

## Full-length article

**Electroporative interleukin-10 gene transfer ameliorates carbon tetrachloride-induced murine liver fibrosis by MMP and TIMP modulation<sup>1</sup>**Wen-ying CHOU<sup>2</sup>, Cheng-nan LU<sup>3</sup>, Tsung-hsing LEE<sup>2</sup>, Chia-ling WU<sup>2,5</sup>, Kung-sheng HUNG<sup>4</sup>, Allan M CONCEJERO<sup>4</sup>, Bruno JAWAN<sup>2</sup>, Cheng-haung WANG<sup>2,6</sup>*Departments of <sup>2</sup>Anesthesiology, <sup>3</sup>Chinese Medicine and <sup>4</sup>Surgery, Kaohsiung Chang Gung Memorial Hospital, Taipei, China; <sup>5</sup>Department of Biological Sciences, National Sun Yat-sen University, Kaohsiung, China***Key words**

interleukin-10; gene therapy; liver cirrhosis; gelatinase A; tissue inhibitor of metalloproteinases; cyclooxygenase 2

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**Abstract**

**Aim:** Liver fibrosis represents a process of healing and scarring in response to chronic liver injury. Effective therapies for liver fibrosis are lacking. Interleukin-10 (IL-10) is a cytokine that downregulates pro-inflammatory responses and has a modulatory effect on hepatic fibrogenesis. The aim of this study was to investigate whether electroporative IL-10 gene therapy has an hepatic fibrolytic effect on mice. **Methods:** Hepatic fibrosis was induced by administering carbon tetrachloride (CCl<sub>4</sub>) for 10 weeks in mice. The human IL-10 expression plasmid was delivered via electroporation after hepatic fibrosis was established. Histopathology, reverse transcription polymerase chain reaction (RT-PCR), immunoblotting, and gelatin zymography were used to investigate the possible mechanisms of action of IL-10. **Results:** Human IL-10 gene therapy reversed CCl<sub>4</sub>-induced liver fibrosis in mice. RT-PCR revealed that IL-10 gene therapy attenuated liver TGF- $\beta$ 1, collagen  $\alpha$ 1, fibronectin, and cell adhesion molecule mRNA upregulation. Following gene transfer, both the activation of  $\alpha$ -smooth muscle actin and cyclooxygenase-2 were significantly attenuated. Furthermore, IL-10 significantly inhibited matrix metalloproteinase-2 (MMP-2) and tissue inhibitors of matrix metalloproteinase (TIMP) activation after CCl<sub>4</sub> intoxication. **Conclusions:** We demonstrated that IL-10 gene therapy attenuated CCl<sub>4</sub>-induced liver fibrosis in mice. IL-10 prevented upregulated fibrogenic and pro-inflammatory gene responses. Its collagenolytic effect may be attributed to MMP and TIMP modulation. IL-10 gene therapy may be an effective therapeutic modality against liver fibrosis with potential clinical use.

**Introduction**

Oxidative stress, an important factor that induces liver fibrosis, represents a key feature of hepatitis induced by various conditions, including anoxic/reoxygenation injury, autoimmune hepatitis, viral hepatitis and alcoholic hepatitis<sup>[1]</sup>. Less severe oxidative stress may sustain fibrosis progression by causing activation and morphological changes in hepatic stellate cells (HSC), including promoting proliferative activity, synthesis and degradation/remodeling of the extracellular matrix (ECM), chemotaxis, contractility, proinflammatory activity and retinoid loss<sup>[1,2]</sup>.

Carbon tetrachloride (CCl<sub>4</sub>) is a xenobiotic used extensively to induce oxidative stress. It is assumed to initiate free radical-mediated lipid peroxidation, leading to the accumulation of lipid-derived oxidation products that cause liver injury and excess collagen deposition in the liver, resulting in liver fibrosis<sup>[3,4]</sup>. During hepatic fibrogenesis, there is an imbalance between excess synthesis of ECM and/or its removal, with consequent fibrosis and scarring<sup>[5,6]</sup>. The pathophysiology of ECM formation during liver fibrosis is multifaceted and complex<sup>[7,8]</sup>. It involves a change in the expression of ECM proteases (matrix metalloproteinases; MMP) and their inhibitors (tissue inhibitors of metalloproteinases;

TIMP) and an increase in the synthesis of collagen and fibronectin driven by signaling pathways mediated by pro-inflammatory cytokines such as transforming growth factor- $\beta$ 1 (TGF- $\beta$ 1) and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ )<sup>[9-12]</sup>.

