after the administration of anti-GBM AS. The kidney sections were stained using the Azan-Mallory method. The overproduction of extracellular matrix proteins led to severe glomerulosclerosis and interstitial fibrosis in the AT1a$^{+/+}$ mice(*top*). These histological changes were markedly reduced in the AT1a$^{-/-}$ mice (*bottom*) ×200. (***b***) The percent of glomerular (*left*) and interstitial(*right*) area occupied by extracellular matrix was semiquantitated as described in Methods. The closed bars represent the AT1a$^{+/+}$ mice, and the hatched bars represent the AT1a$^{-/-}$ mice. Data are mean ± SE. $^{**}P < 0.01$ compared with the AT1a$^{+/+}$ mice.

Sustained expressions of TGF-β1 and collagen type I were observed in the AT1a$^{+/+}$ mice. The role of AII in the induction of these proteins has been reported (37). It was reported (38) that in anti-GBM nephritis, the expression of TGF-β was induced by locally generated intrarenal AII that has been suggested to play an important role in the progression of renal diseases. These observations may imply the role of intrarenal AII in the progression of renal injury we studied. Because examination of the intrarenal AII is often impossible, studies in which the inhibition of AII is performed remain the most important approach to assess the role of intrarenal AII in renal diseases. The AT1a$^{-/-}$ mouse is thus an effective model to achieve this aim and will enable us to elucidate the role of intrarenal AII in the progression of renal injury.

In conclusion, our study of renal injuries in the AT1a$^{+/+}$ and AT1a$^{-/-}$ mice demonstrated that AII plays an crucial role in the induction of anti-GBM nephritis. Further studies are needed to establish the pathophysiological role of AII, including the intrarenal AII in anti-GBM nephritis and immune-mediated renal diseases in the context of disease progression.

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1. Matsusaka, T., Hymes, J., and Ichikawa, I. 1996. Angiotensin in progressive renal diseases: theory and practice. *J. Am. Soc. Nephrol.* **7**:2025–2043.
2. Yoshida, Y., Kawamura, M., Ikomo, A., Fogo, A., and Ichikawa, I. 1989. Effects of antihypertensive drugs on glomerular morphology. *Kidney Int.* **36**:626–635.
3. Lafayette, R.A., Mayer, G., Park, S.K., and Meyer, T.W. 1992. Angiotensin II receptor blockade limits glomerular injury in rats with reduced renal mass. *J. Clin. Invest.* **90**:766–771.
4. de Zeeuw, D., Heeg, J.E., and de Joung, P.E. 1992. The antiproteinuric effect of angiotensin converting enzyme inhibitors in human renal disease. In *International yearbook of nephrology*. V.E. Andreucci and L.G. Fine, editors. Springer-Verlag. London, United Kingdom. 95–113.
5. Brunner, H.R. 1992. ACE inhibitors in renal disease. *Kidney Int.* **42**:463–479.
6. Anderson, S., Rennke, H.G., and Brenner, B.M. 1986. Therapeutic advantages of converting inhibitors in arresting progressive renal disease associated with systemic hypertension in the rats. *J. Clin. Invest.* **77**:1993–2000.
7. Ravid, M., *et al.* 1993. Long term stabilizing effect of angiotensin-converting enzyme inhibition on plasma creatinine and on proteinuria in normotensive type II diabetic patients. *Ann. Intern. Med.* **118**:577–581.
8. Egido, J. 1996. Vasoactive hormones and renal sclerosis. *Kidney Int.* **49**:578–598.
9. Gansevoort, R.T., de Zeeuw, D., and de Jong, P.E. 1993. Long-term benefits of the antiproteinuric effect of angiotensin-converting enzyme inhibition in nondiabetic renal disease. *Am. J. Kidney Dis.* **22**:202–206.
10. Ruiz-Ortega, M., *et al.* 1995. ACE inhibition reduces proteinuria, glomerular lesions and extracellular matrix production in a normotensive rat model of immune complex nephritis. *Kidney Int.* **48**:1778-1791.
11. Wilson, C.B., and Dixon, F.J. 1981. The renal response to immunological injury. In *The kidney*. 2nd ed. B.M. Brenner and F.D. Rector, Jr., editors. W.B. Saunders. Philadelphia, PA. 1247–1255.
12. Timmermans, V., Peake, P.W., Charlesworth, J.A., Macdonald, G.J., and Pawlak, M.A. 1990. Angiotensin II receptor regulation in anti-glomerular basement membrane nephritis. *Kidney Int.* **38**:518–524.
13. Yayama, K., *et al.* 1995. Activation of the renin-angiotensin system in anti-glomerular basement membrane antibody-induced glomerulonephritis. *Biol. Pharm. Bull.* **18**:411–415.
14. Zhou, J., Song, K., Harris, P.J., and Mendelssohn, F.A. 1992. *In vitro* autoradiography reveals predominantly AT1 angiotensin II receptors in rat kidney. *Renal Physiol. Biochem.* **15**:231–239.
15. Du, Y., Yao, A., Guo, D.F., Inagami, T., and Wang, D.H. 1995. Differential regulation of angiotensin II receptor subtypes in rat kidney by low dietary sodium. *Hypertension.* **25**:872–877.
16. Sugaya, T., *et al.* 1995. Angiotensin II type 1a receptor deficient mice with hypotension and hyperreninemia. *J. Biol. Chem.* **270**:18719–18722.
17. Piccini, N., Knopf, J.L., and Gross, K.W. 1982. A DNA polymorphism, consistent with gene duplication, correlates with high renin levels in the mouse submaxillary gland. *Cell.* **30**:205–213.
18. Tanimoto, K., Tamura, K., Sugiyama, F., Murakami, K. and Fukamizu, A. 1993. Isolation of the mouse *Ren-1C* gene and characterization of renin gene expression in both ES-D3 cells and their parental mouse strain. *J. Reprod. Dev.* **39**:19–24.
19. Nagai, H., Takizawa, T., Nishiyori, T., and Koda, A. 1982. Experimental glomerulonephritis in mice as a model for immunopharmacological studies. *Jpn. J. Pharmacol.* **32**:1117–1124.
20. Hatae, T., Takimoto, E., Murakami, K., and Fukamizu, A. 1994. Comparative studies on species-specific reactivity between renin and angiotensinogen. *Mol. Cell. Biochem.* **131**:43–47.
21. Fukamizu, A., *et al.* 1993. Chimeric renin–angiotensin system demonstrate sustained increase in blood pressure of transgenic mice carrying

