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Transforming Growth Factor-β Is a Potent Inhibitor of Extracellular Matrix Degradation by Cultured Human Mesangial Cells

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Abstract. Accumulation of the glomerular extracellular matrix (ECM) is a pivotal event in the progression from acute glomerular injury to end-stage renal disease. Although enhanced ECM synthesis has been demonstrated to contribute to ECM accumulation, the role of decreased ECM degradation is largely unknown. It was previously shown that glomerular ECM degradation is mediated by a plasminogen activator (PA)/plasmin/matrix metalloproteinase 2 (MMP-2) cascade. However, little information is available regarding the factors that regulate the activity of this degradative cascade in normal or pathologic states. Transforming growth factor-β1 (TGF-β1) is shown here to be a potent inhibitor of ECM degradation by cultured human mesangial cells. Using human mesangial cells grown on thin films of ^{125}I-labeled Matrigel, dose-dependent inhibition of ECM degradation in the presence of TGF-β1 was observed, reaching >90% inhibition with 0.4 ng/ml TGF-β1. Addition of anti-TGF-β antibodies (4 μg/ml) in the absence of exogenous TGF-β increased ECM degradation (1.8 $\pm$ 0.2-fold *versus* controls, $P < 0.05$). In contrast, platelet-derived growth factor, at concentrations up to 10 ng/ml, had no effect on ECM degradation. TGF-β completely blocked the conversion of plasminogen to plasmin and markedly reduced the conversion of latent MMP-2 to active MMP-2. TGF-β did not significantly alter the levels of tissue PA, total MMP-2, or tissue inhibitor of metalloproteinase-1, but did increase the levels of PA inhibitor-1 (1.8-fold, $P < 0.05$), the major physiologic inhibitor of PA. These data document that TGF-β is a potent inhibitor of ECM degradation by cultured human mesangial cells, and they suggest that decreased mesangial matrix degradation, caused by TGF-β-mediated decreases in the activity of the PA/plasmin/MMP-2 cascade, may contribute to the glomerular matrix accumulation that occurs in progressive renal disease.

Several diverse lines of evidence suggest a pathogenic role for transforming growth factor-β (TGF-β) in glomerular extracellular matrix (ECM) accumulation and the subsequent development of glomerulosclerosis in progressive renal disease (1–5). For example, increased levels of glomerular TGF-β have been observed in animals with experimentally induced glomerular injury (6–9) and in patients with diabetic nephropathy (9,10), mesangial proliferative glomerulonephritis (GN), or diffuse proliferative lupus nephritis (11,12). Importantly, administration of antiserum raised against TGF-β (13) or a naturally occurring inhibitor of TGF-β (14) prevented matrix accumulation in the anti-Thy-1 model of GN. Other studies supporting a key role for TGF-β in glomerular ECM accumulation include those of Isaka *et al.* (15), who demonstrated that transfection of the TGF-β gene into rat kidneys resulted in glomerulosclerosis, Akagi *et al.* (16), who reported that inhibition of TGF-β expression by antisense oligonucleotides suppressed ECM accumulation in anti-Thy-1 GN, Kanai *et al.* (17), who demonstrated increased urinary excretion of TGF-β by patients with focal glomerulosclerosis, and Kopp *et al.* (18) and Clouthier *et al.* (19), who showed that transgenic mice with increased plasma levels of TGF-β developed progressive renal disease.

The mechanism by which overexpression of TGF-β leads to ECM accumulation and glomerulosclerosis is unclear. *In vitro* studies have demonstrated the ability of TGF-β to increase the synthesis by mesangial and epithelial cells of various ECM components, including proteoglycans (20,21), collagen types I and IV (21–23), and fibronectin (22). In *in vivo* studies, Border and coworkers (12) reported that sustained expression of TGF-β was associated with the development of progressive kidney fibrosis and marked deposition of collagen types I and III. Similarly, Sharma *et al.* (24) reported that anti-TGF-β antibodies were able to prevent renal hypertrophy and increased glomerular ECM gene expression in streptozotocin-induced diabetes in mice. Therefore, one mechanism by which TGF-β contributes to glomerulosclerosis seems to be increased synthesis of ECM components.

