Smad7 Gene Therapy Ameliorates an Autoimmune Crescentic Glomerulonephritis in Mice

Shuk-Man Ka,* Xiao-Ru Huang,† Hui-Yao Lan,‡ Pei-Yi Tsai,‡ Shun-Min Yang,§ Hao-Ai Shui,‡ and Ann Chen*

*Department of Pathology, Tri-Service General Hospital, †Graduate Institute of Medical Sciences, and §Graduate Institute of Life Sciences, National Defense Medical Center, Taipei, Taiwan, Republic of China; and ‡Center for Inflammatory Diseases and Molecular Therapies, University of Hong Kong Li Kat Shing Faculty of Medicine, Hong Kong

Autoimmune crescentic glomerulonephritis is characterized by severe immune response with glomerular crescentic formation and fibrosis in the kidney. Recent studies indicate that overexpression of renal Smad7 attenuates both renal fibrosis and inflammation in rat remnant kidney. However, little attention has been paid to the potential role of TGF-β/Smad signaling in autoimmune kidney disease. This study tested the hypothesis that blocking TGF-β signaling by overexpression of Smad7 may have a therapeutic effect in a mouse model of autoimmune crescentic glomerulonephritis that was induced in C57BL/6 × DBA/2 F1 hybrid mice by giving DBA/2 donor lymphocytes. Smad7 gene was transfected into the kidney using the ultrasound-microbubble–mediated system. Results showed that overexpression of Smad7 blocked both renal fibrosis and inflammatory pathways in terms of Smad2/3 and NF-κB activation (P < 0.01), thereby inhibiting α-smooth muscle actin; collagen I, III, and IV accumulation; and expression of inflammatory cytokines (IL-1β and IL-6), adhesion molecule/chemokine (intercellular adhesion molecule-1, monocyte chemoattractant protein-1), and inducible nitric oxide synthase (all P < 0.01). Leukocyte infiltration (CD4+ cells and macrophages) was also suppressed (P < 0.005). Severe histologic damage (glomerular crescent formation and tubulointerstitial injury) and functional injury including proteinuria were significantly improved (all P < 0.05). This study provides important evidence that overexpression of Smad7 may have therapeutic potential for autoimmune kidney disease.


A

utoimmune crescentic glomerulonephritis (ACGN) is an extremely progressive from of glomerulonephritis and pathologically falls under the group of crescentic glomerulonephritis (1). Unfortunately, the current therapy in crescentic glomerulonephritis, including ACGN, is relatively ineffective and nonspecific with poor clinical outcomes (2–4).

TGF-β is a fundamental growth factor and cytokine in fibrogenesis and inflammation (5,6). TGF-β has been shown to exert its biologic effects by signaling through a heteromeric receptor complex of the type I and type II receptors to activate the downstream intracellular substrates Smad2 and Smad3 (7,8). In addition, TGF-β can induce its downstream inhibitory Smad7, which in turn inhibits Smad2/3 phosphor-ylation via the negative feedback mechanisms (9,10). In the context of immune and inflammation, TGF-β plays a critical role in negatively modulating the immune and inflammatory response (11). This is demonstrated by the observations that mice that lack TGF-β develop uncontrollable systemic inflammation and die in 3 wk after birth (12). It should be noted that the blockade of TGF-β by targeting the upstream of its signaling may cause inflammation, although fibrosis response is inhibited. This concern has been addressed by recent studies that blockade of downstream of the TGF-β/Smad signaling pathway by overexpressing Smad7 is able to inhibit both renal fibrosis and inflammation via the mechanisms of blocking both Smad2/3 and NF-κB signaling pathways in rat remnant kidney and obstructive kidney disease (13–16). However, it remains unknown whether blockade of the TGF-β pathway by Smad7 can inhibit immunologically induced kidney disease.

Recently, we demonstrated that a murine chronic graft versus host disease can progress to an experimental ACGN that is characterized by extensive crescent formation in up to 80% of the glomeruli (17). This study tested whether blocking TGF-β signaling by overexpression of Smad7 is able to inhibit progressive renal injury that is induced immunologically in experimental ACGN. In addition, we extended these findings by presenting a safe, noninvasive, effective, and controllable gene therapy method for treatment of kidney disease with progressive renal injury in a murine ACGN model by transferring a doxycycline-regulated Smad7 using a modified ultrasound-microbubble gene transfer–mediated system.
Materials and Methods

