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Transforming growth factor-β1 antisense oligodeoxynucleotides block interstitial fibrosis in unilateral ureteral obstruction

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Background. Interstitial expression of transforming growth factor-β1 (TGF-β1) is important in tubulointerstitial fibrosis, a common process in most progressive renal diseases. However, no effective therapy for progressive interstitial fibrosis is known. Recently, we developed an artificial viral envelope (AVE)-type hemagglutinating virus of Japan (HVJ) liposome-mediated retrograde ureteral gene transfer method, which allowed us to introduce the genetic material selectively into renal interstitial fibroblasts.

Method. We introduced antisense or scrambled oligodeoxynucleotides (ODNs) for TGF-β1 into interstitial fibroblasts in rats with unilateral ureteral obstruction, a model of interstitial fibrosis, to block interstitial fibrosis by retrograde ureteral injection of AVE-type HVJ liposomes.

Results. TGF-β1 and type I collagen mRNA increased markedly in the interstitium of untreated obstructed kidneys, and those were not affected by scrambled ODN transfection. Northern analysis and in situ hybridization revealed that the levels of TGF-β1 and type I collagen mRNA were dramatically decreased in antisense ODN-transfected obstructed kidneys. Consequently, the interstitial fibrotic area of the obstructed kidneys treated with antisense ODN was significantly less than that of the obstructed kidneys untreated or treated with scrambled ODN.

Conclusion. The introduction of TGF-β1 antisense ODN into interstitial fibroblasts may be a potential therapeutic maneuver for interstitial fibrosis.

Tubulointerstitial inflammation and fibrosis are common features in a variety of renal diseases that may predict renal function and long-term prognosis more accurately than glomerular injury [1, 2]. Fibrotic structural derangement of the tubulointerstitial compartment occurs in virtually progressive renal diseases. Interstitial fibrosis is characterized by the accumulation of matrix proteins in the renal tubulointerstitial compartment. The mechanisms underlying the progression of interstitial fibrosis are not well understood, but this accumulation of matrix is accompanied by infiltrating macrophages and increased numbers of activated fibroblasts in the interstitium [3].

Although several cytokines have fibrogenic potential, transforming growth factor-β (TGF-β) remains the prime fibrogenic cytokine in renal interstitial fibrosis. Active TGF-β evokes a variety of responses relevant to renal fibrosis [4]. TGF-β stimulates synthesis of individual matrix components, including proteoglycans, collagens, and glycoproteins [5]. TGF-β also inhibits matrix degradation by decreasing synthesis of proteases and increasing synthesis of protease inhibitors [6]. TGF-β modulates the expression of integrin receptors and alters their relative abundance on the cell surface in a manner capable of facilitating adhesion to the matrix [7]. TGF-β is also a chemoattractant for fibroblasts [4] and stimulates fibroblast proliferation [8]. The net effect of TGF-β is to shift the balance of matrix synthesis and degradation toward accumulation of matrix proteins.

By inhibiting TGF-β using a neutralizing antiserum [9] or neutralizing proteoglycan decorin [10], Border et al have established a role for TGF-β in the anti–Thy-1 model of glomerulonephritis. We have been investigating the potential of molecular intervention in antagonizing the action of TGF-β in glomerular diseases. One strategy is direct inhibition of TGF-β1 synthesis by application of antisense oligodeoxynucleotides (ODNs) [11]; the other is gene transfer to induce synthesis of inhibitory molecules against TGF-β such as decorin [12] or soluble chimeric TGF-β receptor [13], which competitively block

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the binding of TGF-β to its receptor. Because the pathogenesis of glomerulosclerosis and that of tubulointerstitial fibrosis share certain key mechanisms, targeting interstitial expression of TGF-β1 should be able to prevent progression of interstitial fibrosis [4]. Targeting interstitial cells has proven to be challenging, and inhibition studies in animal models of progressive interstitial fibrosis largely remain to be performed, because effective vehicles have not been available to introduce the genetic material into interstitial fibroblasts.

Recently, we pioneered a new strategy of gene transfer to the renal interstitial fibroblasts [14]. Fluorescein isothiocyanate (FITC)-labeled ODNs were introduced into the kidney of normal rats retrogradely via the ureter by using an artificial viral envelope (AVE)-type hemagglutinating virus of Japan (HVJ) liposome method. FITC-labeled ODNs were accumulated selectively in the nuclei of the interstitial fibroblasts diffusely in the transfected kidney 10 minutes after transfection [14]. Here, we describe the successful introduction of antisense ODN for TGF-β1 into renal interstitial fibroblasts to inhibit interstitial TGF-β1 expression and thereby prevent interstitial fibrosis.