Decreased degradation of the ECM could also contribute to matrix accumulation and glomerulosclerosis. It is now generally accepted that ECM degradation in glomeruli and glomerular cells is mediated by a plasminogen activator (PA)/plasmin/matrix metalloproteinase-2 (MMP-2) (gelatinase A) cascade. For example, we have shown that ECM degradation by human (25), rat (26), and mouse (unpublished observations) mesangial cells is dependent on the production of plasmin and, to a lesser extent, active MMP-2. In addition, a number of studies have

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reported decreased PA activity, decreased plasmin activity, and/or increased levels of PA inhibitor-1 (PAI-1) (the major PA inhibitor) in glomeruli obtained from animals with experimentally induced glomerular injuries known to result in mesangial matrix accumulation (27–31).

A direct link between TGF-β and the PA/plasmin system has also been reported. Border and coworkers demonstrated that the addition of TGF-β to glomeruli isolated from normal rats increased the synthesis of PAI-1 and decreased the activity of PA (29) and that sustained expression of TGF-β was associated with elevated PAI-1 levels in glomeruli isolated from rats with a chronic form of anti-Thy-1-induced GN (12). Similar effects of TGF-β on rat proximal tubular cells were reported by Kanalas and Hopfer (32).

Despite the implications of the aforementioned studies, there is little if any direct evidence that TGF-β actually decreases glomerular ECM degradation. In this report, we provide direct evidence that TGF-β1 is a potent inhibitor of ECM degradation by cultured human mesangial cells.

Materials and Methods

Reagents

TGF-β1 and platelet-derived growth factor (PDGF) (A/B isoform) were obtained from Boehringer. Polyclonal antihuman TGF-β antibodies prepared in turkeys and polyclonal turkey IgG (control antibody) and Matrigel were obtained from Collaborative Research. All other chemicals were reagent grade or higher and were obtained from commercial sources, as described in our previous reports (25,26). Exogenously added agents (*e.g.*, plasminogen, TGF-β, and PDGF) were prepared immediately before use and were dissolved, at the concentrations indicated, in RPMI 1640 containing 0.2% lactalbumin hydrolysate.

Mesangial Cell Culture

Human mesangial cells, which were a generous gift from Dr. Hanna Abboud (University of Texas Health Sciences Center, San Antonio, TX), were cultured in Waymouth's medium containing 15% fetal calf serum, as described by Abboud and coworkers (33). These cells were extensively characterized as follows. All cells were stellate or spindle-shaped, with irregular cytoplasmic projections. The cells did not exhibit contact inhibition and became multilayered if allowed to grow beyond confluence. Immunoperoxidase staining was positive for desmin, myosin, vimentin, fibronectin, and actin and negative for cytokeratin, von Willebrand factor, common leukocyte antigen, and DR antigen. The cells did not express detectable amounts of angiotensin-converting enzyme activity. The human mesangial cells used in these studies were between passages 9 and 15. Mice lacking a functional PAI-1 gene (PAI-1 knockout mice) were obtained from Dr. Thomas Bugge (Childrens Hospital, Cincinnati, OH). PAI-1 null mesangial cells were cultured from glomeruli prepared from PAI-1 knockout mice by a combination of sieving and centrifugation (25,26).

Measurement of ECM Degradation by Cultured Mesangial Cells

ECM degradation was measured as described in detail in our previous report (25) and summarized below. Thin films of ^{125}I-labeled Matrigel were produced in 24-well culture plates as described previously. Wells containing dried Matrigel films (25 μg of protein, 20,000 to 25,000 cpm) were washed three times with 1.0 ml of serum-free RPMI 1640, immediately before the addition of mesangial cells (25,000 cells/well) in Waymouth's medium containing 15% fetal calf serum. Plates were then incubated for 48 h (37°C, 5.0% CO_2) to allow the mesangial cells to attach to the Matrigel films and to recover from the plating procedures. After 48 h, the medium was carefully removed and the cells were washed three times with 1.0 ml of serum-free RPMI 1640 and then incubated, as described above (usually for 72 h), in 500 μl of serum-free RPMI 1640 (without phenol red) containing 0.2% lactalbumin hydrolysate, as described previously.