Interleukin (IL)-10 is a cytokine that downregulates pro-inflammatory responses<sup>[13]</sup>. Human IL-10 is a 160 amino acid protein (molecular weight=18.5 kDa), and murine IL-10 is a 157 amino acid protein with 80% homology to the human form<sup>[14]</sup>. Recombinant human IL-10 has been produced and tested in clinical trials. Studies suggest that IL-10 may be effective against chronic hepatitis C and other liver diseases<sup>[15]</sup>. Further, IL-10 gene therapy has been studied extensively in animal models for autoimmune diabetes, thyroiditis, and colitis<sup>[16-18]</sup>. Because the elimination half-life of recombinant IL-10 is relatively short ( $\approx$ 2 h)<sup>[19]</sup>, it may be possible to utilize its therapeutic properties to develop a gene-based treatment regimen. In the present study, we investigate whether IL-10 gene therapy is effective against CCl<sub>4</sub>-induced liver fibrosis in mice.

## Materials and methods

**Subjects** Male 6- to 8-week-old ICR mice were purchased from the National Science Council, Taiwan, China, and were allowed to acclimatize for 5 d before experimentation. The mice were housed in Kaohsiung Chang Gung Memorial Hospital Animal Facility under standard temperatures, and with a standard light and dark cycle. All procedures performed on the mice were approved by the Kaohsiung Chang Gung Memorial Hospital Animal Care and Use Committee.

**IL-10 expression plasmid preparation** A human IL-10 expression plasmid (pCYIL-10 vector) was used in the present study<sup>[20]</sup>. In brief, full-length human IL-10 cDNAs were subcloned into a pCY4B expression vector driven by a chicken  $\beta$ -actin promoter with a cytomegalovirus immediate early enhancer. pCMV-LacZ was used as the vehicle control. These plasmids were purified using the EndoFree Plasmid Giga Kit (Qiagen, Valencia, CA, USA).

**Liver fibrosis induction and gene therapy** Based on the method used in a previous study, but with some modifications, the mice were administered CCl<sub>4</sub> (1 mL/kg body weight) dissolved in olive oil (1:1) twice a week for 10 weeks<sup>[21]</sup>. Sixteen mice were killed at the end of 6 weeks to confirm that liver fibrosis was established (group I). To evaluate the anti-fibrotic effect of IL-10, gene therapy administration was started at the end of 6 and 8 weeks of CCl<sub>4</sub> treatment. Briefly, 30  $\mu$ L bovine hyaluronidase (0.4 IU/ $\mu$ L) (Sigma-Aldrich, St Louis, MO, USA) was injected into the anterior tibialis (AT) muscle of the mice 2 h before electroporation. pCYIL-10 was

injected into the bilateral AT muscles using a 27G needle (30  $\mu$ L into each leg; 4  $\mu$ g/ $\mu$ L; group II,  $n=16$ ). Electroporation was carried out using electrical pulses (8 pulses of 20 ms, 175 V/cm, and 1 s intervals) with Tweezertrode electrode disks and an electrical pulse generator (T830; BTX, San Diego, CA, USA)<sup>[22]</sup>.

Sixteen mice received gene electro-transfer therapy using the same procedure as described above using pCMV-LacZ (group III) as a vehicle control at the end of 6 and 8 weeks. All surviving mice (group II,  $n=12$ ; group III,  $n=7$ ) were killed at the end of the 10-week CCl<sub>4</sub> treatment. Five mice were killed before CCl<sub>4</sub> intoxication as normal controls (group N).

**Histopathology and immunohistochemistry** For histopathology studies, mice were killed at 0, 6, and 10 weeks after CCl<sub>4</sub> administration. The liver was removed and fixed in 10% formalin solution. Five-micrometer sections were stained with 0.1% Sirius red in picric acid (Sigma-Aldrich). Matrix density was quantified using a computerized image analysis system as previously described<sup>[23]</sup>. For immunohistochemical studies, the sections were washed in phosphate-buffered saline (PBS), and incubated in 3% normal goat serum with 0.3% Triton X-100 in PBS for 1 h. The sections were incubated free-floating at 4 °C with IL-10 (specific for human origin; Santa Cruz Biotechnology, Santa Cruz, CA, USA), cyclooxygenase-2 (COX-2), MMP-2, and TIMP-1 (Abcam, Cambridge, MA, USA) antibodies. Immunoreactivity was visualized using the Vectastain Elite ABC Peroxidase method (Vector Laboratories, Burlingame, CA, USA) with diaminobenzidine (DAB) as the chromagen.

