Therapeutic effect of all-trans retinoic acid on rats with anti-GBM antibody glomerulonephritis

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Background. All-trans retinoic acid (ATRA) has antiproliferative and anti-inflammatory effects and is currently used in the treatment of leukemia and dermatologic diseases. We tested the therapeutic potential of ATRA on anti-glomerular basement membrane (GBM) glomerulonephritis rats.

Methods. Glomerulonephritis was induced in male Wistar-Kyoto rats on day 0 by an intravenous injection of antirat GBM antibody. On day 14 after the induction of anti-GBM glomerulonephritis, some rats were sacrificed (N = 5). Another 10 rats were divided into two groups: the vehicle group (N = 5) and the ATRA treated group (N = 5). ATRA was orally administrated from day 14 to day 27 after disease induction. Blood pressure, body weight, urinary protein excretion, and blood chemistry was determined on days 1, 14, 21, and 27. Kidney samples were obtained on day 28. The kidneys were examined with periodic acid-Schiff staining (PAS) and immunohistochemistry using antibodies against the proliferative cell nuclear antigen (PCNA), rat monocyte and macrophage (ED-1), and α-smooth muscle actin (α-SMA). Glomerular RNA was extracted from isolated glomeruli, and reverse transcription (RT) followed by polymerase chain reaction (PCR) was performed.

Results. ATRA administration produced a 55% reduction of proteinuria in glomerulonephritis rats. Light microscopic analysis revealed severe necrosis/crescent formation (>50% of the glomerulus) affecting 34% of glomeruli in vehicle rats, whereas ATRA treatment reduced the glomeruli showing severe change to 14%. ATRA also significantly reduced PCNA-positive cells, ED-1-positive cells and α-SMA-positive area in the glomeruli. RT-PCR analyses revealed that a wide variety of genes including inflammation related [tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and CCAAT enhancer-binding protein δ (C/EBPδ)], cell proliferation–related [platelet-derived growth factor (PDGF)] and fibrosis-related [transforming growth factor-β1 (TGF-β1), type I collagen, and α-SMA] genes were suppressed in the glomeruli of ATRA-treated rats.

Conclusion. ATRA administration significantly reduced severe necrosis/crescent formation and urinary protein excretion in glomerulonephritis rats. Suppression of a wide variety of gene expression may partly explain the mechanism of ATRA's antiproliferative and anti-inflammatory effects. These data suggest a novel therapeutic application of ATRA toward glomerulonephritis.

Crescentic glomerulonephritis is a disease that rapidly progresses to renal failure in humans. In Wistar-Kyoto rats, administration of a small dose of anti-glomerular basement membrane (anti-GBM) antibody induces severe necrotizing glomerulonephritis with crescent formation [1].

Retinoid acids, which are biologically active derivatives of vitamin A, are necessary for normal growth, maintenance of tissues, reproduction, immune response, and survival [2]. Retinoic acid receptors belong to the supergene family of ligand-inducible transcriptional regulatory factors that includes steroid hormone, thyroid hormone, and vitamin D receptors as well as the peroxisome proliferator–activated receptors (PPAR) and others [3]. Retinoid receptors are nuclear receptors, which enter the cell nucleus only after a ligand has been bound. They bind to specific sequence elements on the promoters of responsive genes, which allow the retinoid receptors to directly modulate gene transcription [4].

Retinoid acids are not complete newcomers in the area of human therapy. In dermatology, retinoids have been used for the treatment of acne, psoriasis, and neoplastic processes [5].

Recently, in anti-Thy1.1 nephritis, treatment of nephritic rats with all-trans retinoic acid (ATRA) or isotretinoin (13-cis RA) effectively limited renal damage and mesangial cell proliferation [6]. The retinoid acids attenuated the increase in glomerular cell proliferation, glomerular transforming growth factor-β1 (TGF-β1) expression, and urinary albumin excretion. These results indicate that the properties of retinoic acids to down-
regulate inflammatory and proliferative responses makes them attractive potential candidates for therapeutic use in renal disease.

In the present study, we tested the therapeutic efficacy of ATRA on anti-GBM antibody–induced renal injury. We started the ATRA therapy at 14 days after disease induction, when the disease is fully developed.

