Clin Exp Immunol 1994; 97:309–314

Intraglomerular expression of transforming growth factor-beta 1 (TGF-β1) mRNA in patients with glomerulonephritis: quantitative analysis by competitive polymerase chain reaction

M. IWANO, Y. AKAI*, Y. FUJII, Y. DOHI*, N. MATSUMURA & K. DOHI *First Department of Internal Medicine and *Department of Public Health, Nara Medical University, Nara, Japan*

(Accepted for publication 11 April 1994)

SUMMARY

TGF-β1 is involved in the pathogenesis of glomerular sclerosis. We studied the intraglomerular expression of TGF-β1 mRNA in patients with glomerulonephritis using competitive polymerase chain reaction (PCR). This method is sensitive enough to quantify cDNA copies of mRNA present in small amounts of samples. Renal biopsy specimens were obtained from 42 patients with various kinds of glomerulonephritis. Ten glomeruli were dissected from renal biopsy specimens. Normal glomeruli were also obtained from the resected kidneys of eight patients with renal cell cancer. Total RNA was extracted from the glomeruli and reverse transcribed into cDNA with reverse transcriptase. To prepare samples containing identical amounts of β-actin cDNA (8 pg), we performed competitive PCR by co-amplifying mutant templates of β-actin with a unique EcoRI site. Next, to measure TGF-β1 cDNA, we performed competitive PCR by co-amplifying mutant templates of TGF-β1. We observed a higher glomerular expression of TGF-β1 mRNA in cases of mesangial proliferative glomerulonephritis having a moderate increase in mesangial matrix, diabetic nephropathy and diffuse proliferative lupus nephritis, compared with normal glomeruli. Results suggest that the intraglomerular synthesis of TGF-β1 may be involved in the progression of glomerulonephritis in humans.

Keywords cytokine diabetes mellitus glomerulonephritis polymerase chain reaction systemic lupus erythematosus

INTRODUCTION

TGF-β1, a multifunctional cytokine, functions in tissue repair by stimulating the synthesis of, and inhibiting the degradation of extracellular matrix. However, overproduction of TGF-β1 can lead to fibrosis via the pathological accumulation of extracellular matrix [1–4].

Accumulation of extracellular matrix in glomeruli is a prominent feature of progressive glomerular sclerosis observed in glomerulonephritis, which advances to end-stage renal failure [5,6]. TGF-β1 stimulates the production of extracellular matrix proteins by glomerular epithelial cells and mesangial cells [7–9]. In experimental models of acute antithymocyte serum-induced glomerulonephritis and antiglomerular basement membrane antibody-mediated crescentic glomerulonephritis, enhanced expression of TGF-β1 in renal cortex was observed [10,11]. Border *et al.* have demonstrated that the administration of anti-TGF-β1 antibody or decorin, which suppresses TGF-β1 activity, to antithymocyte serum-induced glomerulonephritis prevents the production of mesangial matrix *in vivo* [12,13]. A recent report by Isaka *et al.* showed that selective overexpression of the TGF-β1 gene in rat glomeruli induces glomerular sclerosis, using an *in vivo* transfection technique [14]. By immunohistochemical staining, Yoshioka *et al.* showed mature TGF-β1 and the latency-associated peptide of natural TGF-β1 were localized in association with the matrix components in human glomeruli. By *in situ* hybridization, they observed the intraglomerular expression of TGF-β1 mRNA in the mesangial area [15]. Yamamoto *et al.* noted an increased amount of TGF-β1 protein in the glomeruli of patients with diabetic nephropathy [16]. These reports indicate that the intraglomerular overproduction of TGF-β1 is involved in the mesangial matrix increase observed in glomerulonephritis.

No studies have yet quantified the expression of the intraglomerular mRNA encoding for TGF-β1 in human glomerulonephritis, since few renal biopsy specimens are available. Conventional methods such as Northern blot and nuclease protection assay are not sensitive enough to detect minute amounts of mRNA species. *In situ* hybridization allows detec-

Correspondence: Masayuki Iwano, First Department of Internal Medicine, Nara Medical University, 840 Shijo-cho, Kashihara, Nara 634, Japan.

tion of mRNA in a single cell, but is insensitive and non-quantitative. In this study, we performed quantitative analysis of intraglomerular mRNA expression of TGF-β1 in human glomerulonephritis, using the competitive polymerase chain reaction (PCR) technique.