**Soluble collagen measurement** For soluble collagen analysis, the Sircol collagen assay (Biocolor, Belfast, UK) was performed following the manufacturer's instructions as described in a previous study<sup>[24]</sup>. Briefly, 50 mg of liver was homogenized. Total acid pepsin-soluble collagens were extracted overnight using 5 mg/mL pepsin in 500  $\mu$ L of 0.5 mol/L acetic acid. One milliliter of Sircol dye reagent was added to every 100  $\mu$ L of each sample, in duplicate, and the mixture was incubated at 25 °C for 30 min. After centrifugation, the pellet was suspended in 1 mL of alkali reagent. The absorbance was read at 540 nm.

**Immunoblotting** The liver specimens were homogenized in a lysis buffer with complete protease inhibitor cocktail tablets (Roche, Mannheim, Germany). For analysis of  $\alpha$ -smooth muscle actin ( $\alpha$ -SMA) expression after CCl<sub>4</sub> administration, 20  $\mu$ g of protein extracts were electrophoresed on a 10% acrylamide sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gel and immunoblotted onto PVDF membranes. The membranes were blocked for 1 h at

room temperature and incubated overnight with a 1:1000 dilution of  $\alpha$ -SMA, and  $\alpha$ -tubulin antibodies (Abcam). Antibody binding was detected using horseradish peroxidase (HRP)-linked immunoglobulin G (IgG). Bands were visualized using an ECL detection system (Amersham-Pharmacia Biotech, Little Chalfont, UK). Band intensities were quantified using an image analyzer (Densitograph AE-6900M; Atto, Tokyo, Japan).

**Reverse transcription-polymerase chain reaction** Livers were harvested at 0, 6, and 10 weeks after CCl<sub>4</sub> administration. The expression levels of TGF- $\beta$ 1, collagen  $\alpha$ 1, fibronectin, TNF- $\alpha$ , intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), TIMP-1, and TIMP-2 mRNA were analyzed using reverse transcription-polymerase chain reaction (RT-PCR) techniques. The total RNA was extracted and then reverse-transcribed into cDNA. PCR was performed at a final concentration of 1 $\times$  PCR buffer, 1.0  $\mu$ mol/L of each of the 3' and 5' primers, and 10 U of *Advantag* Plus DNA polymerase (Clontech, Palo Alto, CA, USA) in a total volume of 50  $\mu$ L. The mixture was amplified for 32 cycles in a thermal cycler (Stratagene, La Jolla, CA, USA). The  $\beta$ -actin was amplified to verify equal loading. The primer sequence and expected product size were as previously described<sup>[25]</sup>. The amplification products were separated by agarose gel electrophoresis and visualized using ethidium bromide staining. The gel was scanned at a NucleoVision imaging workstation (NucleoTech, San Mateo, CA, USA), and quantified using GelExpert release 3.5.

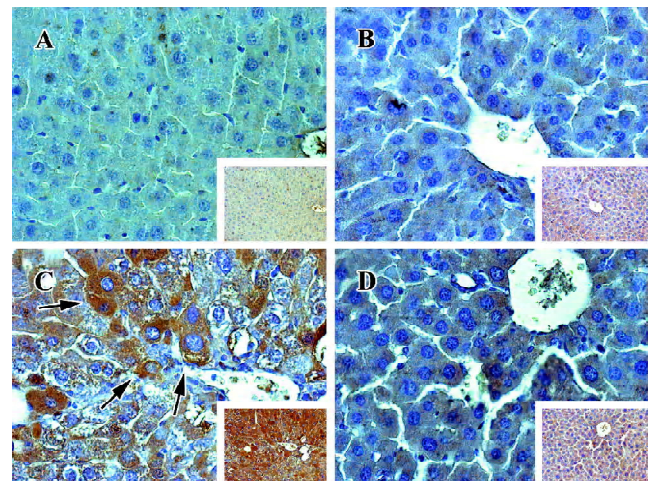
**Gelatin zymography** Gelatin zymography was carried out to explore MMP activity. Briefly, liver tissues were homogenized in a protein extraction buffer. The supernatant of a centrifuged liver sample (20  $\mu$ g of protein extract per line) was mixed 1:3 with a sample buffer and separated by sodium dodecylsulfate (SDS)-polyacrylamide gel electrophoresis in 8% polyacrylamide gel copolymerized with 1 mg/mL gelatin (Sigma-Aldrich) as described elsewhere<sup>[26]</sup>. Gels were incubated at 37 °C overnight in an MMP activation buffer. After Coomassie blue staining, the extent of gel digestion localized to bands of active-MMP-2 (64-kDa) were quantified by densitometry.