METHODS

Synthetic oligodeoxynucleotides

Sequences of phosphorothioate ODNs for rat TGF-β1 used in this study were antisense ODN, 5′-CGAGGGC GGCATGGG-3′, and scrambled ODN, 5′-GCATGGG CGAGGGGC-3′. Antisense TGF-β1 ODNs were complementary to rat TGF-β1 mRNA at the translation initiation region [11]. These ODNs were purchased from Bex (Tokyo, Japan). The ODNs were deprotected on a column, dried, resuspended in balanced salt solution (BSS; 140 mmol/L NaCl, 5.4 mmol/L KCl, and 10 mmol/L Tris-HCl, pH 7.6), and quantitated using a spectrophotometer.

Preparation of HVJ liposome

Artificial viral envelope-type HVJ liposomes were prepared as described previously [15], with minor modifications [14]. Briefly, egg yolk phosphatidyl choline (ePC; Sigma Chemical, St. Louis, MO, USA), dioleoyl phosphatidyl ethanolamine (DOPE; NOF Corporation, Tsukuba, Japan), egg yolk sphingomyelin (eSph; Sigma), bovine brain phosphatidyl serine (bPS; Avanti Polar Lipid, Birmingham, AL, USA), and cholesterol (Sigma) were mixed in a molar ratio of 16.7:16.7:16.7:10:50 and dissolved in chloroform at a concentration of 30 mmol/L. The lipid mixture (500 μL, 15 μmol) was transferred to a glass tube and dried as a thin lipid film by evaporation. Dried lipid was hydrated in 200 μL of BSS containing 50 μg of ODN, mixed vigorously with a vortex agitation, and sonicated to form liposomes. The liposomes were incubated with purified HVJ inactivated by ultraviolet irradiation (110 erg/mm^2/sec) for three minutes immediately before use. The mixture was incubated at 4°C for 10 minutes and gently shaken for 60 minutes at 37°C. Free HVJ was removed from the HVJ liposomes by discontinuous density gradient centrifugation with sucrose.

Transfer of antisense ODNs by the AVE-type HVJ liposome method

We examined the effect of antisense ODNs for TGF-β1 on tubulointerstitial fibrosis in a rat model of unilateral ureteral obstruction. Male Sprague-Dawley (SD) rats at the age of six weeks (Japan SLC, Inc., Hamamatsu, Japan) were separated into three groups: (*1*) TGF-β1 antisense ODN-transfected obstructed rats, (*2*) scrambled ODN-transfected obstructed rats, and (*3*) untreated obstructed rats. Specificity of action of the antisense ODN used here for TGF-β1 has been confirmed in cultured smooth muscle cells and also in vivo; glomerular TGF-β1 expression was suppressed in anti–Thy-1 glomerulonephritic rats treated with antisense ODNs for TGF-β1, but was not affected when treated with scrambled ODNs [11]. All procedures were handled in a humane fashion in accordance with the guidelines of the Animal Committee of Osaka University (Osaka, Japan). SD rats were anesthetized by an intraperitoneal injection of pentobarbital (50 mg/kg). The left kidney and ureter were surgically exposed by a midline incision, and a polyethylene (PE) 10 tubing (Beckton-Dickinson, Sparks, MD, USA) was inserted into the left ureter and advanced in a retrograde manner. Immediately after clamping the left renal vein, 300 μL of AVE-type HVJ liposome suspension, which contained approximately 5 μg of antisense or scrambled ODN, were slowly infused. After 10 minutes of incubation, the catheter was removed, and the clamp was released from the renal vein. Thereafter, ureteral obstruction was induced by ligation. Studies were carried out either four or seven days after the initiation of obstruction.

RNA preparation and Northern blot analysis

To examine the effect of antisense ODN on the level of TGF-β1 and type I collagen mRNA expression, Northern blot analysis was performed. On day 0, antisense or scrambled ODNs were each introduced into the left ureter in six rats with unilateral ureteral obstruction. Seven days later, the kidneys were perfused with cold phosphate-buffered saline (PBS), and the cortex was removed, snap frozen in liquid nitrogen, and kept at −80°C until use. The renal cortex was homogenized with a Polytron homogenizer (Kinematica, Switzerland) in TRIzol reagent (GIBCO BRL, Gaithersburg, MD, USA). Total RNA was prepared by an acid guanidinium thiocyanate-phenol-chloroform extraction procedure according to the manufacturer's instructions [16]. For Northern analy-