both human renin and human angiotensinogen genes. *J. Biol. Chem.* **268**:11617–11621
22. Tanimoto, K., *et al.* 1994. Angiotensinogen-deficient mice with hypotension. *J. Biol. Chem.* **269**:31334–31337.
23. Sakurai, H., *et al.* 1996. Activation of transcription factor NF-kappa B in experimental glomerulonephritis in rats. *Biochem. Biophys. Acta.* **1316**:132–138.
24. Floege, J., *et al.* 1991. Increased synthesis of extracellular matrix in mesangial proliferative nephritis. *Kidney Int.* **40**:477–488.
25. Shih, W., Hines, W.H., and Neilson, E.G. 1988. Effects of cyclosporin A on the development of immune-mediated interstitial nephritis. *Kidney Int.* **33**:1113–1118.
26. Tang, W.W., Qi, M., and Warren, J.S. 1996. Monocyte chemoattractant protein 1 mediates glomerular macrophage infiltration in anti-GBM Ab GN. *Kidney Int.* **50**:665–671.
27. Oliverio, M.I., Best, C.F., Smithies, O., and Coffman, T.M. 1997. A severe kidney phenotype in AT_{1A} receptor-deficient mice. *J. Am. Soc. Nephrol.* **8**:406A. (Abstr.)
28. Boyce, N.W., and Holdsworth, S.R. 1987. Intra-renal haemodynamic alterations induced by anti-GBM antibody. *Kidney Int.* **31**:8–14.
29. Brasier, A.R., and Li, J. 1996. Mechanism for inducible control of angiotensinogen gene transcription. *Hypertension.* **27**:465–475.
30. Rozzo, S.J., Vyse, T.J., Drake, C.G., and Kotzin, B.L. 1996. Effect of genetic background on the contribution of New Zealand Black loci to autoimmune lupus nephritis. *Proc. Natl. Acad. Sci. USA.* **93**:15164–15168.
31. Kimura, M., *et al.* 1993. Interstrain differences in murine daunomycin-induced nephritis. *Nephron.* **63**:193–198.
32. Stahl, R.A.K., *et al.* 1993. Increased expression of monocyte chemoattractant protein-1 in anti-thymocyte antibody-induced glomerulonephritis. *Kidney Int.* **44**:1036–1047.
33. Schlöndorff, D., Nelson, P.J., Luckow, B., and Banas, B. 1997. Chemokines and renal disease. *Kidney Int.* **51**:610–621.
34. Lloyd, C.M., *et al.* 1997. RANTES and monocyte chemoattractant protein-1 (MCP-1) play an important role in the inflammatory phase of crescentic nephritis, but only MCP-1 is involved in crescent formation and interstitial fibrosis. *J. Exp. Med.* **185**:1371–1380.
35. Ruiz-Ortega, M., *et al.* 1998. Angiotensin II participates in mononuclear cell recruitment in experimental immune complex nephritis through nuclear factor-κB activation and monocyte chemoattractant protein-1 synthesis. *J. Immunol.* **161**:430–439.
36. Goetzel, E.J., Klickstein, L.B., Watt, K.W.K., and Wintroub, B.U. 1980. The preferential human mononuclear leukocyte chemotactic activity of the substituent tetrapeptides of angiotensin II. *Biochem. Biophys. Res. Commun.* **97**:1097–1102.
37. Kagami, S., Border, W.A., Miller, D.E., and Noble, N.A. 1994. Angiotensin II stimulates extracellular matrix protein synthesis through induction of transforming growth factor-β expression in rat glomerular mesangial cells. *J. Clin. Invest.* **93**:2431–2437.
38. Yayama, T., Makino, J., Takano, M., and Okamoto, H. 1995. Role of angiotensin II in the transforming growth factor-β1 expression of rat kidney in anti-glomerular basement membrane antiserum-induced glomerulonephritis. *Biol. Pharm. Bull.* **18**:687–690.