**Statistical analysis** All data (from at least 3 separate experiments) are presented as mean $\pm$ SEM. Statistical analysis was performed using one-way ANOVA followed by the *t*-test. *P*<0.05 was considered significant.

## Results

**Long-term IL-10 expression following electroporative gene transfer** There was only scanty staining of cells for

human IL-10 in the non-gene transfer groups (groups N, I, and III; Figure 1). Strong positive staining of cells for human IL-10 was seen in the livers of the gene transfer group II and 4 weeks after electroporation (group II; Figure 1C).



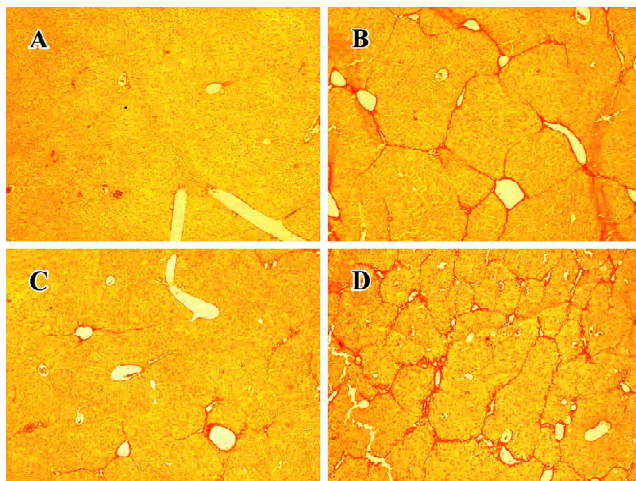
**Figure 1.** Representative photomicrographs of IL-10-stained mouse liver sections. There was scanty staining for human IL-10 in the non-gene therapy groups (groups N, I, and III). Strong positive staining for human IL-10 was detected in the livers of the gene transfer group (group II). (A) Normal control; (B) Group I; (C) Group II; (D) group III. Arrows indicate IL-10-positive cells. Magnification,  $\times$ 400 (inset,  $\times$ 200).

### IL-10 gene therapy reversed CCl<sub>4</sub>-induced liver fibrosis

There was no significant difference in food and water intake throughout the study period between groups. After 6 weeks of CCl<sub>4</sub> administration, liver fibrosis was seen histopathologically. Sirius red staining of liver sections revealed extensive fibrosis, portal-to-portal fibrous bridging, and nodular transformation in groups I and III (Figure 2). Human IL-10 gene therapy (group II) significantly ameliorated hepatic fibrogenesis and reduced matrix density (Figure 2C). These findings were further confirmed by measurements of liver collagen content (Table 1).

**IL-10 gene therapy attenuated COX-2 increment after CCl<sub>4</sub>** COX-2 was not detected immunohistochemically in the normal group. COX-2 expression was upregulated after CCl<sub>4</sub> administration (groups I and III; Figure 3). IL-10 gene therapy significantly diminished this COX-2 expression (Figure 3C).

**IL-10 gene therapy suppressed hepatic stellate cell activation after CCl<sub>4</sub>**  $\alpha$ -SMA [activated hepatic stellate cell (HSC) markers] are known to be activated after acute liver injury<sup>[27,28]</sup>. In the present study, the expression of  $\alpha$ -SMA increased after chronic CCl<sub>4</sub> administration as measured using immunoblotting (Figure 4). IL-10 gene therapy (group II)



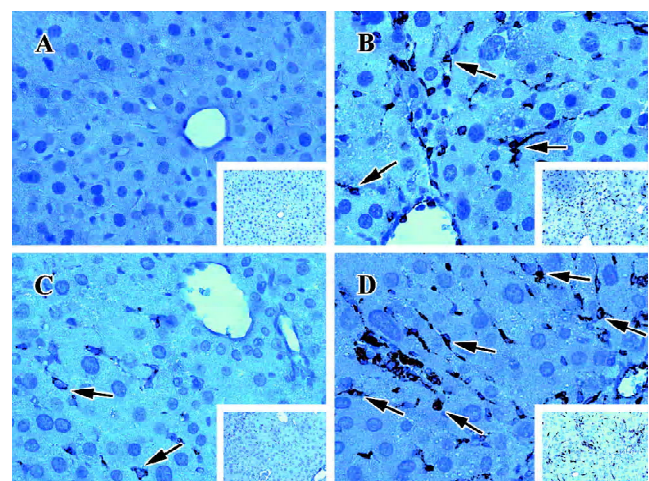
**Figure 2.** Representative photomicrographs of Sirius red-stained mouse liver sections. Extensive fibrosis, portal-to-portal fibrous bridging, and nodular transformation were seen in groups I and III after chronic CCl<sub>4</sub> administration. IL-10 gene therapy (group II) significantly reduced matrix density and abrogated hepatic fibrogenesis. (A) Normal control; (B) Group I; (C) Group II; (D) Group III. Magnification,  $\times 50$ .