PATIENTS AND METHODS

Materials

Renal biopsy specimens were obtained from a total of 42 patients with various kinds of glumerulonephritis. There were 19 males and 23 females, aged 18–69 years (mean 46 years). All patients gave their informed consent to this study. Serum creatinine levels in all patients were less than 2·0 mg/dl. Sixteen patients were diagnosed as mesangial proliferative glomerulonephritis, which included 12 IgA nephropathy and four non-IgA nephropathy, seven membranous nephropathy, three minimal change nephrotic syndrome, two focal glomerular sclerosis, and one membranoproliferative glomerulonephritis (type 1), nine non-insulin-dependent diabetes mellitus with diffuse mesangial expansion (diabetic nephropathy) and four lupus nephritis (two patients WHO class II, two patients WHO class IV). Patients with mesangial proliferative glomerulonephritis were divided into two groups: those with a mild increase in mesangial matrix, and those with more than moderate mesangial matrix increase by the modified criteria of Pirani & Salinas-Madrigal [17]. In four patients with mesangial proliferative glomerulonephritis having a moderate increase in mesangial matrix, we observed focal accentuation of glomeruli such as segmental sclerosis and small crescent formation. As control, we obtained eight specimens, which were the normal part of the resected kidney of either patients with renal cell cancer. These specimens showed minor change in glomeruli by light microscopy (normal glomeruli). As shown in Fig. 1, 10 glomeruli were obtained by dissecting 10–20% of each of the renal biopsy specimens under phase-contrast microscopy [18].

Total RNA extraction and reverse transcription

We extracted total RNA by the method using Nonidet P-40, which is suitable for extracting RNA from small numbers of cells [19]. In order not to lose any glomeruli, we performed all procedures under phase-contrast microscopy. To protect against RNA degradation during experiments, all buffers contained 1 U/μl RNase inhibitor (Wako Pure Chemical Industries, Ltd., Osaka, Japan). The ten glomeruli were washed with PBS pH 7·4 containing 0·1% diethyl pyrocarbonate by pipetting several times at 4°C, and then treated with collagenase type IV (Worthington Biochemical Co., Freehold, NJ) at 37°C for 15 min. After another washing, they were lysed with an ice-cold solution of 0·5% Nonidet P-40, 10 mM Tris buffer pH 8·0, 10 mM NaCl and 3 mM $MgCl_2$. After pipetting on ice for 5 min to disperse the glomeruli throughout the lysis solution, the solution was centrifuged at 12 000 ***g*** for 2 min at 4°C and the supernatant reverse transcribed into cDNA with 1 U/μl of RAV-2 reverse transcriptase (Amersham, Aylesbury, UK). The reaction mixture also contained 100 pM random hexamer, 1 mM of dATP, dCTP, dTTP and dGTP, and 1 U/μl RNase inhibitor (Amersham). The reagents were incubated at 42°C for 1 h, then heated to 95°C for 5 min to denature the RNA–cDNA hybrid and to inactivate the reverse transcriptase.

Primers

The primers were synthesized on a PCR-Mate DNA synthesizer (Applied Biosystems, Inc., Foster, CA). Human β-actin cDNA was amplified with the following sequences: TGACGGGGTCACCCACACTGTGCCCATCTA (bases 509–538) as the sense primer and ACTCGTCATACTCCTGCTTGCTGATCCA (bases 1107–1134) as the anti-sense primer [20]. Human TGF-β1 cDNA was amplified with the following sequences: AGCGACTCGCCAGAGTGGTTATCTT (bases 1411–1435) as the sense primer and TTATGCTGGTTGTACAGGGCCAGGA (bases 1860–1884) as the anti-sense primer [21].

Generation of competitive mutant templates

To circumvent the problem of variability in amplification efficiency, competitive templates were generated by site-specific mutagenesis using two mutant primers, as described by Higuchi *et al.* [22]. Mutant primers of 30mers contained one base pair change which created a unique EcoRI site in the competitive templates of the human β-actin gene and the human TGF-β1 gene. Mutant fragments were subcloned into pUC 18 and verified by sequencing. As shown in Fig. 2, the mutant β-actin cDNA template and the mutant TGF-β1 template were prepared by one point mutation, creating a unique EcoRI site.