ECM degradation by cultured mesangial cells was measured by the release of radioactivity into the cell-free culture medium. At the end of the incubation period (usually 72 h), the medium from each well was carefully removed, counted (in a gamma counter), and stored at −20°C until other biochemical parameters were measured (*e.g.*, using enzyme activity assays, substrate gels, or enzyme-linked immunosorbent assays [ELISA]), as indicated below. ECM degradation was expressed as the mean ± SEM of the micrograms of Matrigel degraded in the number of determinations indicated (each assayed in triplicate). Matrigel degradation was calculated from the decay-corrected specific radioactivity of the Matrigel after correction for the appropriate controls (Matrigel incubated with cells only, Matrigel incubated with medium only, and Matrigel incubated with plasminogen only), which were included on each 24-well plate.

Quantification of Urokinase PA, Tissue PA, PAI-1, MMP-2, and Tissue Inhibitor of Metalloproteinase-1 by ELISA

Quantitative measurement of urokinase PA (uPA), tissue PA (tPA), and PAI-1 was performed as described in our previous report (25), using the following volumes of undiluted conditioned medium obtained from 25,000 cells grown for 72 h in 500 μl of RPMI 1640 containing 0.2% lactalbumin hydrolysate: for uPA, 100 μl; for tPA, 60 μl; for PAI-1, 6 μl. Each sample was measured in duplicate, and the amounts of uPA, tPA, and PAI-1 were determined from standard curves (assayed simultaneously with the samples) after correction for the appropriate blanks (medium obtained from wells containing no cells). MMP-2 and tissue inhibitor of metalloproteinase-1 (TIMP-1) protein levels were measured by ELISA, as described previously (34).

Measurement of Plasmin and MMP-2 Activities

Plasmin activity in medium obtained from mesangial cells cultured on Matrigel films, appropriate controls, and plasmin standards was determined using the synthetic fluorometric plasmin substrate methoxysuccinyl-L-Ala-L-Phe-L-Lys-7-amido-4-methyl-coumarin, as described in detail in our previous report (25). Results are expressed as the mean ± SEM of nanomoles of 7-amido-4-methyl-coumarin produced per well per 60-min incubation, corrected for duplicate blanks (with medium added after the stop solution) assayed for each sample. Semiquantitative analysis of MMP-2 activity was performed using gelatin zymography, as described in our previous reports (25,26).

Statistical Analysis

The significance of differences between means was compared by the *t* test, with *P* values of <0.05 considered significant.

Results

TGF-β Inhibition of ECM Degradation

In our first series of studies, we examined the effects of exogenous TGF-β and PDGF on ECM degradation by cultured human mesangial cells. As shown in Figure 1A, TGF-β caused a marked reduction in ECM degradation. In contrast, PDGF

(10 ng/ml) had no effect on ECM degradation (Figure 1A). The effect of TGF-β was dose-dependent (0.01 to 1.0 ng/ml), with significant inhibition of ECM degradation occurring at TGF-β concentrations of 0.05 ng/ml (Figure 1B). Mesangial cells are known to produce TGF-β (35). Therefore, we examined the effects of anti-TGF-β antibodies on ECM degradation by mesangial cells in the absence of exogenous TGF-β. As shown in Figure 2, anti-TGF-β antibodies (4 μg/ml) caused a marked increase in ECM degradation, compared with the same amount of nonspecific IgG. These data establish that TGF-β is a potent inhibitor of ECM degradation by mesangial cells, and they suggest that endogenous TGF-β exhibits a tonic inhibitory effect on ECM degradation.