**Table 1.** Effects of IL-10 gene therapy on regression of hepatic fibrosis. IL-10 gene therapy significantly reduced the degree of liver fibrosis caused by chronic CCl<sub>4</sub> administration in mice as measured using Sirius red matrix density and collagen content. Mean $\pm$ SEM. <sup>b</sup> $P < 0.05$ , <sup>c</sup> $P < 0.01$  vs group I. <sup>f</sup> $P < 0.01$  vs group III.

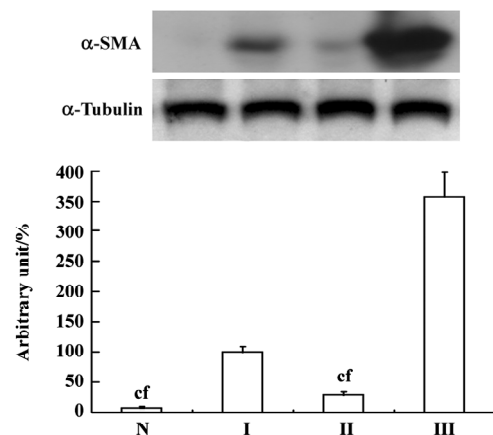
	Sirius red matrix density (%)	Collagen content ( $\mu\text{g}/\text{mg}$ )
Group N	0 <sup>cf</sup>	10.6 $\pm$ 1.7 <sup>cf</sup>
Group I	6.1 $\pm$ 0.5	48.3 $\pm$ 2.0
Group II	4.1 $\pm$ 0.5 <sup>bc</sup>	35.7 $\pm$ 3.0 <sup>bc</sup>
Group III	8.2 $\pm$ 0.6	59.7 $\pm$ 5.0

significantly reduced this upregulation, indicating HSC inactivation ( $P < 0.01$  vs group I;  $P < 0.01$  vs III).  $\alpha$ -Tubulin was used as an internal control.

**IL-10 gene therapy attenuated fibrogenic, proinflammatory, and cell adhesion molecule gene responses after CCl<sub>4</sub> treatment** Expression of TGF- $\beta$ 1, collagen  $\alpha$ 1, fibronectin, TNF- $\alpha$ , ICAM-1, and VCAM-1 mRNA were all upregulated in the fibrotic liver as semi-quantified using RT-PCR (Figure 5).  $\beta$ -actin was amplified as an internal control. IL-10 gene therapy (group II) significantly attenuated these increase. In brief, IL-10 gene transfer suppressed the fibrogenic, proinflammatory, and cell adhesion molecule gene responses after CCl<sub>4</sub> administration.