Modified Ultrasound-Mediated Gene Transfer of Inducible Smad7 Gene-Bearing Microbubbles into the Kidney of Mice

Preparation of a mixture of doxycycline-regulated pTRE-m2Smad7–expressing plasmids and gene transfer into the kidney of mouse using the ultrasound-microbubble–mediated technique were used as described previously (13–15), with modification. Briefly, for achievement of doxycycline-induced (a tetracycline derivative) Smad7 transgene expression, a mixed solution that contained 200 µg of pTRE-m2Smad7 and Tet-On plasmids and Optison (echocardiographic contrast microbubbles; Mallinkrodt, St. Louis, MO) at the ratio of 1:1 (vol:vol) in 300 µl was co-transfected into the kidney via the tail vein followed by ultrasound treatment transcutaneously on the back of both sides of kidney location (Sonopuls 590, 1 MHz; Ernaf-Nonius, Delft, Netherlands). Ultrasound was performed immediately after injection with a continuous-wave output of 1 MHz for 30 s on one side, and then 30 s on the other side, for a total of 10 min. After ultrasound treatment, 200 µg of doxycycline (Sigma, St. Louis, MO) was injected intraperitoneally, followed by the addition of doxycycline in the daily drinking water (200 µg/ml).

For determination of the efficacy of ultrasound-microbubble–mediated gene transfer into the kidney of the mice, groups of six normal mice were given a mixture of Smad7 plasmids (200 µg per mouse) and Optison via the tail vein, followed immediately by ultrasound treatment as described. The mice were killed at days 1, 7, 14, and 28 after Smad7 gene transfer, and their kidneys were collected for examination of Smad7 mRNA expression by real-time PCR and Smad7 protein by Western blot analysis and the expression of Smad7 by immunohistochemistry (IHC) with the flag m2 mAb (Sigma).

Establishment of ACGN Model

A murine ACGN model was induced in 7- to 8-wk-old (C57BL/6 x DBA/2J) F1 hybrid mice by giving DBA/2J donor lymphocytes as described previously (17). Briefly, cell suspensions that contained a mixture of donor cells from the thymus, spleen, and lymph nodes (originating from the neck, axillary, and inguinal regions) were injected intravenously three times at 3- to 4-d intervals. Immediately after the induction, mice were randomly assigned into groups of 10 and received either Smad7 or empty vectors. In addition, 10 (C57BL/6 x DBA/2J) F1 hybrid mice were used as normal control. All mice were killed at week 9 after disease induction. Renal tissues and blood and urine samples were collected for analysis as described next. Two weeks after the induction of ACGN model, the mice were treated biweekly with the ultrasound-mediated mixture (0.3 ml) of pTRE-m2Smad7/Tet-on plasmids plus Optison or the mixture of empty vectors and Optison via the tail vein as described and killed at week 9.

Clinical and Pathologic Evaluation

As described previously (18), 24-h urine samples were collected in metabolic cages at week 0 and weekly for the determination of urinary levels of protein by Pierce BCA protein assay kit (Perbio Science, Etten-Leur, Netherlands). Mouse serum was collected at week 9 for serum urea nitrogen and creatinine determination.

For histopathology, the tissues were fixed in 10% buffer formalin (originating from the neck, axillary, and inguinal regions) were injected intravenously three times at 3- to 4-d intervals. Immediately after the induction, mice were randomly assigned into groups of 10 and received either Smad7 or empty vectors. In addition, 10 (C57BL/6 x DBA/2J) F1 hybrid mice were used as normal control. All mice were killed at week 9 after disease induction. Renal tissues and blood and urine samples were collected for analysis as described next. Two weeks after the induction of ACGN model, the mice were treated biweekly with the ultrasound-mediated mixture (0.3 ml) of pTRE-m2Smad7/Tet-on plasmids plus Optison or the mixture of empty vectors and Optison via the tail vein as described and killed at week 9.

Western Blot Analysis

Each protein sample was run on a 12% SDS-PAGE gel. The gel was electroblotted onto polyvinylidene difluoride nitrocellulose membrane (Amersham Int., Buckinghamshire, UK); incubated for 1 h in 20 ml of blocking buffer (Tris-buffered saline that contained 5% skim milk); and incubated with goat anti-Smad7 (Santa Cruz Biotechnology), goat anti-Smad2 (Santa Cruz, Biotechnology), rabbit anti-pSmad2 (Biosource, Camarillo, CA), rabbit anti-Smad3 (Zymed, San Francisco, CA), or rabbit anti-pSmad3 (Biosource) antibodies at 4°C overnight. The antibody was specific for pSmad3 as described previously (21,22). After washing, the membrane was incubated with horseradish peroxidase–
conjugated rabbit anti-goat or goat anti-rabbit (Pierce, Rockford, IL) antibodies for 1 h at room temperature. The membrane-bound antibody detected was incubated with chemiluminescent reagent plus (PerkinElmer Life Sciences, Boston, MA) and captured on x-ray film.