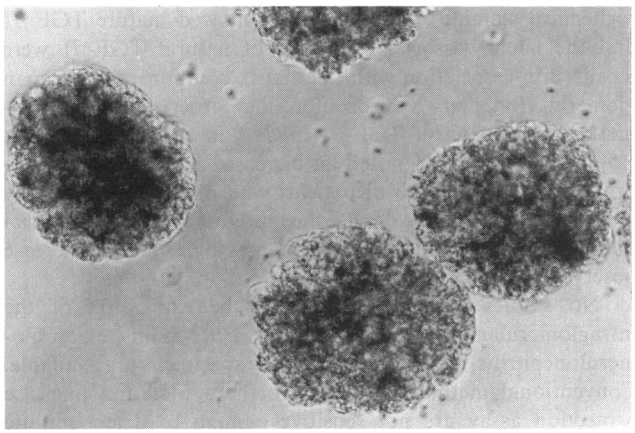

Fig. 1. Glomeruli isolated from one renal biopsy specimen.

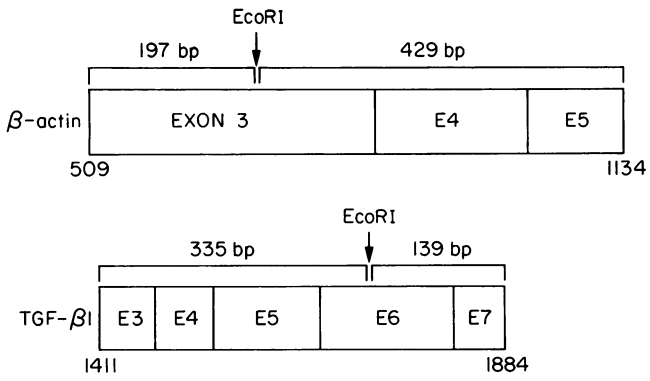

Fig. 2. Scheme of the competitive mutant templates. Mutant β-actin cDNA template and mutant TGF-β1 template were prepared by one point mutation, creating a unique EcoRI site.

Competitive PCR

Competitive PCR was performed as described by Gilliland *et al.* [19]. A master mix containing primers, PCR buffer, Taq polymerase (Perkin Elmer/Cetus, Norwalk, CT) and an appropriate amount of cDNA, was prepared. To 90 μl of this mixture were added 10 μl of the competitive template diluted to a series of known concentrations. The mixture was overlaid with mineral oil and then amplified with the Perkin Elmer/Cetus thermal cycler. The amplification profile consisted of denaturation at 95°C for 1 min, primer annealing at 65°C for 30 s and extension at 72 °C for 1 min. After a 40 cycle amplification, an aliquot of each sample was digested with an appropriate restriction enzyme (EcoRI) and run on a 2% NuSieve/agarose gel (FMC Bioproducts, Rockland, ME). To determine the amount of glomerular β-actin cDNA, the staining intensity of ethidium bromide was analysed by densitometry.

Southern blot hybridization

Southern blot analysis was used to determine the amount of glomerular TGF-β1 cDNA. The gels were electrophoresed, denatured and the DNA transferred to a Hybond-N+ filter (Amersham). After drying at 80°C for 2 h, the filters were prehybridized in 6× SSC, 10× Denhardt's, 1% SDS and 50 μg/ml salmon sperm DNA at 42°C for 3 h, then hybridized with a 5′ end-labelled oligonucleotide probe (GGTAGTGA-ACCCGTTGATGTCCACTTGCA), which was labelled using γ-^{32}P-ATP. This probe was complementary to the sequence on exon 4 and 5 of TGF-β1 gene. The filters were hybridized at 37°C overnight, then washed with 6× SSC and 0·1% SDS at room temperature three times for 10 min and at 60°C for 30 min. After exposure to Kodak X-Omat film (Eastman Kodak Co., Rochester, NY), the amount of cDNA was analysed by densitometry and a computer-based imaging system (Fuji, Bas 2000, Dusseldorf, Germany).

We performed competitive PCR on 10 samples on five different days and found an interassay variation consistently below 15%.

Statistical analysis

Mean differences were analysed by the Mann–Whitney *U*-test. The null hypothesis was rejected at a level of 0·05. All data were expressed as the median (25th percentile, 75th percentile).

RESULTS

Quantification of intraglomerular β-actin mRNA expression in human glomerulonephritis

The initial competitive PCR allowed us to adjust the samples to equal concentrations of β-actin cDNA. As shown in Fig. 3a, samples that contained large amounts of the mutant competitive template showed a higher intensity of 197/429-bp DNA double bands cleaved by EcoRI. Following the dilution of mutant templates, the staining intensity of these double bands decreased, while the intensity of a 626-bp band, which is derived from authentic β-actin mRNA, increased. A plot of the ratio of authentic β-actin to the mutant templates *versus* the known concentration of the input mutant template was linear (Fig. 3b). At the point at which authentic β-actin cDNA and the mutant template cDNA products are equivalent, the starting concentration of β-actin is regarded as equal to the known starting concentration of the competitive mutant template.