We found that urinary protein excretion was reduced in ATRA-treated group along with a significant attenuation of glomerular injury. A wide variety of glomerular gene expression was suppressed in ATRA-treated group, which may partly contribute to the therapeutic efficacy of ATRA on renal disease.

METHODS

Experimental protocol
Male Wistar-Kyoto rats, aged 12 weeks, were used in the present study. Glomerulonephritis was induced in 15 rats on day 0 by a single intravenous injection of 25μL/100 g body weight of anti-rat GBM antiserum as previously reported [7]. Five rats were sacrificed on day 14. We divided the remaining 10 rats into two groups: the vehicle group, the anti-GBM glomerulonephritis without treatment (N = 5), and the ATRA-treated group (N = 5), the anti-GBM glomerulonephritis group treated by ATRA. ATRA was mixed with sesame oil (9 mg/mL) and was orally administrated at a dose of 30 mg/kg body weight using gavage tube once daily from day 14 to day 27. Blood pressure and body weight was determined on days 1, 14, 21, and 27. Blood pressure was measured by blood pressure monitor for rats and mice model MK-1100 (Muromachi Kikai Co., Ltd., Tokyo, Japan).

Proteinuria and creatinine determination
For determination of urinary protein excretion, rats were placed in metabolic cages and urine was collected for 24 hours on days 1, 14, 21, and 27. Blood samples were taken from tail vein on the same time points. Urinary protein concentration was determined by pyrogallol red-molybdate complex method using a Micro TP-test WAKO (Wako Pure Chemical Industries, Ltd., Osaka, Japan) [8]. Serum and urinary creatinine concentration was determined by Jaffe method using a Creatinine-test WAKO (Wako Pure Chemical Industries, Ltd.).

RNA isolation from glomeruli
Both vehicle-treated rats and ATRA-treated rats were sacrificed on day 28. Both kidneys were removed and partially preserved for histologic analysis. Glomeruli were isolated by differential sieving as described previously [9]. Glomerular total RNA was extracted from isolated glomeruli by the acid guanidium thiocyanate-phenol-chloroform method [10].

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**Abbreviations** are: GADPH, glyceraldehyde-3-phosphate dehydrogenase; TNF-α, tumor necrosis factor-α; IL-1β, interleukin-1β; TGF-β1, transforming growth factor β1; MCP-1, monocyte chemoattractant protein-1; -SMA, smooth muscle actin; ICAM-1, intercellular adhesion molecule-1; PDGF, platelet-derived growth factor; C/EBPβ, CCAAT enhancer-binding protein β.
Semiquantitative reverse transcription-polymerase chain reaction

Reverse transcription (RT) was performed as follows: 4 μL first-strand RT buffer was added to 0.4 μg of total RNA from isolated glomeruli [final concentration of 50 mmol/L Tris (hydroxymethyl) aminomethane hydrochloride, pH 8.3, 75 mmol/L KCl, and 3 mmol/L MgCl2], 2.5 μL H2O, 0.5 μL RNase inhibitor (55 U), 1 μL of 10 mmol/L deoxyoligonucleotide mixture, 1 μL random primer [0.02 A260 absorbance units of hexadeoxyribonucleotide mixture (p(dN)6) per reaction], 2 μL of 0.1 mol/L dithiothreitol, and 1 μL Moloney murine leukemia virus reverse transcriptase (MMLV transcriptase) (Gibco BRL, Gaithersburg, MD, USA). Reaction tubes were incubated at 30°C for 10 minutes and 42°C for 40 minutes. At the end of the incubation, the reaction was stopped by heating at 95°C for 5 minutes to inactivate MMLV.

Polymerase chain reaction (PCR) was performed as follows: 0.4 μL of 10 μmol/L forward and reverse primer was added to 1 μL of the RT reaction mixture, 2 μL of 10 × buffer (final concentration of 10 mmol/L Tris HCl, pH 8.3, 50 mmol/L KCl, 1.5 mmol/L MgCl2, and 0.001% gelatin), 14.5 μL H2O, 0.5 μL L of the RT reaction mixture, 2 μL of 2.5 mmol/L deoxyribonucleotide mixture, 1 μL of 0.1 mol/L dithiothreitol, and 0.1 μL of Taq polymerase.