sis, 20 μg aliquots of total RNA were separated on 1% agarose-formaldehyde gels, transferred onto nylon membranes (Hybond-N+; Amersham, Boston, MA, USA), and cross-linked by ultraviolet wave irradiation. Partial clones of rat TGF-β1 cDNA [11], type I collagen cDNA [mouse α1(I) collagen; a kind gift from Dr. T. Tanaka, Kyoto University, Japan] [17], and rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA [11] were labeled by a random priming method using [α-^{32}P] dATP (3000 Ci/mmol; Amersham). Hybridization was carried out at 42°C overnight in 50% formamide, 10 × Denhardt's solution, 1% sodium dodecyl sulfate (SDS), 5 × standard saline citrate (SSC), 50 mmol/L sodium phosphate, and 200 μg/mL salmon sperm DNA. The blots were washed three times at 50°C in 0.5 × SSC with 0.1% SDS, and the signals were quantitated by laser densitometry (Scanningimager; Molecular Dynamics, Sunnyvale, CA, USA).

In situ hybridization

Since evidence is conflicting as to whether interstitial cells [18] or tubules [19] are a major source of increased TGF-β1 in a rat model of obstructive nephropathy, the effects of antisense ODN for TGF-β1 on interstitial TGF-β mRNA expression were further evaluated at the histologic level using in situ hybridization. Antisense and sense RNA probes of rat TGF-β1 [11] and α1(I) (type I collagen) [17] cDNA were labeled with digoxigenin (DIG)-labeled uridine triphosphate (UTP) as described [20]. All tissues were fixed in freshly prepared 4% paraformaldehyde and processed and embedded in paraffin utilizing conventional techniques. Paraffin-embedded sections from obstructed kidneys obtained seven days after disease induction were deparaffinized with chloroform and graded ethanol solutions and then were incubated in 0.2 N HCl for 10 minutes, in proteinase K (5 mg/mL) for 20 minutes, and in 0.25% acetic anhydride/0.1 mol/L triethanolamine for 10 minutes. Hybridization was performed at 53°C for 16 hours in a solution containing 50% formamide, 2 × SSC, 10% dextran sulfate, 10 mmol/L Tris-HCl (pH 7.5), 1 mmol/L ethylenediaminetetraacetic acid (EDTA), 0.1% sarcosyl, 0.02% SDS, 4 × Denhardt's solution, 500 ng/mL denatured salmon sperm DNA, 500 ng/mL yeast transfer RNA, and DIG-labeled riboprobe (2.5 mg/mL). Sections were placed in a high-stringency wash (HSW) buffer (50% formamide, 2 × SSC) for 30 minutes at 60°C and then were treated with 20 ng/mL of ribonuclease A (Sigma) at 37°C for 10 minutes again placed in HSW buffer for 10 minutes, at 60°C, in 2 × SSC at room temperature for 10 minutes and in 0.2 × SSC at room temperature for 10 minutes. Immunologic detection of DIG was performed using a Genius nonradioactive DNA detection kit (Boehringer Mannheim, Mannheim, Germany) according to the manufacturer's protocol. As a negative control, a DIG-labeled sense RNA probe was used instead of an antisense probe.

Immunohistochemical study

Glomerular macrophages were stained with the monoclonal antibody ED-1 (Serotec, Oxford, UK). To stain smooth muscle α-actin (SMαA), a monoclonal antibody designated clone 1A4 was used (Sigma). Polyclonal rabbit anti-mouse type I collagen antiserum was obtained from Chemicon International (Temecula, CA, USA). Immunohistochemical staining was performed with paraffin-embedded sections when anti–ED-1 or anti-SMαA antibody was used as the first antibody and with frozen sections when antitype I collagen antibody was used as the first antibody.