Effects of TGF-β and PDGF on Plasmin and Gelatinase Activities and TIMP-1 Levels

We have shown that ECM degradation by cultured mesangial cells is mediated by plasmin and MMP-2 (25,26). Therefore, we examined the effects of TGF-β and PDGF on the activity of these two proteinases in medium obtained from mesangial cells that had been incubated with or without TGF-β or PDGF for 72 h. As shown in Figure 3, plasmin activity in medium obtained from mesangial cells that had been incubated in the absence of TGF-β was similar to that reported in our previous study (0.85 $\pm$ 0.08 nmol/well) (25). In contrast, plasmin activity was undetectable in medium obtained from mesangial cells that had been incubated for 72 h in the presence of 1.0 ng/ml TGF-β. Semiquantitative determination of MMP-2 activity was performed by zymography of medium obtained from cells that had been incubated in the presence or absence of TGF-β or PDGF. As shown in Figure 4, TGF-β markedly reduced the amount of active MMP-2, as indicated by the absence of the lower MMP-2 band. However, ELISA demonstrated the same amount of total MMP-2 protein (latent plus active) in medium obtained from cells incubated in the presence or absence of TGF-β (Figure 5). Mesangial cells also produce MMP-2 inhibitors, called TIMP (36). Therefore, we also measured the effect of TGF-β and PDGF on the levels of TIMP-1, the major TIMP produced by mesangial cells. Neither TGF-β nor PDGF had any effect on TIMP-1 protein levels (Figure 5). In addition, PDGF (10 ng/ml) had no effect on the conversion of latent MMP-2 to active MMP-2 (Figure 4).

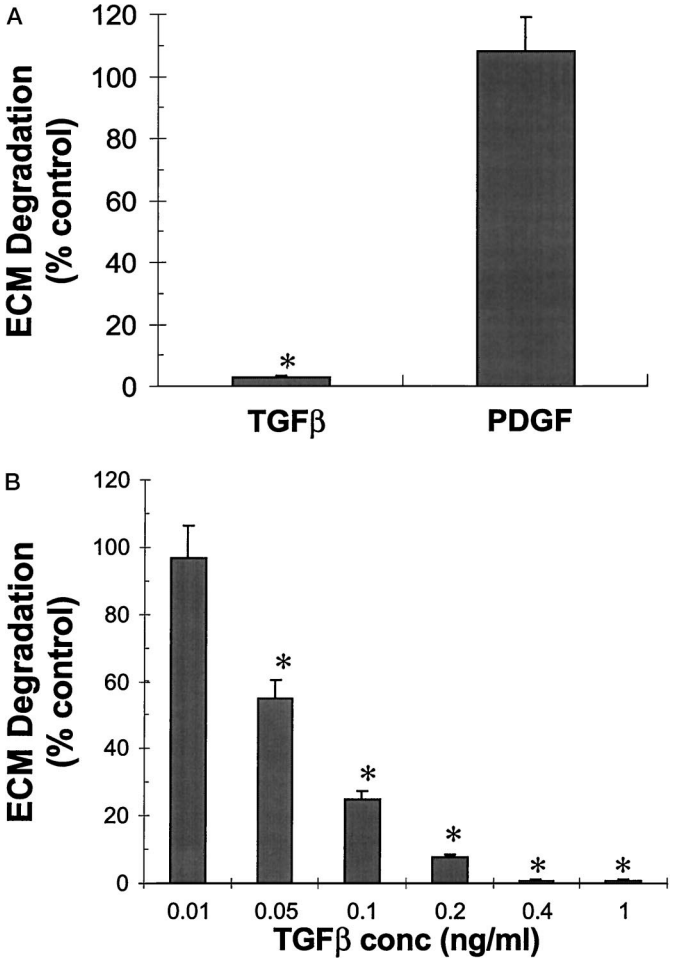

Figure 1. Effects of transforming growth factor-β (TGF-β) and platelet-derived growth factor (PDGF) on extracellular matrix (ECM) degradation by cultured human mesangial cells. Mesangial cells were incubated for 72 h in the presence of TGF-β (1 ng/ml) or PDGF (10 ng/ml). Results are presented as the mean $\pm$ SEM of at least four determinations (assayed in triplicate). *$P < 0.05$. For more details, see Materials and Methods.