The primers that we used are listed in Table 1. The primers for glyceraldehyde-3-phosphate dehydrogenase (GAPDH), interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), TGF-β1, and monocyte chemoattractant protein-1 (MCP-1) were used as described previously [11]. The primers for type I collagen (collagen I) were used as previously reported [12]. The primers for intercellular adhesion molecule-1 (ICAM-1) were used as previously reported [13]. The primers for platelet-derived growth factor (PDGF) and CCAAT enhancer-binding protein β (C/EBPβ) were designed based on mouse PDGF and C/EBPβ nucleotide sequences.

All PCR was performed using a thermal cycler, PCR System 9700 (Perkin Elmer, Wellesley, MA, USA), using the following parameters. After initial denaturation for 5 minutes at 95°C, 25 to 32 cycles of sequential steps denaturation was performed at 95°C for 1 minute, annealing at 55 to 64°C for 1 minute, extension at 72°C for 2 minutes, followed by a final incubation at 72°C for 7 minutes. The primers and PCR conditions for each primer set are summarized in Table 1.

The PCR products were separated by electrophoresis on 2.0% agarose gels and visualized by ethidium bromide staining. Each experiment included the amplification of GAPDH, and the intensities of cDNA bands were quantified with the computing densitometry Image Quant (Molecular Dynamics, Sunnyvale, CA, USA), and were normalized to those of the GAPDH band as reported previously [12].

Histologic examination

The kidneys were perfused with cold autoclaved phosphate-buffered saline (PBS) and were removed. Tissues for microscopic examination were fixed with 4% paraformaldehyde overnight and then dehydrated by graded ethanol, then paraffin embedded. Thin section was examined with periodic acid-Schiff (PAS) staining as described previously [14]. Fifty glomeruli per section were randomly selected and were assessed. Glomerular appearance was graded as normal, mild to moderate, or severe injury (>50% of glomerulus affected by necrosis/crescent formation), and results were expressed as percentage of glomeruli examined. At the same time, glomerular area and necrosis/crescent formation area were quantitatively measured under high-power magnification (×400) by using computer-aided manipulator program (Maccscope; Mitani Corporation, Fukui, Japan), and the percentage of the glomerular necrosis/crescent formation area was calculated.

Immunohistochemistry

PBS-perfused slices (4 μm) of renal tissue obtained from comparable renal areas in all rats were fixed in methacarn solution (methanol 60%, chloroform 30%, and acetic acid 10%) and processed using the direct or indirect immunoperoxidase technique. The endogenous peroxidase activity in tissue sections was blocked by incubating in PBS with 3% hydrogen peroxide for 30 minutes. Tissue sections were preincubated with goat or horse serum (diluted 1:20 with PBS) for 30 minutes to block the nonspecific staining and were then incubated with the primary antibodies for 60 minutes at room temperature. Glomerular cell proliferation was assessed by staining with 19A2 (Coulter Corp., Hialeah, FL, USA), a mouse monoclonal immunoglobulin M (IgM) antibody to the proliferative cell nuclear antigen (PCNA). To assess the invasion of macrophages, mouse IgG antirat monocye and macrophage (ED-1) antibody was used (Serotec, Inc., Raleigh, NC, USA). To stain α-SMA, mouse IgG anti-SMA monoclonal antibodies were used (Immuno-tech S.A., Cedex, Marseilles, France). To stain C/EBPβ, rabbit IgG anti-C/EBPβ monoclonal antibodies (M-17) was used (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA). Sections were then processed using an avidin-biotinylated peroxidase complex method (Vectorstain ABC kit; Vector Laboratories, Inc., Burlingame, CA, USA) with diaminobenzidine as the chromogen. The sections were counterstained with methyl green. Sections labeled with PCNA and ED-1 antibody were then incubated with the nonspecific staining and were then incubated with the primary antibodies for 60 minutes at room temperature. Glomerular appearance was graded as normal, mild to moderate, or severe injury (>50% of glomerulus affected by necrosis/crescent formation), and results were expressed as percentage of glomeruli examined. At the same time, glomerular area and necrosis/crescent formation area were quantitatively measured under high-power magnification (×400) by using computer-aided manipulator program (Maccscope; Mitani Corporation, Fukui, Japan), and the percentage of the glomerular necrosis/crescent formation area was calculated.
ing of the marker positive cells was performed under
high-power ($\times$ 400) microscopy. The number of PCNA-
positive cells and ED-1–positive cells per glomerulus was
determined by the observation of randomly selected 50
glomeruli for each animal. All scoring was performed on
blinded slides by one of the authors. Glomerular $\alpha$-SMA–
positive and C/EBP$\delta$ area was quantitatively analyzed by
using computer-aided manipulator program, Macscope
(Mitani Corporation), as described previously [15, 16].