Paraffin-embedded sections from obstructed kidneys obtained four and seven days after disease induction, as well as contralateral kidneys as normal controls, were deparaffinized with xylene and graded ethanol solutions. Frozen sections from obstructed kidneys obtained after seven days were fixed in ice-cold acetone for 30 minutes. The frozen sections then were incubated for five minutes at room temperature in 0.1% trypsin. The various sections then were incubated in a 99:1 methanol/H_2O_2 solution for 30 minutes at room temperature to inactivate the endogenous peroxidase activity, washed three times in PBS for 10 minutes, preincubated at room temperature in blocking solution for 30 minutes [PBS containing 5% goat serum and 1% bovine serum albumin (BSA) for rabbit antiserum or PBS containing 1% BSA for mouse monoclonal antibody], and finally washed in PBS for 3 minutes three times. The first antibodies (anti–ED-1 monoclonal antiserum, 1:100; anti-SMαA mouse monoclonal antibody, 5 mg/mL; or anti-mouse type I collagen polyclonal antiserum, 1:100) were then applied to the sections. Incubation with the first antibody was performed for 16 hours at 4°C. After removal of unbound first antibody by three serial washes in ice-cold PBS, the sections were incubated with the second antibody (a biotinylated goat anti-rabbit antibody, 1:150, or biotinylated goat anti-mouse monoclonal antibody, 1:150, Vector ABC kit; Vector Laboratories, Inc., Burlingame, CA, USA) for one hour at room temperature, followed by three washes in ice-cold PBS. After an avidin-biotinylated horseradish peroxidase complex (Vector) incubation for 40 minutes at room temperature, peroxidase activity was visualized with a solution containing 0.6 mg/mL of p-dimethyl aminobenzaldehyde and 10 mg/mL of H_2O_2 in 50 mmol/L Tris-HCl (pH 6.85) using DAB-buffer tablets (Merck, Darmstadt, Germany). After counterstaining with methyl green, the sections were mounted. Negative control studies were performed using nonimmunized normal rabbit serum for rabbit polyclonal antibody and normal mouse IgG (Santa Cruz Biotechnology, Santa Cruz, CA, USA) for mouse monoclonal

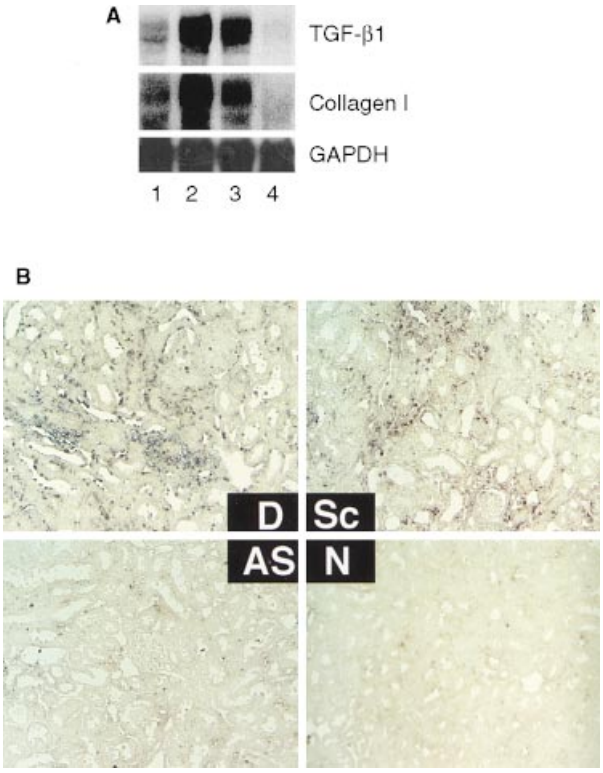

Fig. 1.

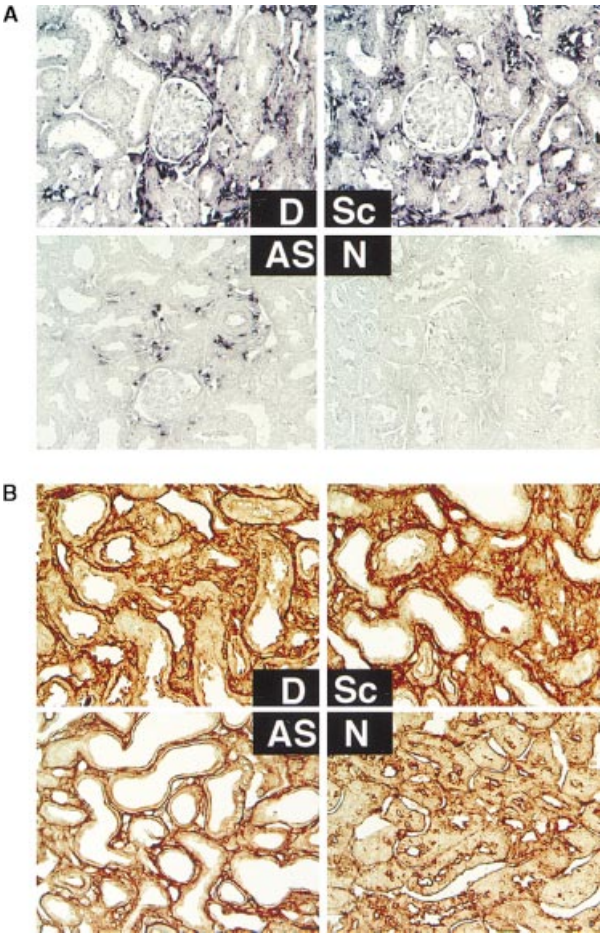

Fig. 2.