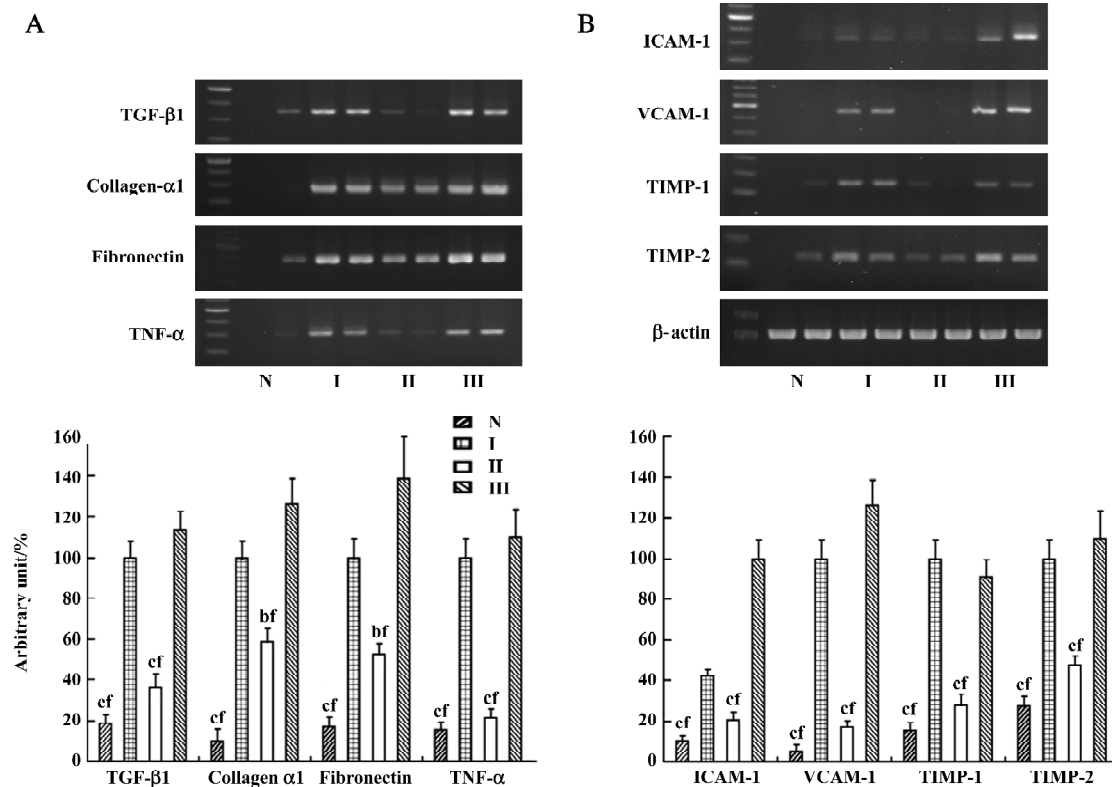
**Figure 3.** Representative photomicrographs of COX-2-stained mouse liver sections. COX-2 was not detected in the normal control group. COX-2 expression was increased in groups I and III after CCl<sub>4</sub> administration. IL-10 gene therapy (group II) significantly attenuated this increase. (A) Normal control; (B) Group I; (C) Group II; (D) Group III. Arrows indicate COX-2 positive cells. Magnification  $\times 400$  (inset  $\times 200$ ).



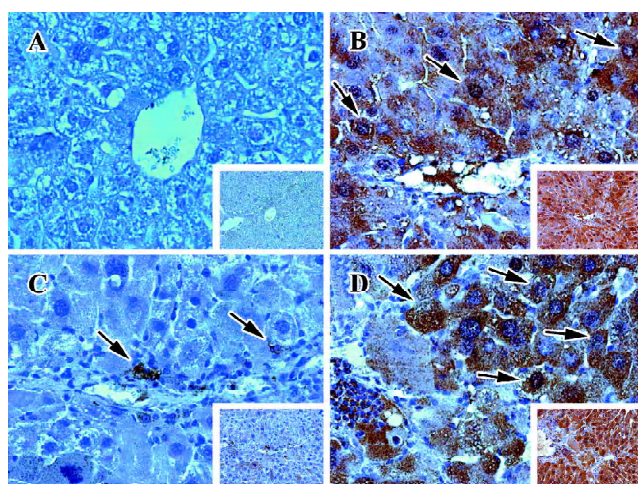
**Figure 4.** Representative immunoblots showing  $\alpha$ -SMA expression in CCl<sub>4</sub>-treated mouse liver. When compared with normal controls,  $\alpha$ -SMA activation was seen in groups I and III after CCl<sub>4</sub> administration. IL-10 gene therapy (group II) significantly abrogated this activation. Mean $\pm$ SEM. <sup>c</sup> $P < 0.01$  vs group I, <sup>f</sup> $P < 0.01$  vs group III. The arbitrary units are defined as  $\alpha$ -SMA/ $\alpha$ -tubulin band density.

**IL-10 gene therapy attenuated MMP-2 activation in the fibrotic liver** The expression of MMP after CCl<sub>4</sub> treatment was evaluated by using immunohistochemical and gelatin zymography methods. Immunohistochemical studies showed that when compared with normal livers, MMP-2 levels were significantly increased in the fibrotic livers (groups I and III; Figure 6). IL-10 gene therapy attenuated this upregulation





**Figure 5.** Representative photographs of semi-quantified RT-PCR in the CCl<sub>4</sub>-treated mouse liver. IL-10 gene therapy (group II) significantly attenuated TGF-β1, collagen α1, fibronectin, TNF-α, ICAM-1, VCAM-1, TIMP-1, and TIMP-2 mRNA activation after CCl<sub>4</sub> administration when compared with groups I and III (<sup>b</sup>*P*<0.05, <sup>c</sup>*P*<0.01 vs group I. <sup>f</sup>*P*<0.01 vs group III). β-Actin was amplified to verify equal loading. The arbitrary units are defined as target gene/β-actin. Mean±SEM.