**ELISA**

The TGF-β1 protein levels in renal tissue were measured using the commercial ELISA kits (R&D Systems), according to the manufacturer’s instructions. Briefly, protein samples were acidified with 1 N HCl and neutralized with 1.2 N NaOH/0.5 M HEPES to assay for the amount of TGF-β1. The absorbance was determined at 450 nm using an ELISA plate reader (Bio-Tek, Winooski, VT).

NF-κB p65 was measured in renal tissue nuclear protein extracts using Trans-AM ELISA assay kits (Active Motif, Carlsbad, CA), according to the manufacturer’s instructions. Briefly, nuclear proteins were extracted using a nuclear extract kit (Active Motif) and were measured using a Pierce BCA protein assay kit (Perbio Science, Bezons, France). The absorbance was determined at 450 nm using an ELISA plate reader (Bio-Tek).

**Statistical Analyses**

Values were presented as means ± SEM. Individual experimental group means of data were compared with controls using *t* test. *P* < 0.05 was considered statistically significant.

**Results**

**Ultrasound-Microbubble–Mediated Gene Transfer to the Kidney**

As shown in Figure 1, Smad7 mRNA was rapidly increased at day 1 (*P* < 0.05) and then declined to normal levels by day 14 (Figure 1A) after ultrasound treatment. Western blot analysis showed that Smad7 protein expression was increased at day 1, peaked at day 7 (*P* < 0.005), and became NS by day 14 (Figure 1B). The flag m2 Smad7 gene transfection rate and transgene expression were further detected by IHC with the anti–flag m2 mAb. As shown in Figure 1C (day 0), whereas the anti–flag m2 mAb did cross-react to normal tubular cells as a result of the antibody cross-reactivity, it was negative in glomeruli as described previously in the normal kidney (13). Thus, the Smad7 gene transfection rate and transgene expression were evaluated in the glomerulus. As shown in Figure 1C (days 7, 14, and 28), ultrasound-mediated Smad7 gene transfer resulted in a marked increase in Smad7 transgene expression as identified by the anti–flag m2–positive cells in the majority of glomerular cells, peaked at day 7, and then declined from day 14 onward. Therefore, to maintain a high level of Smad7 within the kidney, Smad7 gene transfer was repeatedly given every 14 d after the previous treatment.

**Smad7 Gene Transfer Improves Proteinuria, Renal Function, and Pathology**

All ACGN mice survived until they were killed, although mice that were treated with empty vector showed moderate or severe ascites. As shown in Figure 2, ACGN mice that were...
treated with empty vectors developed progressive renal injury as demonstrated by an increase in proteinuria over weeks 3 to 9 after ACGN induction. In contrast, mice that were treated with Smad7 exhibited a significant reduction of proteinuria over weeks 6 to 9 (Figure 2A). This was associated with a significant reduction in both serum levels of serum urea nitrogen (Figure 2B) and creatinine (Figure 2C). Histologically, as shown in Figure 3, diseased mice that were treated with the empty vectors revealed extensive crescent formation, glomerulosclerosis, and remarkable interstitial mononuclear cell infiltration (mainly periglomerular; Figure 3B). In contrast, as shown in Figure 3, C and J, ACGN mice that were treated with Smad7 substantially inhibited crescent formation, glomerulosclerosis, and interstitial mononuclear cell infiltration (all P < 0.01, respectively). It is interesting that there was no significant difference in the intensity of IgG and C3 deposition in the glomerulus between the empty vector and the Smad7-treated ACGN mice (Figure 3, D through I and K), indicating that treatment with Smad7 had no effect on immune complex deposition.