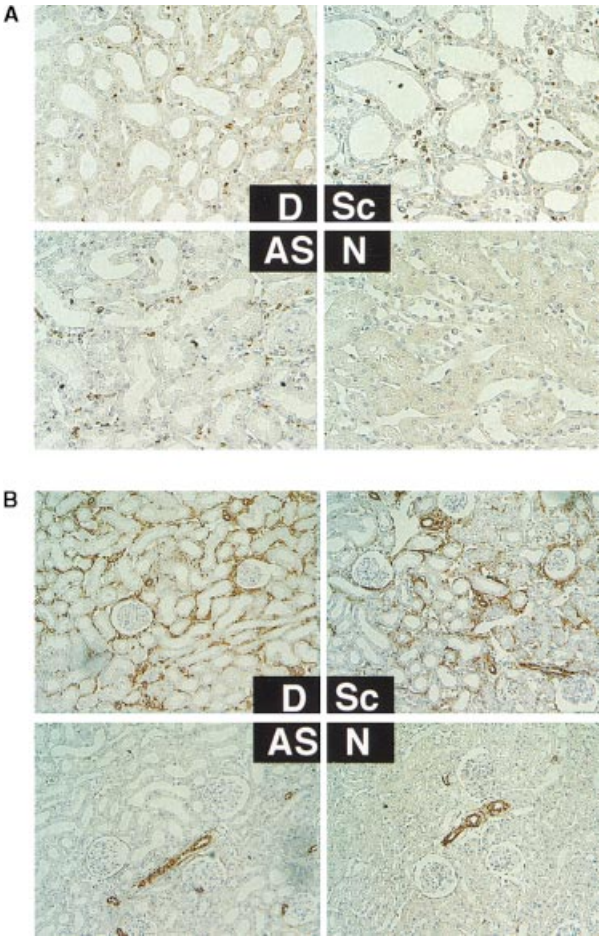

Fig. 3.

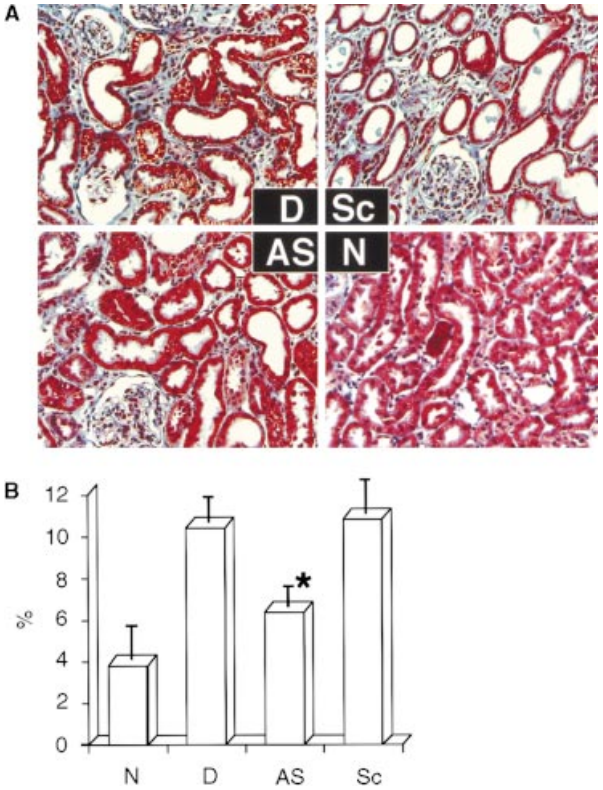

Fig. 4.

Fig. 1. (*A*) Northern blot of cortical transforming growth factor-β1 (TGF-β1), type I collagen, and GAPDH mRNA in obstructed kidneys from rats treated with antisense oligodeoxynucleotides (ODN) for TGF-β1 (lane 1) or scrambled ODN (lane 2) untreated disease control rats (lane 3) or in contralateral normal kidneys (lane 4). (*B*) In situ hybridization of TGF-β1 mRNA in obstructed kidneys from untreated disease control rats (D), unilateral ureteral obstruction model rats treated with scrambled ODN (Sc) or antisense ODN (AS), or in contralateral normal kidneys (N; ×100).

