Expression of PDGF and PDGF Receptor mRNA in Glomeruli in IgA Nephropathy

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Abstract. This study investigated the mRNA expression of the platelet-derived growth factor (PDGF) A-chain and B-chain and PDGF-β receptor in glomeruli of 15 immunoglobulin A (IgA) nephropathy kidneys and those with minimal-change lesion (N = 7), membranous nephropathy (N = 3), and focal segmental glomerulonephritis (N = 5), by using competitive RT-PCR methods. The level of PDGF B-chain and β receptor mRNA expression in IgA nephropathy was significantly higher than in the other forms of glomerulonephritis, but mRNA expressions of PDGF A-chain were not significantly different. Significant correlations were observed between the urinary protein level and the mRNA level of PDGF-β receptor expression and PDGF B-chain expression, and between the serum creatinine level and the mRNA level of PDGF-β receptor expression. The PDGF B-chain and β receptor may be upregulated and accelerate cell proliferation in a paracrine or autocrine manner and may play a role in the pathogenesis of IgA nephropathy. (J Am Soc Nephrol 8: 817-819, 1997)

Platelet-derived growth factor (PDGF) has been found to be a potent mitogen for mesangial cells in culture and is expressed in both experimental and human forms of glomerulonephritis in which mesangial cell proliferation occurs (1-7). Recently, Gesualdo et al. and Niemir et al. reported that PDGF-β receptor and PDGF B-chain expression are increased in immunoglobulin A (IgA) nephropathy and other types of human glomerulonephritis (8,9). However, these reports did not include quantitative analyses of PDGF A-chain and B-chain expression. Quantitative analysis of mRNA expression of PDGF A-chain, PDGF B-chain, and PDGF-β receptor in human glomerulonephritis is important in considering the pathogenesis of IgA nephropathy. In this study, we report that the expression of PDGF B-chain and β-receptor mRNA is increased in IgA nephropathy when competitive RT-PCR methods are used, and that the expression of PDGF-B chain and β-receptor is a potential indicator of disease activity.

Materials and Methods

Portions of kidney biopsy tissue were obtained with informed consent from 30 patients. Histological diagnosis of these biopsies were IgA nephropathy (N = 15), minimal-change lesion (N = 7), membranous nephropathy (N = 3), and focal segmental glomerulonephritis (N = 5). Five glomeruli were microdissected and transferred into the RT-PCR tube. In each reaction tube, point-mutated standard cDNA was added and coamplified by 30 to 35 PCR cycles by using a programmed thermocycler (Astec, Tokyo, Japan). In this study, competitive PCR was performed to quantitate mRNA for PDGF A-chain, PDGF B-chain, and PDGF-β receptor using point-mutated internal standards (10). The primers and probe for PDGF A-chain were GCCCTAGGGAGTCAGGTATAA, TTTACCTGACTCCCTAGGCC, and AAGCATCGAGGAACGTGTCCT. The primers and probe for PDGF B-chain are CACGCATGACAAAGCGCC, ACAGGAAGACCGCCAC, and ATCTCCGCGCGCTCTAGA. The primers and probe for PDGF-β receptor were GTTCTTCTACCTGCGCTGA, ATGGGCA- CATAGTCCACCGA, and AAGATCTGTTGACTTTGCGCT. The primers and probe for GAPDH are AGATCCACAGCGATACCT, TCCTCAAAGATTTCACG, and ACCGCTCTTCAGCCCTGAT. The PCR products derived from the point-mutated standard cDNA could be readily distinguished after the digestion by restriction enzymes. The gels were Southern-blotted to nitrocellulose filters and hybridized with 32P-labeled specific probe (10). The relative amounts of PCR products were determined by densitometer scanning of autoradiographs of Southern hybridization. To test the relationship between the quantities of starting amounts of mRNA and those of amplification product (as reflected by densitometry values), we compared amplification products from 0.01 to 1.0 μg of total RNA. There was good correlation between amounts of RNA and densitometric values (N = 12, for each set of primers; r = 0.978 for PDGF A-chain, r = 0.985 for PDGF B-chain, r = 0.987 for PDGF-β receptor, r = 0.989 for GAPDH). The mRNA amounts were corrected by the mRNA amount of the GAPDH in each sample.