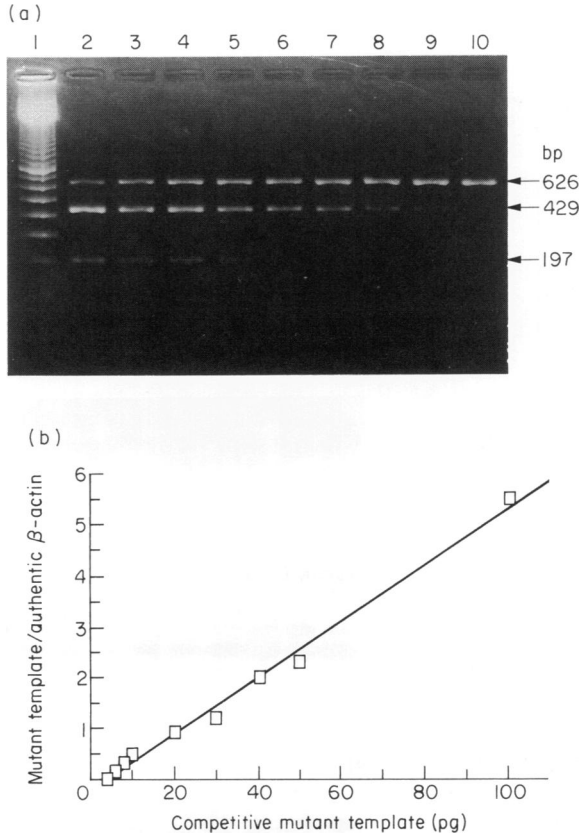

Fig. 3. Quantitative analysis of glomerular β-actin mRNA. (a) Ethidium bromide-stained gel after amplification. Lane 1, base size marker (100 bp ladder); lanes 2–10, the starting concentrations of competitive mutant template in each tube (left to right) were 100, 50, 40, 30, 20, 10, 8, 6, 4 pg, respectively. (b) Densitometric data, plotted as the ratio of authentic β-actin to mutant competitive template *versus* the known input concentration of competitive template, were linear.

We found that the levels of glomerular β-actin cDNA were significantly higher in the patients with diabetic nephropathy (16 (7·3, 19) pg/glomerulus) and in those with mesangial proliferative glomerulonephritis having a moderate increase in mesangial matrix (16 (4·9, 63) pg/glomerulus) than in the normal glomeruli (8·0 (5·0, 11·5) pg/glomerulus).

Intraglomerular expression of TGF-β1 mRNA in various kinds of glomerulonephritis

We used samples containing 8 pg of β-actin cDNA to determine the amount of intraglomerular TGF-β1 mRNA. We again performed competitive PCR by co-amplifying the mutant templates of TGF-β1. Figure 4a shows the result of competitive PCR in normal glomeruli. Raising amounts of the mutant TGF-β1 cDNA from 0·5 fg to 10 fg increasingly inhibited amplification of authentic TGF-β1 cDNA. Figure 4b shows the Southern blots when co-amplifying 4 fg of the mutant template in various kinds of glomerulonephritis. In all normal glomeruli, in all patients with minimal change nephrotic syndrome, in six out of seven patients with membranous nephropathy, and in nine out of 10 patients with mesangial proliferative glomerulonephritis having a mild increase in mesangial matrix, we observed a higher intensity of the band derived from the mutant template compared with that from authentic TGF-β1 (the ratio of authentic TGF-β1/mutant