Fig. 3. Immunohistochemical staining for infiltrated macrophages (*A*; ×200) and transformed myofibroblast (*B*; ×100) in obstructed kidneys from untreated disease control rats (D), unilateral ureteral obstruction model rats treated with scrambled ODN (Sc) or antisense ODN (AS) or in contralateral normal kidneys (N).

antibody instead of the first antibodies. No staining was observed in these negative controls.

Morphometric analysis of the interstitial fibrosis

The area of the fibrotic lesion of cortical interstitium was determined in sections stained by Masson's trichrome method to stain collagen fibers (in light blue) in the tubular basement membrane, glomeruli, and interstitial space. Under high-power magnification (×400), 10 nonoverlapping fields from the cortical region were selected at random and analyzed by an observer unaware of the experimental protocol. The fibrotic areas in the interstitium and tubular basement membrane were highlighted on digitized images using a computer-aided manipulator (microscope; Leitz DM IRB, software, Quantimet 500+; Leica, Tokyo, Japan). The fibrotic area relative to the total area of the field was calculated as a percentage. Glomeruli and large vessels were not included in the microscopic fields for image analysis. The scores of 10 fields per kidney were averaged, after which the mean scores from six separate animals per group were averaged.

Statistical analysis

All values are expressed as the mean ± SD. Statistical significance (defined as P values of less than 0.01) was evaluated using the one-way analysis of variance (ANOVA).

RESULTS

Effect of antisense TGF-β1 ODN transfer on TGF-β1 expression

We examined the effect of antisense ODN for TGF-β1 on tubulointerstitial fibrosis in rats with unilateral ureteral obstruction, a well-characterized model of renal injury leading to tubulointerstitial fibrosis that has been linked to up-regulation of interstitial TGF-β1 expression [21, 22]. Obstructed rats were divided into three groups that received either TGF-β1 antisense ODN (antisense group), scrambled ODN (scrambled group), or no treatment (untreated group). In the present experiments, antisense or scrambled ODN-bearing AVE-type HVJ liposomes were slowly instilled immediately after clamping the left renal vein, followed after 10 minutes by ureteral catheter removal, unclamping of the vein, and ureteral ligation. Northern blot analysis demonstrated that densitometric values of the TGF-β1 mRNA transcript levels were 14.8 ± 4.4-fold increased over those in normal control kidneys in the untreated group seven days after obstruction. However, treatment with antisense ODN significantly reduced the levels of TGF-β1 mRNA expression in the renal cortex to 30% of those levels in untreated group (4.4 ± 2.3-fold increase over normal control levels, $P \leq 0.01$ vs. untreated group; Fig. 1A). In contrast, no significant differences in the level of TGF-β1 mRNA were seen between the scrambled group (16.3 ± 5.3-fold increase) and the untreated group. GAPDH mRNA expression remained unaffected in all transfected rats. In addition, in situ hybridization confirmed that TGF-β1 mRNA was up-regulated in the interstitium and tubules of the untreated group and the scrambled group, mainly in the interstitial area, as previously reported [23]. This up-regulation was also suppressed in the antisense group (Fig. 1B). No signal was detected when serial sections from the same kidney were hybridized with the sense probe (data not shown).

Effect of antisense TGF-β1 ODN transfer on type I collagen expression

Given the effective suppression of TGF-β1 mRNA, we assessed type I collagen expression by Northern blot

Fig. 2. Interstitial expression of type I collagen mRNA and protein seven days after ureteral obstruction. In situ hybridization of type I collagen mRNA (*A*; ×200) and immunohistochemical localization of type I collagen protein (*B*; ×200) in obstructed kidneys from untreated disease control rats (D), unilateral ureteral obstruction model rats treated with scrambled ODN (Sc) or antisense ODN (AS) or in contralateral normal kidneys (N).

Fig. 4. (*A*) Masson's trichrome stained kidneys in obstructed kidneys from untreated disease control rats (D), unilateral ureteral obstruction model rats treated with scrambled ODN (Sc) or antisense ODN (AS), or in contralateral normal kidneys (N; ×400). (*B*) Morphometric analysis of relative interstitial fibrotic area. Data are expressed as mean ± SD. $^*P < 0.01$ vs. D and Sc by analysis of variance.

analysis (Fig. 1A), in situ hybridization (Fig. 2A), and immunohistochemistry (Fig. 2B). Renal cortical mRNA levels of type I collagen were low in contralateral normal kidneys. In contrast, type I collagen mRNA levels were 15.8 ± 4.8-fold increased in the untreated group seven days after obstruction. However, treatment with antisense ODN significantly reduced the levels of type I collagen mRNA expression (6.5 ± 2.9-fold increase over normal control levels, $P \leq 0.01$ vs. untreated group; Fig. 1A). In contrast, no significant differences were seen between the scrambled group (18.3 ± 5.7-fold increase) and untreated group. Transcript levels of GAPDH mRNA expression remained unaffected in all transfected rats.

