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Effects of Circular Type TGF-β1 Antisense Oligonucleotides on Anti-Thy-1 Glomerulonephritis

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Objectives

Overproduction of transforming growth factor (TGF)- β1 has been implicated in the pathogenesis of fibrotic diseases. TGF- β1 plays a crucial role in the accumulation of extracellular matrix (ECM) in human and experimental glomerular diseases. However, it remains unclear whether inhibition of TGF- β1 overproduction would suppress TGF- β1 induced ECM accumulation. To inhibit the overproduction of TGF- β1 in experimental glomerulonephritis induced by anti-Thy 1.1 antibody, we introduced antisense oligodeoxynucleotides (ODN) for TGF- β1 into the nephritic kidney by the HVJ-liposome-mediated gene transfer method.

Materials & Method

1. Materials : Antibody - Rat Thymocyte IgG (Accurate chemical & scientific corp. USA)
2. Experimental Design
   1) ATS injection
      - Male Sprague-Dawley rats
      - injected intravenous with 0.1 ml saline contaning ATS
        a single dose; 0.1 mg/100 g body weight
   2) Transfection of Circular type TGF-β1 antisense ODN
      - vector : Hemagglutinating Virus of Japan (HVJ)-liposome- TGF-β1 antisense ODN (10 ug/ rat)
      - liposome : egg yolk phosphatidylcholin : dioleoly phosphatidylethanolamine : egg yolk sphingomyelin : bovine brain phosphatidylserine : Concentraion ; 30 mmol/L
3. Assay : H&E, Manson's trichrom, Imunohistochemistry, RT-PCR, Northern blot

Results and Discussion

In the circular type antisense TGF- β1 ODN transfected rats, a marked decrease in expression of TGF- β1 mRNA was confirmed by Northern analysis. Consequently, the expression of TGF- β1 protein in the glomerulus was markedly reduced in the circular type antisense ODN transfected kidney with a comparable effect in preventing glomerular ECM expansion in experimental glomerulonephritis. In contrast, sense ODNs failed to suppress TGF- β1 expresion an ECM accumulation. Thus, these results

suggested that inhibion of TGF- β1 overproduction could suppress progression to glomerulosclerosis.

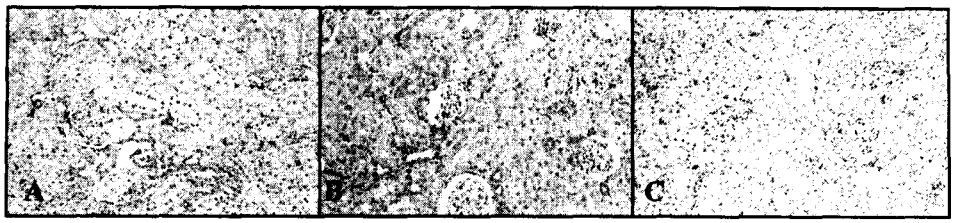

Fig. 1. Immunohistochemistry for TGF-β1 of a control (A), sense TGF-β1 ODN treated (B), and antisense TGF-β1 ODN treated kidney (C). Killed on the fifth day of the experiment.

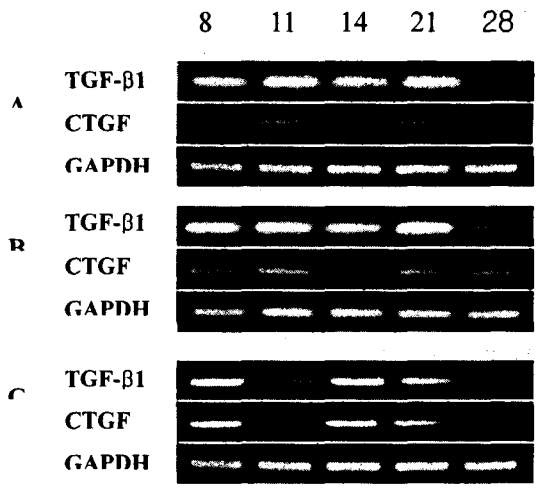

Fig. 2. TGF-β1 and CTGF mRNA expression level a control (A), sense TGF-β1 ODN treated (B), and antisense TGF-β1 ODN treated kidney (C). Killed on 8, 11, 14, 21, and 28 days of the experiment.

References

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