Renal Fibrosis Is Inhibited by Treatment with Smad7

We first examined the therapeutic effect of Smad7 on renal fibrosis. As shown in Figure 4A, real-time PCR showed that

---

**Figure 3.** Effects of Smad7 gene therapy on renal pathology and immune complex deposition in the glomeruli. Mice were killed at week 9 after disease induction. Hematoxylin and eosin (H&E) stain: Normal control (A), empty vector-treated mice with ACGN (B), Smad7-treated mice with ACGN (C). Immunofluorescence (IF) with IgG: Normal control (D), empty vector–treated mice with ACGN (E), Smad7–treated mice with ACGN (F). IF with C3: Normal control (G), Empty vector–treated mice with ACGN (H), Smad7–treated mice with ACGN (I). (J) Semiquantitative analysis of H&E. (K) Semiquantitative analysis of IF. Each bar represents the mean ± SEM for a group of 10 mice. **P < 0.01; ***P < 0.005; #Not detectable. Magnification, ×400.
Smad7 treatment resulted in a significant increase in the level of renal Smad7 mRNA. All fibrogenic markers including mRNA were upregulated in ACGN that were mice treated with empty vector. In contrast, this was associated with the marked inhibition of $\alpha$-SMA (Figure 4B), collagen I (Figure 4C), collagen III (Figure 4D), and collagen IV (Figure 4E) mRNA expression. Similarly, IHC demonstrated that increased expression of $\alpha$-SMA and collagens I, III, and IV within the diseased kidney of ACGN mice that were treated with empty vectors was inhibited by gene transfer with Smad7 (Figure 5).

**Blockade of the TGF-β/Smad2/3 Signaling Is a Mechanism by Which Smad7 Inhibits Renal Fibrosis**

We next investigated the mechanisms by which Smad7 inhibits renal fibrosis in ACGN. As shown in Figure 6, A and B, real-time PCR and ELISA analyses showed that compared with normal control mice, renal TGF-β1 mRNA and protein were significantly upregulated in ACGN mice that were treated with empty vectors ($P < 0.05$), which resulted in a significant increase in phosphorylation of Smad3 (Figure 6, C and D) and Smad2. However, overexpression of Smad7 abrogated an increase in both TGF-β1 mRNA (Figure 6A) and protein (Figure 6B), thereby blocking Smad3 (Figure 6, C and D) and Smad2 activations. To confirm the expression of these proteins in renal tissues, we performed IHC. As shown in Figure 7, A through F, whereas mice that were treated with Smad7 revealed a moderate to strong positivity for the flag m2 in both the glomerulus and tubulointerstitial tissues (Figure 7F), both normal control and diseased mice that were treated with empty vectors showed negative signals for the flag m2 in the glomerulus, although the tubular staining remained strong (Figure 7, D and E). The latter may be associated with the cross-reactivity of the anti–flag m2 antibody to an isoform of Mg2+-dependent protein phosphatase as previously reported (13).

By IHC, low levels of TGF-β1 and phosphorylated Smad2/3 within the nuclei were found in the normal control kidney with moderate Smad7 expression (Figure 7, A, B, and C). However, TGF-β1 was upregulated in the diseased mice that were treated with empty vectors (Figure 7K), resulting in a marked activation of Smad2/3 (Figure 7H), whereas renal Smad7 remained lower (Figure 7, B and E). In contrast, gene transfer with Smad7 resulted in a marked upregulation of Smad7, which was associated with inhibition of renal TGF-β1 expression and Smad2/3 activation (Figure 7, C, D, E, and F).

**Smad7 Gene Transfer Inhibits Renal Inflammation in ACGN**

It is known that T cell (23,24) or macrophage infiltration (25–27) may play an important role in the development and progression of crescentic glomerulonephritis. In the context of renal immune injury, we first tested whether overexpression of Smad7 inhibits renal inflammation in mouse with ACGN. IHC showed that a profound T cell and macrophage infiltration was noted in the kidney of ACGN mice that were treated with empty vectors (Figure 8, B, E, and F), which was abrogated by Smad7 treatment (Figure 8, C, F, and G). It seemed that the effect of Smad7 overexpression was domi-
nant in blocking monocyte/macrophage over T cell infiltrates. Further studies with real-time PCR showed that inhibition of T cell and macrophage infiltration was associated with suppression of inflammatory cytokines (IL-1β and IL-6; Figure 9, A and B), adhesion molecule and chemokine (ICAM-1 and MCP-1; Figure 9, C and D), and iNOS mRNA expression (Figure 9E). Although Smad7 treatment resulted in a great suppression of inflammatory cytokines in ACGN mice, MCP-1 levels remained high when compared with normal control mice (Figure 9C). Similarly, Smad7 treatment inhibited the protein levels of IL-1β, IL-6, MCP-1, iNOS, and ICAM-1 in the glomerulus of the ACGN model as demonstrated by IHC (Figure 10).