Type I collagen expression, which increases in interstitial fibrotic area, was enhanced in interstitial fibrous spaces in the untreated group and the scrambled group as reported previously [24]. When cortical sections of kidneys from untreated and scrambled group were hybridized with the antisense riboprobe for type I collagen, strong signals were exclusively observed in the interstitial area (Fig. 2A). A slight signal was detected when cortical sections from antisense ODN-treated obstructed kidneys or when the contralateral normal kidney was probed with the antisense riboprobe for type I collagen. No signal was detected when serial sections from the same kidney were hybridized with the sense probe (data not shown).

In addition, immunostaining of type I collagen in the interstitial area was faint in normal kidneys, but strong in untreated obstructed kidneys and scrambled ODN-treated obstructed kidneys. In antisense ODN-treated obstructed kidneys, immunostaining was weak (Fig. 2B). Thus, introduction of antisense ODN markedly suppressed enhanced expression of type I collagen in obstructed kidneys.

Effect on macrophage infiltration and SMαA expression

Macrophage infiltration of the interstitium and increased numbers of activated fibroblasts (myofibroblasts) accompany interstitial matrix accumulation. Infiltrated interstitial macrophages and SMαA, a myofibroblast marker, were stained with monoclonal antibodies designated ED-1 and clone 1A4, respectively. The number of ED1-positive cells in the interstitium increased in obstructed kidneys compared with the opposite kidney four days after disease induction (Fig. 3A), but no significant differences were seen in the number of ED1-positive cells between the three treatment groups either on day 4 (23.9 ± 5.9, 23.8 ± 6.5, and 25.5 ± 6.5 cells per high-power fields in the untreated group, the scrambled group, and the antisense group, respectively) or on day 7 (39.5 ± 9.4, 41.5 ± 10.4, and 44.3 ± 12.1, respectively).

Phenotypic plasticity of resident fibroblasts is becoming increasingly evident [25, 26]. Alterations in the phenotype and behavior of pre-existing renal fibroblasts may be one of the most important events associated with increased matrix synthesis. As shown in Figure 3B, interstitial SMαA expression was markedly increased in the untreated group and the scrambled group, and this distended expression was blocked in the antisense group. The observation that TGF-β1 can transform fibroblasts to myofibroblasts [27] complements our results indicating that a therapeutic strategy targeting interstitial TGF-β1 could inhibit phenotypic changes in the interstitium.

Effect on interstitial fibrosis

Kidneys with ureteral obstruction for seven days showed a greatly expanded interstitium with marked increases in cellular infiltration (Fig. 4A). Note that the blue staining of the interstitium in the obstructed kidney indicates the interstitial fibrosis. The obstructed kidneys from either the untreated or scrambled group exhibited increased tubular dilation, with a marked expansion of the interstitium. In contrast, obstructed kidneys from the antisense group showed a minimal interstitial expansion, although they exhibited the same extent of tubular dilation. In the untreated group or the scramble group, obstruction for seven days resulted in a significant relative increase in fibrotic area compared with the opposite kidneys (Fig. 4B). Obstructed kidneys from the antisense group showed a significantly smaller extent of fibrotic area than those from the untreated group or the scrambled group.

DISCUSSION

This report provides the first clear demonstration, to our knowledge, that introduction of antisense ODN for TGF-β1 into interstitial fibroblasts by the AVE-type HVJ liposome gene delivery system can suppress interstitial TGF-β1 expression and thereby block consequent interstitial fibrosis in rats with unilateral ureteral obstruction. Despite various approaches and techniques, few reports of success have emerged concerning in vivo transfection targeting interstitial fibroblasts, which have been highlighted as the source of increased extracellular matrix synthesis [24]. Recently, Zhu et al have reported successful adenoviral vector transfection into the interstitial compartment. In that work, expression of the β-galactosidase gene was reported in the interstitial vasculature including arteries of the outer medulla in both the outer and inner stripes and in periglomerular and peritubular capillaries of the cortex [28]. In contrast, retrograde introduction via the ureter using an AVE-type HVJ liposome method enabled us to introduce ODN selectively into interstitial fibroblasts.