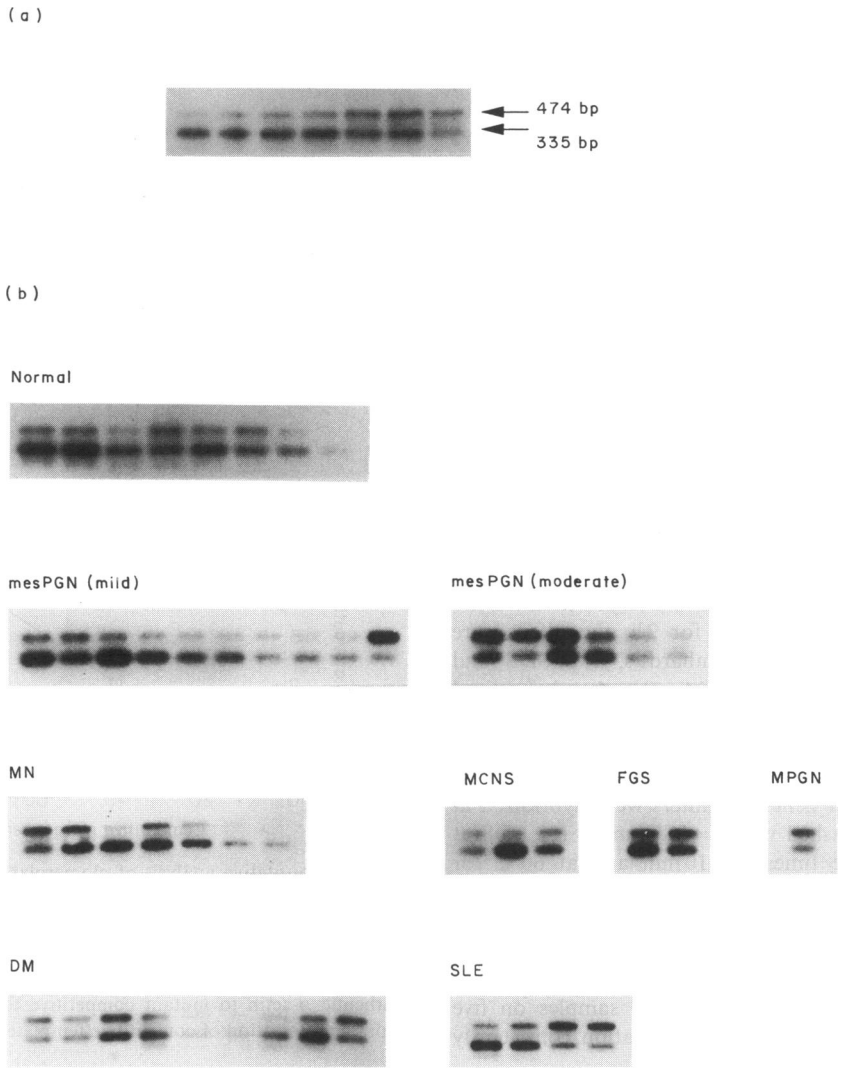

Fig. 4. Quantitative analysis of glomerular TGF-β1 mRNA. Upper bands (474 bp) were amplified from the authentic TGF-β1. Lower bands (335 bp) were amplified from the mutant TGF-β1 template. (a) Southern blot of amplified sequences by competitive polymerase chain reaction (PCR). The starting concentrations of competitive mutant template in each tube (left to right) were 10, 8, 6, 4, 2, 1, 0·5 fg, respectively. (b) Southern blot of amplified sequences when co-amplifying the 4-fg mutant TGF-β1 template in various kinds of glomerulonephritis. Glomeruli were obtained from biopsy specimens of patients with mesangial proliferative glomerulonephritis (mesPGN), membranous nephropathy (MN), minimal change nephrotic syndrome (MCNS), focal glomerular sclerosis (FGS), membranoproliferative glomerulonephritis type 1 (MPGN), diabetic nephropathy (DM) and lupus nephritis (SLE). Patients with mesPGN were divided into two groups: those with a mild increase in mesangial matrix (mild) and those with more than moderate mesangial matrix increase (moderate).

TGF-β1 was less than one). In four out of six patients with mesangial proliferative glomerulonephritis having a moderate increase in mesangial matrix, in three out of nine patients with diabetic nephropathy, in two out of four patients with lupus nephritis, and in one patient with membranoproliferative glomerulonephritis (type 1), the intensity of the band from authentic TGF-β1 exceeded that of the mutant template (the ratio was more than one). Histologic study of these two patients with lupus nephritis showed diffuse proliferative lupus nephritis (WHO class IV).

Results of quantitative analysis of the intraglomerular TGF-β1 mRNA in patients with various kinds of glomerulonephritis appear in Fig. 5. The amount of intraglomerular TGF-β1 mRNA was significantly elevated in patients with mesangial proliferative glomerulonephritis having a moderate increase in mesangial matrix (0·50 (0·38, 0·53) fg/pg β-actin), diabetic nephropathy (0·47 (0·41, 0·60) fg/pg β-actin), and lupus nephritis (0·71 (0·28, 1·31) fg/pg β-actin) compared with normal glomeruli (0·18 (0·13, 0·25) fg/pg β-actin). Levels of TGF-β1 mRNA expression in patients with diffuse proliferative lupus nephritis (1·50 and 1·13 fg/pg β-actin) were more than five times higher than those in normal glomeruli.