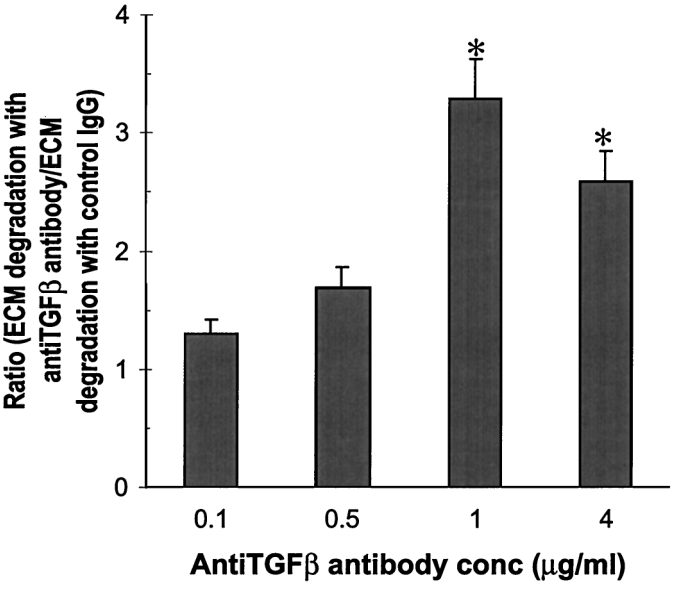

Figure 2. Effects of anti-TGF-β antibodies on ECM degradation by cultured human mesangial cells. Mesangial cells were incubated for 72 h in the presence of 4 μg/ml anti-TGF-β antibodies. Nonspecific turkey IgG (4 μg/ml) served as a control. Results are presented as the mean $\pm$ SEM of four determinations (assayed in triplicate). *$P < 0.05$. For more details, see Materials and Methods.

Effects of TGF-β on the Production of tPA, uPA, and PAI-1

Cultured human mesangial cells produce tPA, uPA, and PAI-1 (25,37,38). Using ELISA, we measured the levels of tPA, uPA, and PAI-1 antigens in medium obtained from mesangial cells that had been cultured for 72 h in the presence or

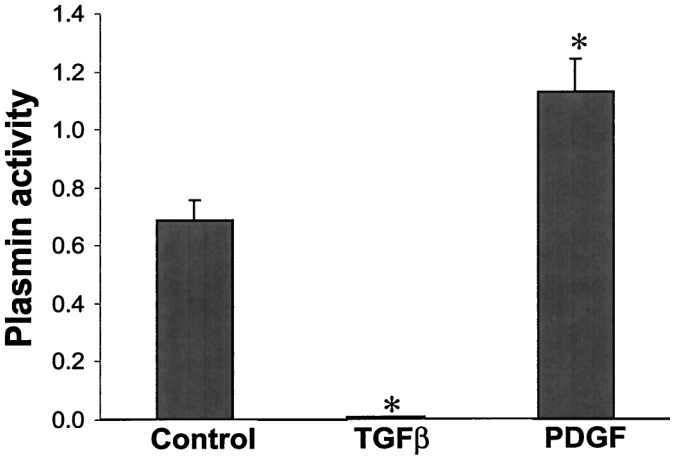

Figure 3. Plasmin activity in medium obtained from human mesangial cells incubated in the presence of TGF-β**.** Mesangial cells were incubated for 72 h in the presence of 1 ng/ml TGF-β or 10 ng/ml PDGF. Plasmin activity was determined using the fluorometric plasmin substrate methoxysuccinyl-L-Ala-L-Phe-L-Lys-7-amido-4-methyl-coumarin, as described in detail in our previous report (25). Results are expressed as the mean ± SEM of nanomoles of 7-amido-4-methyl-coumarin produced per well per 60-min incubation, corrected for duplicate blanks (with medium added after the stop solution) assayed for each sample. $*P < 0.05$. For more details, see Materials and Methods.