**Figure 5.** Effects of Smad7 gene therapy on protein expression of renal fibrogenic markers by IHC. Mice were killed at week 9 after disease induction. (A through C) Anti-α-SMA. (D through F) Anti-collagen I. (G through I) Anti-collagen III. (J through L) Anti-collagen IV. Magnification, ×400.

**Blockade of NF-κB Activation Is a Key Mechanism by Which Smad7 Inhibits Renal Inflammation**

We further investigated whether inhibition of NF-κB activation is a central mechanism whereby overexpression of Smad7 inhibits renal inflammation in the ACGN model. As shown in Figure 10, NF-κB p65 was markedly activated in the diseased mice that were treated with empty vectors, compared with the normal control kidney, as evident by its nuclear location in the glomeruli and tubulointerstitium as well as in mononuclear cells that infiltrated the periglomerular regions (Figure 10, M, N, and P). In contrast, NF-κB p65 activation was virtually blocked by gene transfer of Smad7 (Figure 10, O and P). Consistent with the IHC, ELISA assay for renal tissue nuclear protein extracts also demonstrated that the Smad7-treated ACGN mice produced a significant inhibition of NF-κB p65 activation, compared with the empty vector-treated ACGN mice (16.46 ± 2.48 versus 40.92 ± 11.10 ng/ml; P < 0.01; Figure 10Q).

**Discussion**

This study provides the first demonstration that gene therapy with inducible Smad7 can effectively ameliorate ACGN, a model of autoimmune kidney disease. This was demonstrated
by the inhibition of renal inflammation and fibrosis with crescentic glomerulonephritis. Blocking of TGF-β/Smad signaling and NF-κB activation is the central mechanism by which gene transfer of Smad7 attenuated progressive renal injury in a mouse model of ACGN.

In this study, Smad7 gene was successfully transfected into the kidney using the noninvasive ultrasound-microbubble-mediated system after intravenous injection of a mixture of microbubbles (Optison) and pTRE-m2Smad7 plasmids. The Smad7 transgene expression was identified in the majority of glomerular cells with the anti–flag m2 mAb, although Smad7 transgene expression by tubular cells remains unidentified because of the antibody cross-reactivity to the tubule cells as described previously (13). Ultrasound-mediated microbubble cavitation could be a key mechanism by which ultrasound treatment largely enhances Smad7 gene transfection rate and transgene expression (13).

As expected, overexpression of Smad7 blocked Smad2/3 activation and renal fibrosis in ACGN, which is consistent with previous reports in obstructive kidney disease (16) and remnant kidney diseases (13). However, inhibition of immune-mediated kidney disease, particularly crescentic glomerulonephritis by overexpression of Smad7, is novel, although it is noted that expression of Smad7 is able to block renal inflammation in nonimmunologically mediated renal injury in a mouse obstructive kidney model and in a rat model of kidney disease (13–16). It is generally believed that TGF-β is an anti-inflammatory cytokine and immune modulator (28). Thus, blockade of TGF-β may promote inflammation and immune-mediated injury (29). Lessons learned from the TGF-β knockout mice with massive systemic inflammation reveal the critical role for TGF-β in anti-inflammation. The use of neutralizing antibodies to block TGF-β is likely to prevent the development of fibrosis but may also enhance inflammatory response and renal injury as reported in a db/db diabetic mouse model and a puromycin aminonucleoside nephropathy in rats (30,31), suggesting the complexity of TGF-β in the pathophysiologic progression of renal disease. The findings that blockade of downstream of TGF-β signaling with Smad7 inhibited renal fibrosis and immune-mediated renal injury indicate an additional role for Smad7 in anti-renal inflammation in ACGN. It is generally accepted that glomerular crescent formation is a severe form of immune-mediated glomerulonephritis as demonstrated by the participation of macrophages, T cells, and neutrophils (27,32,33). In past decades, many studies have shown that deletion of T cells as well as macrophages attenuates crescentic glomerulonephritis (23,25,34), demonstrating an essential role for T cells and macrophages in the pathogenesis of crescentic glomerulonephritis. In addition, NF-κB–dependent proinflammatory cytokines, including IL-1 and TNF-α, have been shown to play a critical role in the pathogenesis of crescentic glomerulonephritis (35,36). Blockade of NF-κB inhibits crescentic glomerulonephritis in a rat model of anti-
glomerular basement membrane disease, demonstrating a critical role for the NF-κB pathway in progressive renal injury (37). Thus, blockade of NF-κB activation in both glomeruli and interstitial tissues could be a key mechanism by which Smad7 inhibits immune-mediated renal injury in ACGN. It is likely that Smad7 inhibited renal inflammation through a combined suppression of both NF-κB activity and mononuclear leukocyte infiltration in the kidney.