DISCUSSION

Several cytokines and growth factors have been implicated in

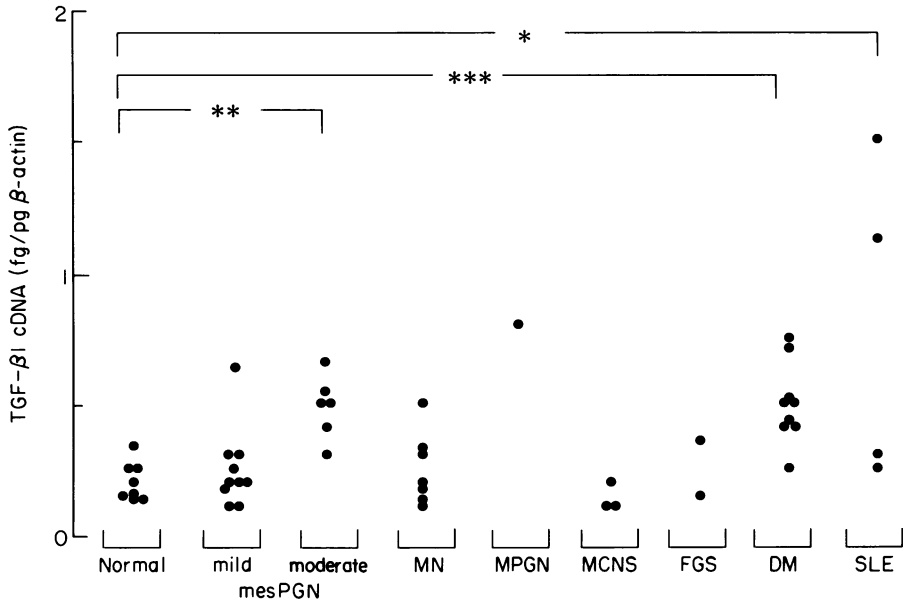

Fig. 5. Quantitative analysis of glomerular TGF-β1 in patients with various kinds of glomerulonephritis. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.005$ *versus* normal glomeruli. Glomeruli were obtained from biopsy specimens of patients with mesangial proliferative glomerulonephritis (mesPGN), membranous nephropathy (MN), minimal change nephrotic syndrome (MCNS), focal glomerular sclerosis (FGS), membranoproliferative glomerulonephritis type 1 (MPGN), diabetic nephropathy (DM) and lupus nephritis (SLE). Patients with mesPGN were divided into two groups: those with a mild increase in mesangial matrix (mild) and those with more than moderate mesangial matrix increase (moderate).

the progression of glomerulonephritis [23–27]. To our knowledge, this study is the first report to demonstrate directly the glomerular overexpression of a cytokine mRNA in human glomerulonephritis. Quantifying the expression of mRNA can provide important information about protein levels, and thus biological activity. We used competitive PCR to measure the expression of intraglomerular TGF-β1 mRNA in patients with glomerulonephritis. This procedure uses a competitive mutant template of the same sequence as the target, except for the addition of a restriction site by a single base substitution. Since the sequence of the competitor is almost identical to that of the target, difference of amplification efficiency should be negligible. However, there are two problems in analysing the intraglomerular expression of mRNA for cytokines in human glomerulonephritis by using competitive PCR: a difficulty in quantifying the amount of total RNA in 10 glomeruli, and an inconsistency in the efficiency of reverse transcription. To circumvent these problems, we first quantified the amounts of mRNA expression of the 'housekeeping' gene, β-actin, then diluted the samples so that they contained equal amounts of β-actin cDNA. Several reports indicated that β-actin mRNA is expressed at varying levels in various pathological conditions [28–30]. In the present study, levels of glomerular β-actin mRNA expression were significantly elevated in patients with glomerulonephritis. Thus, β-actin mRNA itself may be enhanced in glomerular disease. Because we adjusted our samples to an equal concentration of glomerular β-actin mRNA, however, we can conclude that the elevated amounts of glomerular TGF-β1 mRNA in several kinds of glomerulonephritis were not due to enhanced β-actin mRNA expression, but rather to the enhanced growth factor mRNA itself.