Statistical analysis

The results were given as means $\pm$ standard deviation.
The differences between vehicle-treated and ATRA-
treated groups were tested using the Student $t$ test. Statisti-
cally significant differences between groups were de-
defined as $P$ values less than 0.05.

RESULTS

ATRA reduced renal injury

We examined whether ATRA administration could ameliorate the renal injury of anti-GBM glomerulone-
phritis rats. On day 14, light microscopy of kidney tissue of anti-GBM glomerulonephritis rats showed diffuse
necrotizing glomerulonephritis affecting 81% of glomer-
uli, with severe segmental necrosis/crescent formation
($>50\%$ of the glomerulus) affecting 14% of glomeruli.
On day 28, vehicle-treated rats showed diffuse necrotiz-
ing glomerulonephritis affecting 89% of glomeruli, with
severe necrosis/crescent formation affecting 34% of glo-
meruli. ATRA-treated rats showed diffuse necrotizing
glomerulonephritis affecting 89% of glomeruli, with
severe necrosis/crescent formation affecting 34% of glo-
meruli. ATRA-treated rats showed diffuse necrotizing
glomerulonephritis affecting 67% of glomeruli, with se-
vere change in 14% (Fig. 1D). Computer-aided quantita-
tive analysis revealed that glomerular necrosis/crescent
formation area was also reduced from 33.1 $\pm$ 5.7% to
17.9 $\pm$ 4.4% ($P < 0.01$) (Fig. 1E). We examined whether
ATRA could decrease the glomerular cell proliferation
assessed by the PCNA expression. The number of PCNA-
positive cells per glomerulus in ATRA-treated rats was
significantly reduced (2.2 $\pm$ 0.43) compared to that in
vehicle-treated glomerulonephritis rats (5.2 $\pm$ 2.2) (Fig. 2).

Effects of ATRA on glomerular gene expression

Histologic examination demonstrated that ATRA ad-
melioration of glomerular injury in anti-GBM
glomerulonephritis. To explore the underlying mecha-
nisms of this therapeutic efficacy, we examined the effects
of ATRA administration on glomerular gene expression
of wide variety of genes, including inflammation-related,
cell proliferation–related, and fibrosis-related genes by
semi-quantitative PCR. TNF-\(\alpha\) and IL-1$\beta$ mRNAs were
significantly reduced in ATRA-treated rats (Fig. 6),
TGF-\(\beta\)1, type I collagen, and $\alpha$-SMA mRNA was reduced
in ATRA-treated rats compared with vehicle-treated rats
(Fig. 7). PDGF, MCP-1, and ICAM-1 mRNA was also
significantly suppressed in ATRA-treated rats (Fig. 8).
Additionally, c-fos and c-jun mRNAs, which are compo-
nents of activated protein 1 (AP-1), were significantly
reduced in ATRA-treated rats (Fig. 9). C/EBP$\delta$ mRNA
was significantly reduced in ATRA-treated rats (Fig. 10),

Fig. 1. Effect of all-trans retinoic acid (ATRA) administration on anti-glomerular basement membrane (anti-GBM) glomerulonephritis. Periodic
acid-Schiff (PAS) staining of kidney on day 14 in anti-GBM glomerulonephritis rats (A), on day 28 in vehicle-treated rats (B), and ATRA-treated
rats (C). The severity of glomerular injury was graded as normal, mild to moderate, or severe (>50% of glomeruli affected by necrosis/crescent
formation) (D). Vehicle-treated rats showed 89% injured glomeruli, with severe segmental necrosis/crescent formation affecting 34% of glomeruli.
ATRA-treated rats showed 67% injured glomeruli, with severe change in 14%. Glomerular necrosis/crescent formation area was quantitatively
analyzed by computer-aided manipulator program (E). The area was 24.9 $\pm$ 3.9% on day 14. The area on day 28 in vehicle rats was 33.1 $\pm$ 5.7%
and 17.9 $\pm$ 4.4% in ATRA-treated rats.
which coincides with the changes in glomerular immunostaining of C/EBPβ as shown in Figure 5.