Activation of Smad3 has been shown to play a critical role in fibrosis. This is further delineated by the findings from Smad3 knockout mice. Many studies have shown that mice that are null for Smad3 are protected against fibrosis in a number of disease settings, including tubulointerstitial fibrosis in obstructive kidney disease (38,39). Beyond the context of fibrosis, mice that lack Smad3 also exhibit reduced inflammatory response, such as macrophage and T cell infiltration in obstructive kidney and in skin wound healing (39,40), indicating an additional role for Smad3 in inflammation. This is further demonstrated by the recent studies that TGF-β signals through Smad3 to exert its inhibitory effect on T cell proliferation, MCP-1–driven leukocyte infiltration, adhesion molecules (41), and Th1-cytokine IL-2 production (42). On the basis of our observations that overexpression of Smad7 is able to block Smad2/3 activation and inhibition of NF-κB activation, this study demonstrated that there are three major mechanisms by which Smad7 inhibits progressive renal injury in ACGN. First, overexpression of Smad7 may act as a negative regulator of TGF-β signaling to inhibit renal fibrosis by blocking the TGF-β/Smad-dependent pathway. Second, Smad7 may block renal inflammation via inhibition of the NF-κB–mediated inflammatory pathway, although inhibition of Smad3 activation by Smad7 may also have an impact on renal inflammation. Finally, one potential mechanism that should also be considered is the possible effect of Smad7 gene therapy on the circulating and/or tissue inflammatory cells themselves. Inhibition of T cell and macrophage infiltrations...
Figure 8. Effects of Smad7 gene therapy on renal T cell and macrophage infiltration by IHC. Mice were killed at week 9 after disease induction. (A through C) Anti-CD4+ T cells. (D through F) Anti-F4/80 (macrophages). (G) Semiquantitative analysis. ***P < 0.005. Magnification, ×400.

Figure 9. Effects of Smad7 gene therapy on renal inflammatory mRNA gene expression by real-time PCR. Mice were killed at week 9 after disease induction. (A) IL-1β mRNA. (B) IL-6 mRNA. (C) Monocyte chemoattractant protein-1 (MCP-1) mRNA. (D) Intercellular adhesion molecule-1 (ICAM-1) mRNA. (E) Inducible nitric oxide synthase (iNOS) mRNA. Each bar represents the mean ± SEM for a group of 10 mice that were treated with Smad7 (□), empty vector (■), or normal control (□). **P < 0.01; ***P < 0.005; †P < 0.05 versus normal control.
Figure 10. Effects of Smad7 gene therapy on renal inflammatory cytokine (IL-1β and IL-6), MCP-1, iNOS, and NF-κB p65 expression. Mice were killed at week 9 after disease induction. (A through C) Anti–IL-1β. (D through F) Anti–IL-6. (G through I) Anti–MCP-1. (J through L) Anti–iNOS. (M through O) Anti–NF-κB p65. (P) Semiquantitative analysis of NF-κB p65 (IHC). (Q) NF-κB p65 as detected by ELISA (renal tissue nuclear protein extracts). Each bar represents the mean ± SEM for a group of 10 mice treated with Smad7 (□), empty vector (■), or normal control (□). **P < 0.01; ***P < 0.005. Magnification, ×400 each.
tiation and activation could account for the fact that gene therapy with Smad7 blocks renal fibrosis and immune-mediated renal injury in ACGN.

Acknowledgments
This study was supported by grants from the Ministry of Economy (95-EC-17-A-20-S1-028), Tri-Service General Hospital (TSCH-C92-4-002), Taiwan, Republic of China, and the Research Grant Council of Hong Kong (RGC CERG HKU7992/06M).

Disclosures
None.

References
30. Ziyadeh FN, Hoffman BB, Han DC, Iglesias-De, La Cruz MC, Hong SW, Isono M, Chen S, McGowan TA, Sharma K:


42. McKarns SC, Schwartz RH, Kaminke NE: Smad3 is essential for TGF-beta1 to suppress IL-2 production and TCR-induced proliferation, but not IL-2-induced proliferation. *J Immunol* 172: 4275–4284, 2004