Glomerular resident cells possess high-affinity receptors for TGF-β and can produce TGF-β *in vivo* and *in vitro* [31–33]. Several studies have demonstrated that, in cultured glomerular cells and in experimental glomerulonephritis, TGF-β induces production of collagen, fibronectin, and proteoglycans which are components of the mesangial matrix [7–9,11]. In addition, TGF-β1 decreases synthesis of proteolytic enzymes that degrade matrix proteins and increases synthesis of protease inhibitors that block the activity of these enzymes [34,35]. Inhibition of TGF-β1 activity prevents the accumulation of the mesangial matrix, and introduction of TGF-β1 gene induces the mesangial matrix expansion *in vivo* [12–14]. We found that significantly higher levels of glomerular TGF-β1 mRNA were observed in patients with mesangial proliferative glomerulonephritis having a moderate increase in mesangial matrix, diabetic nephropathy and lupus nephritis. The mesangial matrix was markedly increased in these glomerular diseases [36]. Therefore, it is conceivable that TGF-β1 is closely associated with the pathological accumulation of mesangial matrix, i.e. glomerular sclerosis in human glomerulonephritis. In conclusion, the quantification of TGF-β1 mRNA in glomeruli allows one to evaluate the disease activity of glomerulonephritis and its prognosis. In addition, it is useful in evaluating the response to treatment.

An elucidation of the mechanisms that induce the expression of TGF-β1 mRNA in glomeruli and activate the latent form of TGF-β1 in glomeruli is important in gaining a better understanding of the pathogenesis of glomerulonephritis and in improving the therapeutic approach.

ACKNOWLEDGMENTS

This study was supported by a Research Grant for 'Progressive Renal Disease' from 'Specially selected diseases by the Ministry of Health and

Welfare Research Project', Ministry of Health and Welfare, Japan, and by a Program Project Grant of Health and Welfare of Japan.

REFERENCES

1 Roberts AB, Sporn MB. The transforming growth factor-βs. In: Sporn MB, Roberts AB, eds. Handbook of experimental pharmacology. New York: Springer-Verlag, 1989:419–72.

2 Sporn MB, Roberts AB. TGF-β: problems and prospects. Cell Regul 1990; **1**:875–82.

3 Border WA, Ruoslahti E. Transforming growth factor-β in disease: the dark side of tissue repair. J Clin Invest 1992; **90**:1–7.

4 Roberts AB, Sporn MB, Assoain RK *et al.* Transforming growth factor type β: rapid induction of fibrosis and angiogenesis *in vivo* and stimulation of collagen formation *in vitro*. Proc Natl Acad Sci USA 1986; **83**:4167–71.

5 Brenner BM. Nephron adaptation to renal injury or ablation. Am J Physiol 1985; **249**:F324–37.

6 Olson JL, Heptinstall RH. Biology of disease: nonimmunologic mechanisms of glomerular injury. Lab Invest 1988; **59**:564–78.

7 Natori Y, O'meara YM, Manning EC *et al.* Production and polarized secretion of basement membrane components by glomerular epithelial cells. Am J Physiol 1992; **262**:F131–7.

8 Nakamura T, Miller D, Ruoslahti E *et al.* Production of extracellular matrix by glomerular epithelial cells is regulated by transforming growth factor-β1. Kidney Int 1992; **41**:1213–21.

9 Border WA, Okuda S, Languino LR *et al.* Transforming growth factor-β regulates production of proteoglycans by mesangial cells. Kidney Int 1990; **37**:689–95.

10 Coimbra T, Wiggins R, Noh JW *et al.* Transforming growth factor-β production in anti-glomerular basement membrane disease in the rabbit. Am J Pathol 1991; **138**:223–34.

11 Okuda S, Languino LR, Ruoslahti E *et al.* Elevated expression of transforming growth factor-β and proteoglycan production in experimental glomerulonephritis. J Clin Invest 1990; **86**:453–62.

12 Border WA, Okuda S, Languino LR *et al.* Suppression of experimental glomerulonephritis by antiserum against transforming growth factor β1. Nature 1990; **346**:371–4.

13 Border WA, Noble NA, Yamamoto T *et al.* Natural inhibitor of transforming growth factor-β protects against scarring in experimental kidney disease. Nature 1992; **360**:361–4.

14 Isaka Y, Fujiwara Y, Ueda N *et al.* Glomerulosclerosis induced by *in vivo* transfection of transforming growth factor-β or platelet-derived growth factor gene into the rat kidney. J Clin Invest 1993; **92**:2597–601.

