Cytokines and glomerulosclerosis

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Glomerulonephritis (GN), diabetic nephropathy, and hypertension are the major causes of chronic renal failure that finally require renal replacement therapy. Different aetiopathological factors lead to renal injury, however, these diseases share the common histological pathway of a pathological accumulation of extracellular matrix in the glomeruli. These features are clinically referred to as glomerulosclerosis.

TGF-β plays an important role in regulating tissue repair and remodelling following injury [1,2]. One of the most important biological actions of TGF-β is the regulation of extracellular matrix accumulation. TGF-β stimulates the synthesis of individual matrix components including proteoglycans, collagens, and glycoproteins [3]. TGF-β also inhibits matrix degradation by decreasing the synthesis of proteases and increasing the synthesis of protease inhibitors [4]. Finally, TGF-β modulates the expression of integrin receptors and alters their relative proportions on the cell surface in a manner that could facilitate adhesion to the matrix [5]. All of these actions lead to increased deposition and accumulation of extracellular matrix surrounding the cells in a tissue. All of these events have largely been demonstrated in vitro in cultured cells. In an experimental model of GN, TGF-β has also been shown to be responsible for the accumulation of the pathological matrix in the glomeruli following immunological injury. That is, all three actions of TGF-β actions on the extracellular matrix; (1) increased synthesis; (2) decreased degradation; and (3) modulation of integrin receptors, have now been demonstrated to be involved in matrix deposition in GN.

It was not known, however, whether or not the overproduction of a single growth factor in the glomerulus resulted in glomerulosclerosis. Therefore, the haemagglutinating virus of Japan (HVJ) liposome method was applied to create a new animal model expressing TGF-β. The expression vectors carrying cDNA for TGF-β were generated and introduced into the kidney by the HVJ-liposome method. Consequently, the selective overexpression of TGF-β was observed in the glomeruli. The expression efficiency into the glomeruli was approximately 35%. Over-expression of TGF-β caused extracellular matrix accumulation with a mild increase in glomerular cell numbers 5 to 7 days after transfection [6]. Over-expression of TGF-β also altered the phenotype of mesangial cells since type I and type III collagens were expressed in the mesangial area.

Pathophysiological TGF-β actions have now been demonstrated. The discovery that blocking TGF-β with either neutralizing antibodies [7] or the proteoglycan decorin [8] stopped extracellular matrix accumulation offers the potential of developing agents that modulate or antagonize TGF-β as anti-fibrotic drugs.

We have also reported that inhibition of TGF-β gene expression by antisense oligonucleotides could suppress the development of experimental GN [9]. Furthermore, continuous delivery of decorin, which was accomplished by in vivo gene transfer into the skeletal muscle, also inhibited the extracellular matrix expansion in experimental GN [10]. This evidence strongly suggests the hypothesis that the inhibition of over-expressed TGF-β can ameliorate the progression of renal diseases.

Recently it has been shown that TGF-β signals are transduced by contacting two transmembrane serine/threonine kinases known as type I and type II receptors simultaneously [11]. The type II receptor recognizes the active TGF-β ligand, whereas the type I receptor does not. Thus, TGF-β binds directly to the type II receptor, which is a constitutively active kinase. The TGF-β binding type II receptor is then recognized by the type I receptor which is recruited into the complex and becomes phosphorylated by the type II receptor. Both receptors are required for TGF-β action in mammalian cells [12] because mutations in either receptor type disrupts signalling in each case. Based on the differences between their ligand-binding properties, the type II receptor acts upstream of the type I receptor, and so these components may be thought of as primary receptor and transducer, respectively [12]. These results motivated us to produce an inactive type II receptor construct to impede the activities of TGF-β. However, the soluble type II receptor was reported to have about a 10-fold less binding affinity for TGF-β than does the cell-surface type II receptor [13]. This may partly depend on the fact that the soluble type II receptor is
feasibility of chimeric gene transfer

We therefore constructed an expression vector for a chimera of an extracellular domain of the TGF-β type II receptor fused to the IgG-Fc domain called TGF-βRII/Fc, which is expected to form a dimeric receptor and efficiently neutralize the activities of TGF-β in vitro and in vivo.