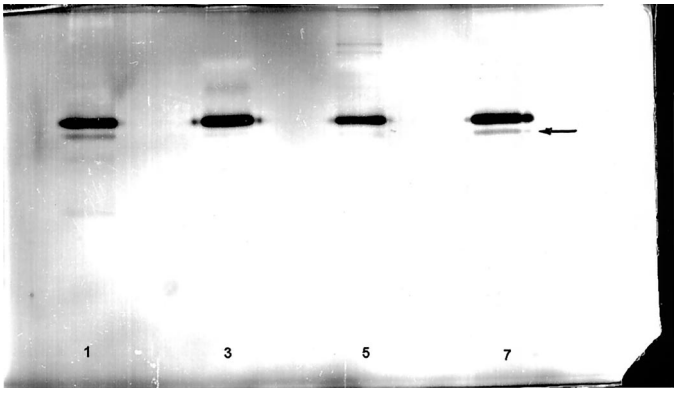

Figure 4. Gelatin zymography of medium obtained from human mesangial cells incubated in the presence of TGF-β or PDGF. Mesangial cells (25,000/well) were incubated for 72 h in the presence of TGF-β or PDGF. Twenty microliters of cell-free medium was mixed with 10 μl of gel sample buffer, and 20 μl of this mixture was used for zymography. Lane 1, medium from cells incubated in the presence of plasminogen and PDGF (10 ng/ml); lane 3, medium from cells incubated in the presence of plasminogen and TGF-β (1 ng/ml); lane 5, medium from cells only (no plasminogen); lane 7, medium from cells incubated in the presence of plasminogen (control). Lanes 2, 4, 6, and 8, empty lanes. Molecular weight standards included in the original gel cannot be seen in this photograph of the computer-generated inverted image. Arrow, active matrix metalloproteinase 2 (MMP-2). There were no significant differences (<10%) in cell density, as indicated by measurements of DNA content. Results shown are from a typical experiment. For more details, see Materials and Methods.

absence of TGF-β or PDGF (Figure 6). TGF-β had no significant effect on the level of tPA antigen. In contrast, the level of PAI-1 antigen was increased approximately twofold in the presence of TGF-β. TGF-β also increased the level of uPA (see Discussion). PDGF had no effect on the levels of tPA or PAI-1 but also increased uPA levels (Figure 6).

These data document that TGF-β inhibits the degradation of ECM by mesangial cells, and they suggest that this effect is mediated by increased production of PAI-1, the major physiologic inhibitor of PA. This suggestion is supported by preliminary studies indicating that TGF-β (1 ng/ml) has no effect on ECM degradation by mesangial cells obtained from mice in which the PAI-1 gene has been disrupted (PAI-1 knockout cells, 3.6 ± 0.6 μg/well, $n = 3$; PAI-1 knockout cells with TGF-β, 3.7 ± 0.4 μg/well, $n = 6$; assayed in triplicate).

Discussion

Our results indicate that TGF-β is a potent inhibitor of ECM degradation by cultured human mesangial cells. This inhibitory effect is mediated by decreases in the conversion of plasminogen to plasmin (Figure 3), the protease that is primarily responsible for ECM degradation in this system (25,26). In contrast, PDGF has no effect on ECM degradation (Figure 1), despite increasing plasmin levels (Figure 3). We have no explanation for this anomalous effect of PDGF. The PA/plasmin/MMP-2 cascade is a complex pathway. The amount of ECM degradation is determined by the relative amounts of the active and inactive forms of each protease and protease inhibitor involved, as well as the time frame in which each of these forms is produced. In addition, it is possible that, under the conditions of our assay, unstimulated cells degrade maximal amounts of ECM, precluding detection of increased ECM degradation. Whatever the explanation, it does not invalidate our major observation that TGF-β inhibits ECM degradation by cultured mesangial cells.

Neither TGF-β nor PDGF had any effect on the levels of TIMP-1 or total MMP-2 protein (latent plus active) (Figure 5). In addition, PDGF had no effect on the production of active MMP-2 (Figure 4). In the presence of TGF-β (1 ng/ml), the amount of active MMP-2 (as measured by zymography) was markedly decreased (Figure 4). This observation is in keeping with our previous observations (25,26) that the conversion of latent MMP-2 to active MMP-2 is dependent on the presence of plasmin. However, in contrast to our original hypothesis, which proposed a direct role for plasmin in MMP-2 activation, the role of plasmin in the activation of latent MMP-2 may be explained by a recent study demonstrating that plasmin can activate a membrane-bound MMP, which in turn could activate latent MMP-2 (39).