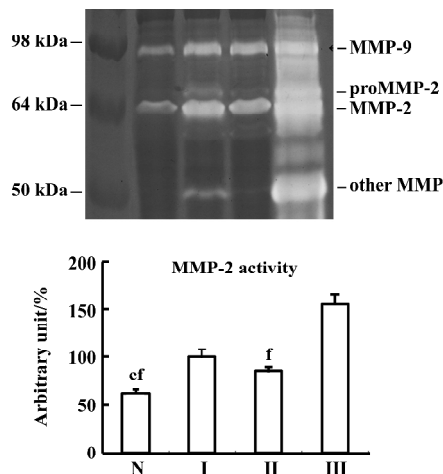
**Figure 6.** Representative photomicrographs of MMP-2-stained mouse liver sections. After CCl<sub>4</sub> administration, MMP-2 staining was significantly accentuated in groups I and III. IL-10 gene therapy (group II) decreased this upregulation. (A) Normal control; (B) Group I; (C) Group II; (D) Group III. Arrows indicate MMP-2-positive cells. Magnification, ×400 (inset, ×200).

(group II; Figure 6C). The collagenolytic activity of MMP protein in liver homogenates was examined by zymography (Figure 7). Gelatin zymography showed that the concentration of the 64 kDa active MMP-2 molecule increased in groups I and III after CCl<sub>4</sub> administration, and IL-10 gene therapy (group II) abrogated this increase (*P*<0.01).

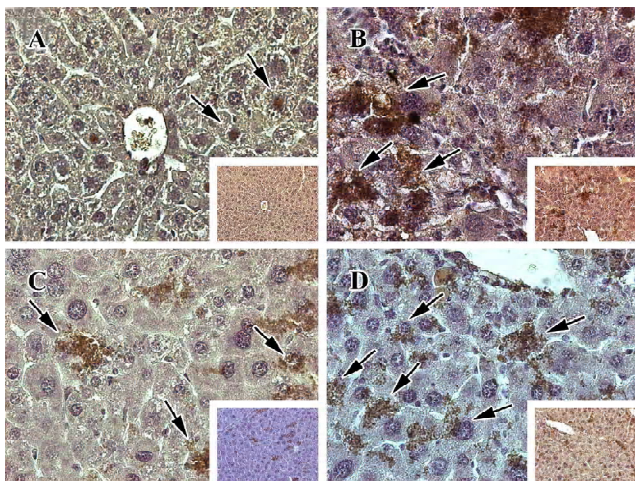
**IL-10 gene therapy attenuated TIMP activation after CCl<sub>4</sub> treatment** Expression of TIMP in the fibrotic livers was also evaluated by RT-PCR and immunohistochemical methods. RT-PCR showed that TIMP-1 and TIMP-2 mRNA were significantly upregulated in the fibrotic liver. IL-10 gene therapy (group II) significantly attenuated these increase (*P*<0.01, Figure 5). Immunohistochemical studies revealed that the level of TIMP-1 was increased after chronic CCl<sub>4</sub> administration (Figure 8). IL-10 gene therapy (group II) significantly attenuated this activation (Figure 8C).

## Discussion

Animal models of hepatic fibrosis provide a means to study the cellular and molecular mediators of fibrosis in a



**Figure 7.** Representative photograph of gelatin zymography in  $\text{CCl}_4$ -treated mouse liver. When compared with the normal control group, levels of the active 64 kDa MMP-2 molecule were increased in groups I and IV after  $\text{CCl}_4$  treatment. IL-10 gene therapy attenuated MMP-2 activation significantly ( $^cP < 0.01$  vs group I.  $^fP < 0.01$  vs group III).



**Figure 8.** Representative photomicrographs of TIMP-1-stained mouse liver sections. After  $\text{CCl}_4$  administration, TIMP-1 staining was significantly accentuated in groups I and III. IL-10 gene therapy (group II) attenuated this upregulation. (A) Normal control; (B) Group I; (C) Group II; (D) Group III. Arrows indicate TIMP-1-positive cells. Magnification,  $\times 400$  (inset,  $\times 200$ ).

serial manner during both progression and recovery. Several approaches to the induction of fibrosis have been described. Of these,  $\text{CCl}_4$  intoxication in rats and mice is probably the most widely studied<sup>[29]</sup>. In addition, the  $\text{CCl}_4$  model is the best characterized with respect to histological, biochemical, cellular, and molecular changes associated with

the development of fibrosis<sup>[30,31]</sup>.  $\text{CCl}_4$  can be given intraperitoneally or by oral gavage; it induces hepatocyte necrosis and apoptosis with associated HSC activation and tissue fibrosis. With ongoing treatment  $\text{CCl}_4$  can be used to induce bridging hepatic fibrosis (4 weeks of twice-weekly treatment), cirrhosis (8 weeks of twice-weekly treatment) and advanced micronodular cirrhosis (12 weeks of twice-weekly treatment)<sup>[31]</sup>.