**Body weight**

There was no significant difference in body weight between the vehicle-treated and ATRA-treated rats (day 1, 300 ± 9.6 g vs. 301 ± 9.0 g; day 14, 339 ± 11.8 g vs. 334 ± 7.4 g; day 27, 350 ± 14.6 g vs. 342 ± 11.0 g).

**Urinary protein excretion**

The rate of urinary protein excretion was markedly increased after injection of anti-rat GBM antibody both in vehicle- and ATRA treated glomerulonephritis rats before the commencement of ATRA treatment. In ATRA-treated group, the rate of urinary protein excretion was significantly reduced. On day 21, 7 days after the commencement of ATRA therapy, the rate of proteinuria was reduced to 46% of that of vehicle-treated rats in ATRA-treated rats and the suppression was continued until day 27 (Fig. 11). Creatinine clearance or serum creatinine levels were not different between vehicle- and ATRA-treated groups (data not shown).

**Blood pressure**

There was no significant difference in blood pressure between the vehicle-treated and ATRA-treated groups just before the commencement of ATRA administration. On day 21, 7 days after ATRA treatment was started, blood pressure was not different, but on day 27, 14 days after the beginning of ATRA administration, blood pressure was significantly lower in ATRA-treated rats compared to vehicle-treated rats (Fig. 12).

**DISCUSSION**

In the present study, intravenous injection of antirat GBM antibody induced necrotizing glomerulonephritis affecting 81% of glomeruli and severe segmental necrosis/crescent formation affecting 14% of glomeruli on day 14, and severe segmental necrosis/crescent formation increased to 34% of glomeruli on day 28 in vehicle-treated rats. In this anti-GBM glomerulonephritis rat model, acute glomerular injuries show a peak around day 15 and then sclerotic/fibrotic glomerular lesions develop thereafter [17]. The percentages of affected glomeruli in the present study are similar to those in the previous report [18]. Anti-GBM glomerulonephritis rats showed about 100 mg/day of proteinuria on day 14, which is comparable to the level of proteinuria induced by the same anti-GBM antibody [19]. In our experiment, ATRA administration significantly reduced renal injury and urinary protein excretion. In immunohistochemical study, ATRA reduced the number of PCNA or ED-1–positive cells in glomeruli. We also found that ATRA suppressed mRNA expression of proliferation and inflammatory-related genes, which may partly contribute to ATRA’s antiproliferative and anti-inflammatory effects on glomerular injury.

The substantial local macrophage proliferation within Bowman’s space in crescentic lesion has already been documented in anti-GBM glomerulonephritis rats by double staining of ED-1 and PCNA [20]. We stained PCNA and ED-1 on consecutive sections and found considerable overlapping of ED-1–positive cells in PCNA-positive cells, although we did not perform double staining. We speculate that some population of PCNA-positive cells in glomeruli was ED-1–positive macrophages.

It is now well established that glomerular macrophage infiltration is closely related to the progression of renal injury [21]. We found that ATRA administration significantly suppressed the glomerular macrophage infiltration. Glomerular expression of ICAM-1 and MCP-1 was also significantly suppressed in ATRA-treated rats. Up-regulation of glomerular ICAM-1 and MCP-1 expression was previously demonstrated in anti-GBM glomerulonephritis rats [7, 22]. Administration of anti-ICAM-1 antibody [22] or anti-MCP-1 antibody [7] significantly suppressed macrophage infiltration and urinary protein excretion in this model. These results suggest that enhanced expression of ICAM-1 and MCP-1 play a crucial role in renal injury.
Fig. 5. Effect of all-trans retinoic acid (ATRA) administration on glomerular CCAAT enhancer-binding protein β (C/EBPβ) expression. Representative immunohistochemical photomicrographs of glomeruli stained for C/EBPβ on day 28 in normal rats (A), vehicle-treated rats (B), and ATRA-treated rats (C). Five animals were analyzed for each group and 50 randomly selected high-power fields were quantitated with a computer-aided image manipulator and averaged to obtain the value for each animal (D). C/EBPβ-positive area in anti-glomerular basement membrane (anti-GBM) glomerulonephritis on day 14 was 30.4 ± 3.9%. The area in vehicle-treated rats was 21.9 ± 4.2% and 14.2 ± 3.5% in ATRA-treated rats \( (P < 0.01) \).