Statistical Analysis

The differences were tested using the non-paired t test. The values were expressed as mean ± SD. P < 0.05 was considered significant.

Results and Discussion

The histological findings revealed that IgA deposits were present in every case; five cases showed slight proliferative changes in glomeruli (group A), six cases showed mild to
moderate proliferative changes (group B), and four cases revealed severe proliferative changes and partial crescent formation in glomeruli (group C). Clinical laboratory findings were as follows: urinary protein excretion rate was 0.9 to 2.3 g/day, serum creatinine level was 0.7 to 1.6 mg/dl, blood urea nitrogen level was 17.5 to 30.2 mg/dl, and creatinine clearance rate was 64 to 98 ml/min. As shown in Figure 1A, the serum creatinine level and blood urea nitrogen level were significantly high and the creatinine clearance was significantly low in group C, in comparison with the other-glomerulonephritis group.

RT and competitive PCR analysis demonstrated that the level of PDGF B-chain mRNA expression in IgA nephropathy was significantly higher in IgA nephropathy than in the other-glomerulonephritis group (5.9 ± 1.9 attomol/five glomeruli versus 4.1 ± 1.0 attomol/five glomeruli, mean ± SD, P < 0.05). A marked increase of PDGF B-chain mRNA expression was observed in samples from group C. On the other hand, PDGF A-chain mRNA expression was not significantly increased in IgA nephropathy (2.5 ± 1.0 attomol/five glomeruli versus 2.0 ± 0.6 attomol/five glomeruli, mean ± SD) (Figure B). Statistical analysis showed that the mean level of PDGF-β receptor expression in IgA nephropathy was significantly higher than in the other-glomerulonephritis group (6.2 ± 2.4 attomol/five glomeruli versus 3.5 ± 1.5 attomol/five glomeruli, mean ± SD, P < 0.05). A marked increase of PDGF B-chain mRNA expression was observed in samples from group C. Significant correlations were observed between the urinary protein level and the mRNA level of PDGF-β receptor expression (r = 0.80) and PDGF-B chain expression (r = 0.76), and between the serum creatinine level and the mRNA level of PDGF-β receptor expression (r = 0.72). Every case of IgA nephropathy in group C showed increased expression of PDGF-B chain and PDGF-β receptor.

It has previously been reported that mesangial cells in vitro express PDGF A-chain, PDGF B-chain, and PDGF-β receptor.

**Figure 1.** Renal-function data of patients (A) and quantitation of mRNA expression of platelet-derived growth factor (PDGF) A-chain (B), B-chain (C), and β-receptor (D) of glomeruli in immunoglobulin A (IgA) nephropathy and other types of glomerulonephritis. (A) Comparisons of renal-function parameters between the other-glomerulonephritis group and groups A, B, and C (mean ± S.D., *P < 0.05 versus other-glomerulonephritis group). (B, C, D) Open circles represent glomeruli from the other-glomerulonephritis group. Open triangles represent group A of IgA nephropathy. Closed circles represent group B of IgA nephropathy. Closed triangles represent group C of IgA nephropathy. The mRNA amounts were corrected by the mRNA amount of the GAPDH in each sample.
mRNA (1,3). PDGF is believed to modulate proliferation of mesangial cells in an autocrine fashion. Johnson et al., Floege et al., and Iida et al. reported that gene expression of PDGF A-chain, PDGF B-chain, and PDGF-β receptor was increased in anti-Thy-1 glomerulonephritis (3,4,7). The evidence indicating a pathological role of PDGF in the stimulation of mesangial proliferation in IgA nephropathy has accumulated (2,6,8,9). Our observation of abundant PDGF-B-chain and β-receptor mRNA in renal biopsies of patients with more severe histological lesions indicates that the expression of PDGF-B-chain and β-receptor is an indicator of disease activity. PDGF B-chain and β-receptor may be upregulated and accelerate cell proliferation in a paracrine or autocrine manner in IgA nephropathy. This evidence suggested that PDGF plays a role in the pathogenesis of IgA nephropathy and that the expression of PDGF-B chain and β-receptor represents a potential indicator of disease activity.

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