IL-10 is a potent anti-inflammatory cytokine that inhibits the synthesis of pro-inflammatory cytokines<sup>[32]</sup>. IL-10 has been shown to downregulate the synthesis of collagen type I and TIMP in previous investigations<sup>[33,34]</sup>. It also plays an anti-fibrogenic role by decreasing the levels of pro-fibrogenic cytokines, including TGF- $\beta$ 1 and TNF- $\alpha$ <sup>[33]</sup>. In the present study, we demonstrated that electroporative IL-10 gene therapy provided an effective expression method for long-term use. This treatment reversed established liver fibrosis and reduced collagen synthesis in mice. IL-10 gene therapy also inhibited HSC activation after  $\text{CCl}_4$  administration. The fibrogenic gene (TGF- $\beta$ 1 and TNF- $\alpha$ ) response attenuation may be responsible for the hepatoprotective effect of IL-10.

COX-2 is a key executor of uncontrolled inflammation<sup>[35]</sup>. Overexpression of COX-2 has been demonstrated in  $\text{CCl}_4$ -induced liver fibrosis and post-viral human cirrhosis<sup>[36,37]</sup>. Further, COX-2 can contribute to hepatic carcinogenesis by increasing necroinflammatory activity, promoting proliferation, and enhancing angiogenesis<sup>[38,39]</sup>. Selective COX-2 blockers are known to reduce  $\text{CCl}_4$ -induced liver fibrosis<sup>[36]</sup>. Hence, COX-2 may be a new therapeutic target for treatments for liver cirrhosis. IL-10 is known as the central regulator of COX-2<sup>[40]</sup>. Therefore, IL-10 gene therapy might have exerted its anti-hepatic fibrogenesis effect through COX-2 inactivation.

Cell adhesion molecules are known as prognostic markers of liver fibrosis<sup>[41]</sup>. Expression of ICAM-1 and VCAM-1 modulated by TNF- $\alpha$  are upregulated in alcoholic hepatitis,  $\text{CCl}_4$ -induced liver injury, and nutritional fibrosis<sup>[42-44]</sup>. A previous study showed that ICAM-1 and VCAM were upregulated in IL-10 knockout-colitis in mice<sup>[45]</sup>. In addition, IL-10 can attenuate ICAM-1 activation in cisplatin nephrotoxicity<sup>[46]</sup>. Therefore, cell adhesion molecule regulation may be involved in the anti-fibrotic effect of IL-10.

The imbalance between MMP and TIMP in the ECM contributes to the pathogenesis of liver fibrosis. Matrix metalloproteinases are a family of zinc-dependent proteases capable of degrading hepatic ECM, thereby playing a central role in tissue remodeling and repair after injury<sup>[47]</sup>; however, persistent overexpression of MMP may contribute to the pathogenesis of liver diseases. Inhibition of MMP-2 produced by activated stellate cells blocks lethal hepatitis

and apoptosis induced by TNF- $\alpha$ <sup>[48]</sup>. Furthermore, MMP-2-deficient mice have decreased hepatocyte apoptosis and necrosis, and enhanced survival in this model. Recent studies have also revealed a strong correlation between MMP-2 activity and severity of human liver disease<sup>[49]</sup>. MMP activity is regulated by the TIMP, which binds in a substrate- and tissue-specific manner to MMP, blocking their proteolytic activity<sup>[50]</sup>. Antibodies and antisense oligonucleotides directed at TIMP-1 attenuate rat liver fibrosis<sup>[50,51]</sup>. IL-10 is known to suppress MMP-2 and TIMP-1 expression in HSC during liver fibrosis<sup>[52]</sup>. In the present study, we demonstrated that IL-10 gene therapy attenuated MMP-2 and TIMP activation in the fibrotic liver. Therefore, its collagenolytic effect might be attributed to MMP and TIMP modulation.

In the present study, we demonstrated the anti-hepatic fibrogenic effect of IL-10 in mice. IL-10 gene therapy reversed established CCl<sub>4</sub>-induced liver fibrosis in mice through fibrogenic gene response attenuation. In conclusion, IL-10 gene therapy may be a new therapeutic modality for liver cirrhosis with potential clinical use.

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