Fig. 6. Effects of all-trans retinoic acid (ATRA) administration on proinflammatory glomerular gene expression in anti-glomerular basement membrane (anti-GBM) glomerulonephritis rats. (A) Ethidium bromide–stained gels. Vehicle-treated rats and ATRA-treated rats. The level of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was not of anti-GBM glomerulonephritis. The reduction of renal macrophage infiltration and proteinuria in our experiment may partly be due to the attenuation of ICAM-1 or MCP-1 mRNA expression. Proinflammatory cytokines such as TNF-α, IL-1β, and nitric oxide are known to activate macrophages. ATRA decreased the level of nitric oxide and TNF-α in macrophage cell line [23]. In our study, glomerular TNF-α and IL-1β mRNA expression was reduced in ATRA-treated rats. This result suggests that ATRA reduces such proinflammatory cytokines and attenuates macrophage activity. The attenuation of macrophage infiltration and activation may be one of the un-

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significantly different. Tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) mRNA expression was significantly reduced by ATRA administration. The intensities of cDNA bands were quantified with the computing densitometry and normalized by GAPDH. (B) TNF-α mRNA was decreased to 55% and (C) IL-1β mRNA was decreased to 44% in ATRA-treated rats compared with vehicle-treated rats.
derlying mechanisms of the therapeutic effect of ATRA on anti-GBM antibody glomerulonephritis.

ATRA was shown to down-regulate H₂O₂-induced [24] and fetal bovine serum (FBS)-stimulated [25] expression of c-fos and c-jun in cultured mesangial cells. In our study, glomerular c-fos and c-jun mRNAs were reduced in ATRA-treated rats in vivo. Previous study demonstrated that down-regulation of c-fos and c-jun mRNA levels suggests a mechanism for anti-AP-1 activity by ATRA [25]. Thus, the blunted gene expression of c-fos and c-jun mRNA in ATRA-treated rats is supposed to result in the down-regulation of AP-1 activity in the glomeruli. MCP-1 expression is reported to be partly regulated by AP-1 activation [26, 27]. MCP-1 production stimulated by IL-1β was suppressed via inhibition of nuclear factor-kappa B (NF-κB) and AP-1 activation [28]. On the other hand, overexpression of AP-1 protein induced ICAM-1 gene expression [29]. Taken together, down-regulation of ICAM-1 and MCP-1 in ATRA-treated rats may at least partly be explained by the suppressive effects of ATRA on c-fos and c-jun mRNAs. There have been some reports demonstrating that AP-1 regulates TGF-β gene expression in variety of cells, including mesangial cells and tubular cells [30–33]. TGF-β1 mRNA expression was decreased in ATRA-treated rats in our study, which might be related to the suppression of AP-1 component genes. Morath et al [34] also suggested that the beneficial effects of ATRA on anti-Thy1.1 nephritis may be due to a suppression of renal TGF-β1. TGF-β is supposed to play a central role in the progression of tissue fibrosis via stimulation of extracellular matrix components (ECM) synthesis and cellular phenotypic change to myofibroblasts. α-SMA is one of the typical molecular markers of myofibroblasts and is known to be induced by TGF-β [35]. The reduction of TGF-β1 mRNA expression may result in the reduction of glomerular...

**Fig. 7. Effects of all-trans retinoic acid (ATRA) administration on fibrosis related glomerular gene expression in anti-glomerular basement membrane (anti-GBM) glomerulonephritis rats.** (A) Ethidium bromide–stained gels. ATRA administration significantly reduced transforming growth factor-β1 (TGF-β1) (−49%) (B), type I collagen (−47%) (C), and α-smooth muscle actin (α-SMA) (−38%) (D) mRNA expression. GAPDH is glyceraldehyde-3-phosphate dehydrogenase.

**Fig. 8. Effect of all-trans retinoic acid (ATRA) administration on proliferation-related and macrophage infiltration–related glomerular gene expression in anti-glomerular basement membrane (anti-GBM) glomerulonephritis rats.** (A) Ethidium bromide–stained gels. ATRA administration significantly reduced platelet-derived growth factor (PDGF) (−63%) (B), monocyte chemotactic protein-1 (MCP-1) (−42%) (C), and intercellular adhesion molecule-1 (ICAM-1) (−56%) (D) mRNA expression. GAPDH is glyceraldehyde-3-phosphate dehydrogenase.
myofibroblast expansion revealed by α-SMA expression and reduction of type I collagen mRNA expression.