Plasmin is a serine proteinase that is produced by the action of PA on plasminogen. Because plasminogen is exogenously added in this system, the decreased plasmin activity observed in the presence of TGF-β must reflect decreased PA activity. Human mesangial cells produce two PA forms (uPA and tPA) (25,37,38) and PAI-1 (25,37,38). Therefore, decreased plasmin activity could result from decreased activity of PA, increased production of PAI-1, or both. As shown in Figure 6, TGF-β

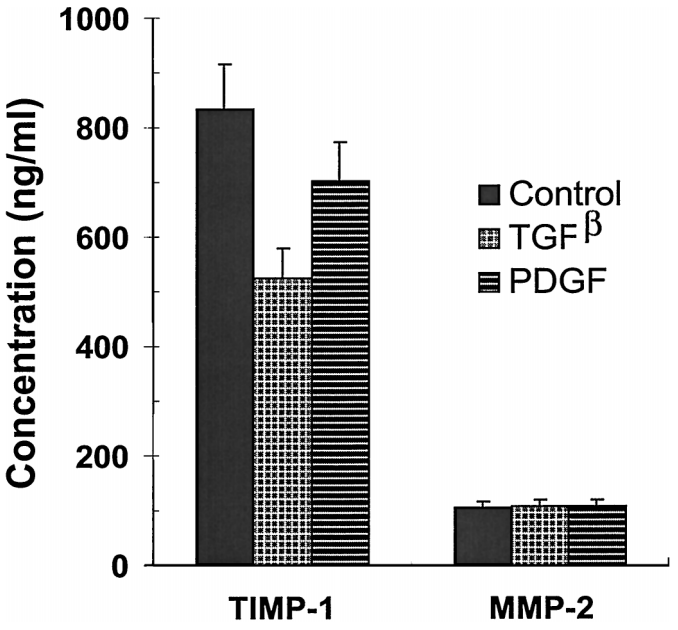

Figure 5. Effects of TGF-β and PDGF on MMP-2 and tissue inhibitor of metalloproteinase 1 (TIMP-1) antigen production by cultured human mesangial cells. MMP-2 and TIMP-1 antigen levels in medium obtained from mesangial cells that had been incubated for 72 h in the presence of 1 ng/ml TGF-β or 10 ng/ml PDGF were determined by enzyme-linked immunosorbent assay (ELISA). Results are expressed as the mean ± SEM for at least four determinations performed in duplicate. *$P < 0.05$. For more details, see Materials and Methods.

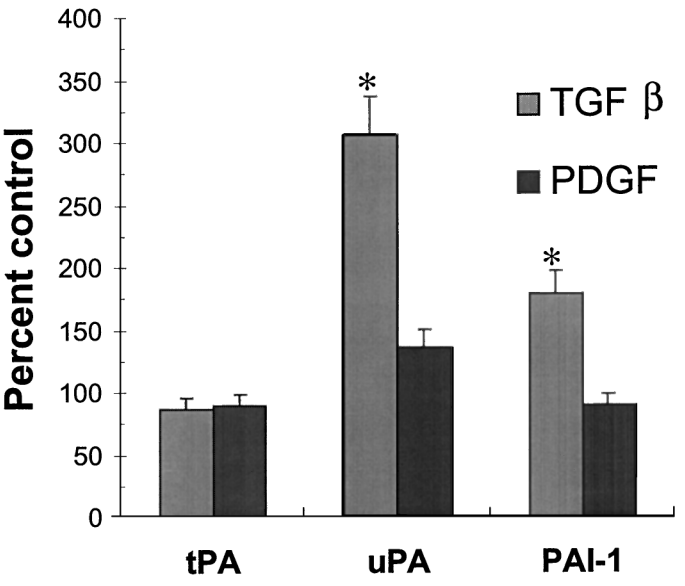

Figure 6. Effects of TGF-β and PDGF on tissue plasminogen activator (tPA), urokinase plasminogen activator (uPA), and plasminogen activator inhibitor-1 (PAI-1) antigen production by cultured human mesangial cells. tPA, uPA, and PAI-1 antigen levels in medium obtained from mesangial cells that had been incubated for 72 h in the presence of 1 ng/ml TGF-β or 10 ng/ml PDGF were determined by ELISA. Results are expressed as the mean ± SEM for eight or more determinations. *$P < 0.05$. For more details, see Materials and Methods.