Based on the primary structure of the extracellular domain connected with the IgG-Fc molecule, our model predicts that these molecules will behave as homodimers by virtue of the IgG hinge region. Analysis of the purified molecules by SDS–PAGE under both reducing and non-reducing conditions demonstrated the purity of the preparations and confirms the predicted homodimer structure of the native chimeric molecules.

To assess the effects of the chimeric molecule on TGF-β’s growth inhibition activity, BNul-7 cells were treated with TGF-β1 and cell growth was measured by 13-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay in the presence of either TGF-βRII/Fc (1.25 μg/ml) or anti-TGF-β antibodies (1.25 μg/ml). At concentrations from 0.01 ng/ml to 1.25 ng/ml of TGF-β, the growth of BNul-7 cells was inhibited by TGF-β1 in a dose-dependent manner. Both anti-TGF-β antibodies and chimeric TGF-βRII/Fc completely blocked the growth inhibitory action of TGF-β1. To study the effects of the chimeric molecule on the alternative splicing of fibronectin pre-mRNA induced by TGF-β1, we designed specific probes complementary to the sequence of pre-mRNA encompassing the EIIIA exon for RT-PCR.

Exposed to TGF-β1, EIIIA+ fibronectin mRNA increased over control NRK cells, while EIIIA- fibronectin mRNA increased slightly. There was a clear reduction in EIIIA+ after treatment with the purified chimeric molecule, the reduction of EIIIA− was much less dramatic. Thus, the chimeric TGF-βRII/Fc molecule also inhibits TGF-β1-induced extracellular matrix synthesis.

Given the competitive properties of chimeric TGF-βRII/Fc against TGF-β1 in vitro, we assessed the feasibility of chimeric gene transfer in vivo. HVJ-liposome solution containing 5 or 20 μg of pCAGGS–TGF-βRII/Fc was introduced into the gluteal muscles of nephritic rats 1 day after injection of OX-7 antibody. HVJ-liposome solution without DNA was also examined in nephritic rats as untreated disease controls. On day 7, we obtained glomerular RNA to examine whether the chimeric TGF-βRII/Fc gene transfection could reduce the message of TGF-β1 in the glomeruli. Glomeruli were isolated by the graded sieving technique, and Northern blot analysis was performed to compare TGF-β1 mRNA. The TGF-β1 mRNA was elevated by more than 10-fold in untreated disease controls 7 days after OX-7 injection. Laser densitometric analysis revealed that TGF-β1 mRNA expression in glomeruli from nephritic rats transfected with 5 and 20 μg of the chimeric genes, and was reduced to 48 and 39% of that in untreated disease controls, respectively (Figure 1). The level of GAPDH mRNA remained unaffected in all transfected kidneys.

We then assessed the inhibitory action of chimeric gene transfection on the histological changes in the kidney on day 7. In the kidneys from the untreated nephritic rats, the glomeruli exhibited marked increases in mesangial extracellular matrix. In contrast, chimeric gene transfection markedly suppressed the increase in mesangial matrix expansion. The mesangial matrix indices of glomeruli from the kidneys untransfected, 5-μg-transfected, and 20-μg-transfected were 3.1 ± 0.4, 2.1 ± 0.5, and 1.9 ± 0.6, respectively. These results demonstrated that chimeric TGF-βRII/Fc gene transfer 1 day after disease induction could suppress extracellular matrix expansion.

Finally, we constructed a chimeric molecule specifically designed to inhibit TGF-β1-mediated glomerular matrix accumulation. The TGF-βRII/Fc binds TGF-β1, thereby blocking the biological activities of TGF-β1 in vitro. Moreover, the transfection of the TGF-βRII/Fc gene into the muscles of nephritic rats prevented glomerular matrix expansion in vivo. These results suggest that TGF-βRII/Fc may prove to be clinically useful, given that blocking of TGF-β over-expression in the glomeruli provides a potentially powerful therapeutic approach to the treatment of TGF-β1-mediated glomerular diseases.

References