Myofibroblasts are recognized as the key to understand the reconstruction and excessive matrix formation in injured tissue. We have been investigating the phenotypic change from renal cells to myofibroblasts in the process of progressive renal diseases in animal models and human glomerulonephritis [15, 35, 36]. α-SMA is a typical molecular marker of myofibroblasts, and we hypothesized that the molecular mechanisms underlying the induction of α-SMA in myofibroblasts are closely related to the molecular pathophysiology of progressive renal disease leading to renal fibrosis. We speculated any factor that promotes α-SMA expression might play an important role for transdifferentiation to myofibroblast and progression of tissue injury. Then, we identified C/EBPδ as a major transcription factor that induces phenotypic change in renal disease. We also found that C/EBPδ-deficient mice show a significant reduction in

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**Fig. 9.** Effect of all-trans retinoic acid (ATRA) administration on c-fos and c-jun mRNA in anti-glomerular basement membrane (anti-GBM) glomerulonephritis rats. (A) Ethidium bromide-stained gels. The level of c-fos (B) and c-jun (C) mRNA expression was significantly reduced by ATRA administration (−69% and −49%, respectively). GAPDH is glyceraldehyde-3-phosphate dehydrogenase.

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**Fig. 10.** Effect of all-trans retinoic acid (ATRA) administration on CCAAT enhancer-binding protein δ (C/EBPδ) mRNA in anti-glomerular basement membrane (anti-GBM) glomerulonephritis rats. (A) Ethidium bromide-stained gels. The level of C/EBPδ mRNA (B) expression was significantly reduced by ATRA administration (−56%). GAPDH is glyceraldehyde-3-phosphate dehydrogenase.

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**Fig. 11.** Effect of all-trans retinoic acid (ATRA) administration on urinary protein excretion in anti-glomerular basement membrane (anti-GBM) glomerulonephritis rats. Urinary protein excretion was decreased to 46% of untreated levels 7 days after ATRA administration. *P < 0.05 vehicle vs. ATRA treated rats.

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**Fig. 12.** Effect of all-trans retinoic acid (ATRA) administration on blood pressure in anti-glomerular basement membrane (anti-GBM) glomerulonephritis rats. There was no difference between two groups 7 days after ATRA administration, but blood pressure was significantly lower in ATRA-treated group 14 days after the beginning of ATRA administration. *P < 0.05 vehicle vs. ATRA-treated rats.
α-SMA expression along with substantial amelioration in renal damage in Habu venom glomerulonephritis or unilateral ureteral obstruction. In the present study, we demonstrated a significant reduction of both C/EBPα and α-SMA mRNA or protein expression in glomeruli from ATRA-treated anti-GBM glomerulonephritis rats. This observation and our results of other study using C/EBPα-deficient mice further support the role of C/EBPα in myofibroblast formation ([manuscript in preparation] abstract; Takeji M, et al, J Am Soc Nephrol 13:295A, 2002). We speculate that the suppression of C/EBPα mRNA is at least part of the molecular mechanism of therapeutic effect of ATRA in anti-GBM glomerulonephritis.

It was already demonstrated that ATRA lowered the high blood pressure in anti-Thy1.1 glomerulonephritis rats [6]. In our experiment, blood pressure of ATRA-treated rats was significantly lower compared with vehicle-treated rats 14 days after ATRA administration, although no significant difference was observed after 7 days of treatment. Interestingly, it was recently shown that ATRA might have an inhibitory effect on renin-angiotensin system (RAS) in the kidney [37]. Thus, the lower blood pressure at 14 days after ATRA administration may be related to the suppression of RAS component genes, and alternatively, be secondary to the amelioration of renal injury by ATRA treatment.

CONCLUSION

We have demonstrated therapeutic effects of ATRA on anti-GBM antibody glomerulonephritis rats revealed by histologic changes, urinary protein excretion, and blood pressure. A wide variety of disease-related gene expression in glomeruli was blunted in ATRA-treated rats, which may explain the molecular mechanisms of therapeutic effects of ATRA in glomerulonephritis rats. Clinical feasibility of ATRA treatment in progressive renal diseases is expected to be established with further investigation.

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