As we previously observed [14], TGF-β1 antisense

ODN were introduced into neither macrophages nor tubular cells, but fibroblasts, suggesting that TGF-β1 antisense ODN could directly affect TGF-β1 expression in interstitial fibroblasts. Importantly, Northern blot analysis and in situ hybridization demonstrated that TGF-β1 antisense ODN transfection into interstitial fibroblasts significantly reduced interstitial TGF-β1 mRNA expression and prevented interstitial fibrosis in obstructed kidneys. Evidence is conflicting as to whether interstitial cells [18] or tubules [19] are a major source of increased TGF-β1 in a rat model of obstructive nephropathy. In situ hybridization studies performed to date have highlighted interstitial cells as the source [29]. A recent report showed that there appeared to be substantially more TGF-β mRNA in the interstitium of obstructed kidneys of wild-type mouse as opposed to those of osteopontin-null mutant mouse at day 7 [23]. Kaneto et al reported the immunoreactivity for TGF-β increased markedly in the interstitium of human obstructed kidneys, but not in tubular cells [30]. Our observations were compatible with the previous impressions that fibroblasts and myofibroblasts are the major TGF-β1–producing cells [31, 32]. Yamamoto et al showed that TGF-β–positive interstitial cells exhibit immunoreactivity for fibroblastic/myofibroblastic cells but not for monocytes/macrophages [32]. We demonstrated that TGF-β1 mRNA was up-regulated in the interstitium and tubules of the untreated group and the scrambled group, mainly in the interstitial area, while this up-regulation was also suppressed in antisense group. Taken together with our observations, both resident renal tubular cells and interstitial cells may be responsible for TGF-β production; however, interstitial fibroblast cells may be the major source of TGF-β in the formation of interstitial fibrosis. As we demonstrated that the introduction of antisense TGF-β1 ODN into the interstitial cells suppressed the up-regulation of TGF-β1 expression and thereby blocked interstitial fibrosis, targeting TGF-β in interstitial fibroblasts—not tubular cells—may be a feasible strategy in the interstitial fibrosis at least in this model.

Recruitment of macrophages becomes apparent during the few hours following onset of obstruction [33]. Antisense ODN introduction into interstitial cells had no significant effects on macrophages infiltration. The factors involved in macrophage infiltration are not well characterized, but monocyte chemoattractant peptide-1 (MCP-1) might participate in macrophage recruitment [18]. TGF-β also plays a role in macrophage recruitment [22, 34]; however, no significant differences were seen in the number of ED1-positive cells between the untreated and antisense-treated obstructed kidneys. Osteopontin is also reported to be a macrophage adhesive protein that is expressed by renal tubules in tubulointerstitial diseases. Ophascharoensuk et al reported that osteopontin mediates early interstitial macrophage influx and interstitial fibrosis in unilateral ureteral obstruction, and that there appeared to be substantially more TGF-β mRNA in the interstitium of obstructed kidneys of wild-type mouse as opposed to those of osteopontin-null mutant mouse at day 7 [23]. They suggested that the reduction in TGF-β in osteopontin-null mutant mice could account for the tubulointerstitial fibrosis. In this disease model, interstitial TGF-β1 may have less effect on macrophage infiltration than the chemotactic factors released by tubular cells. In contrast, interstitial SMαA expression was markedly increased in the untreated group and the scrambled group, whereas this extensive expression was prevented in antisense group. Whether TGF-β1 is directly mediating interstitial SMαA expression or is acting indirectly by producing and/or activating other factors, which might be responsible for the activation of fibroblasts, was not determined in this study. In addition, antisense ODN blocked the interstitial up-regulation of type I collagen and interstitial fibrosis. These results support our hypothesis that interstitial fibroblasts play an important role in TGF-β1 expression and the consequent progression of interstitial fibrosis, and that inhibiting interstitial TGF-β1 expression can directly and/or indirectly prevent the phenotypic alteration of fibroblast and thereby block interstitial fibrosis.

This study demonstrated that interstitial expression of TGF-β1 is a key stimulus for interstitial fibrosis. The development of therapies targeted against interstitial TGF-β should be considered. Although a number of issues such as safety and side effects were not addressed in this study, we should point out that retrograde ureteral catheterization is a common clinical procedure. Therefore, this new molecular strategy of gene transfer to interstitial cells could represent a powerful investigative and potentially therapeutic strategy in interstitial renal disease.

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