15 Yoshioka K, Takemura T, Murakami K *et al.* Transforming growth factor-β protein and mRNA in glomeruli in normal and diseased human kidneys. Lab Invest 1993; **68**:154–63.

16 Yamamoto T, Nakamura T, Noble NA *et al.* Expression of transforming growth factor β is elevated in human and experimental diabetic nephropathy. Proc Natl Acad Sci USA 1993; **90**:1814–8.

17 Pirani CL, Salina-Madrigal L. Elevation of percutaneous renal biopsy. Pathol Annu 1968; **3**:249–96.

18 Bergstein JM, Riley M, Bang NU. Analysis of the plasminogen activator activity of the human glomerulus. Kidney Int 1988; **33**:868–74.

19 Gilliland G, Perrin S, Bunn HF. Competitive PCR. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, eds. PCR protocols: a guide to methods and applications. San Diego: Academic Press, 1990:60–69.

20 Ponte P, Ng S, Engel J *et al.* Evolutionary conservation in the untranslated regions of actin mRNAs: DNA sequence of a human beta-actin cDNA. Nucleic Acids Res 1984; **12**:1687–96.

21 Derynck R, Jarrett JA, Chen EY *et al.* Human transforming growth factor-β complementary DNA sequence and expression in normal and transformed cells. Nature 1985; **316**:701–5.

22 Higuchi R, Krummel B, Saiki RK. A general method of *in vitro* preparation and specific mutagenesis of DNA fragments: study of protein and DNA interactions. Nucleic Acids Res 1988; **16**:7351–67.

23 Horii Y, Muraguchi A, Iwano M *et al.* Involvement of interleukin 6 in mesangial proliferative glomerulonephritis. J Immunol 1989; **143**:3949–55.

24 Johnson RJ, Raines EW, Floege J *et al.* Inhibition of mesangial cell proliferation and matrix expansion in glomerulonephritis in the rat by antibody to platelet-derived growth factor. J Exp Med 1992; **175**:1413–6.

25 Gesualdo L, Pinzani M, Floriano JJ *et al.* Platelet-derived growth factor expression in mesangial proliferative glomerulonephritis. Lab Invest 1991; **65**:160–7.

26 Werber HI, Emancipator SN, Tykocinski ML *et al.* The interleukin 1 gene is expressed by rat glomerular mesangial cell and is augmented in immune complex glomerulonephritis. J Immunol 1987; **138**:3207–12.

27 Floege J, Eng E, Young BA *et al.* Infusion of platelet-derived growth factor or basic fibroblast growth factor induces selective glomerular mesangial cell proliferation and matrix accumulation in rats. J Clin Invest 1993; **92**:2952–62.

28 Peten EP, Striker LJ, Carome MA *et al.* The contribution of increased collagen synthesis to human glomerulosclerosis: a quantitative analysis of α21V collagen mRNA expression by competitive polymerase chain reaction. J Exp Med 1992; **176**:1571–6.

29 Funk CD, FitzGerald GA. Eicosanoid forming enzyme mRNA in human tissues. J Biol Chem 1991; **266**:12508–13.

30 Feldman AM, Ray PE, Bristow MR. Expression of α-subunits of G proteins in failing human heart: a reappraisal of utilizing quantitative polymerase chain reaction. J Mol Cell Cardiol 1991; **23**:1355–8.

31 Mackay K, Striker LJ, Stauffer JW *et al.* Transforming growth factor-β. Murine glomerular receptors and responses of isolated glomerular cells. J Clin Invest 1989; **83**:1160–7.

32 Kaname S, Uchida S, Ogata E *et al.* Autocrine secretion of transforming growth factor-β in cultured rat mesangial cells. Kidney Int 1992; **42**:1319–27.

33 Choi ME, Kim E, Huang Q *et al.* Rat mesangial cell hypertrophy in response to transforming growth factor-β1. Kidney Int 1993; **44**:948–58.

34 Edwards DR, Murphy G, Reynolds J *et al.* Transforming growth factor-β modulates the expression of collagenase and metalloproteinase inhibitor. EMBO J 1987; **6**:1899–904.

35 Laiho M, Saksela O, Keski-Oja J. Transforming growth factor-β induction of type-1 plasminogen activator inhibitor. J Biol Chem 1987; **262**:17467–74.

36 Churg J, Solbin LH. Renal diseases: classification and atlas of glomerular diseases. Tokyo: Igaku Shoin, 1982.