had no effect on tPA levels. However, the levels of PAI-1, the major PA inhibitor, were elevated almost twofold by TGF-β (Figure 6). These data are in good agreement with a previous study by Tomooka and coworkers (29), who reported increased PAI-1 production and decreased total PA activity by isolated rat glomeruli exposed to exogenous TGF-β. The failure of TGF-β to reduce ECM degradation by mesangial cells obtained from PAI-1 knockout mice also supports this conclusion. Taken together, these data suggest that TGF-β-induced inhibition of ECM degradation by mesangial cells is mediated by enhanced production of PAI-1. TGF-β also increased the levels of uPA, by approximately threefold (Figure 6). However, the virtual lack of plasmin activity in the presence of TGF-β, despite the threefold increase in uPA production, supports our previous suggestion that tPA is the major source of plasmin in this system (25).

Our data indicate that the prosclerotic actions of TGF-β are mediated by decreased degradation of ECM components, as well as increased ECM synthesis (as shown by others). The identification of TGF-β as a potent inhibitor of ECM degradation by cultured mesangial cells increases our understanding of the pathogenic mechanisms involved in the development of glomerulosclerosis and emphasizes the key role of TGF-β in progressive renal disease.

Acknowledgments

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References

1. Klahr S, Schreiner G, Ichikawa I: The progression of renal disease. *N Engl J Med* 318: 1657–1666, 1988
2. Peten EP, Striker LJ: Progression of glomerular diseases. *J Intern Med* 236: 241–249, 1994
3. Bruijn JA, Roos A, de Geus B, de Heer E: Transforming growth factor-β and the glomerular extracellular matrix in renal pathology. *J Lab Clin Med* 123: 34–47, 1994
4. Sharma K, Ziyadeh FN: The emerging role of transforming growth factor-β in kidney diseases. *Am J Physiol* 266: F829–F842, 1994
5. Border WA, Noble NA: Transforming growth factor β in tissue fibrosis. *N Engl J Med* 331: 1286–1292, 1994
6. Okuda S, Nakamura T, Yamamoto T, Ruoslahti E, Border WA: Elevated expression of transforming growth factor β and proteoglycan production in experimental glomerulonephritis. *J Clin Invest* 86: 453–462, 1990
7. Coimbra T, Wiggins R, Noh JW, Merritt S, Phan SH: Transforming growth factor β production in antiglomerular basement membrane disease in the rabbit. *Am J Pathol* 138: 223–234, 1991
8. Lianos E, Liu J, Guglielmi K: Quantification of changes in transforming growth factor-β1 gene expression in experimental crescentic glomerulonephritis. *Proc Soc Exp Biol Med* 214: 180–186, 1996

9. Yamamoto T, Nakamura T, Noble NA, Ruoslahti E, Border WA: Expression of transforming growth factor β is elevated in human and experimental diabetic nephropathy. *Proc Natl Acad Sci USA* 90: 1814–1818, 1993
10. Iwano M, Kubo A, Nishino T, Sato H, Nishioka H, Akai Y, Kurioka H, Fujii Y, Kanauchi M, Shiki H, Dohi K: Quantification of glomerular TGFβ-1 mRNA in patients with diabetes mellitus. *Kidney Int* 49: 1120–1126, 1996
11. Iwano M, Akai Y, Fujii Y, Dohi Y, Matsumara N, Dohi K: Intraglomerular expression of transforming growth factor-beta 1 (TGF-β1) mRNA in patients with glomerulonephritis: Quantitative analysis by competitive polymerase chain reaction. *Clin Exp Immunol* 97: 309–314, 1994
12. Yamamoto T, Noble NA, Miller DE, Border WA: Sustained expression of TGF-β1 underlies development of progressive kidney fibrosis. *Kidney Int* 45: 916–927, 1994
13. Border WA, Okuda S, Languino LR, Sporn MB, Ruoslahti E: Suppression of experimental glomerulonephritis by antiserum against transforming growth factor β1. *Nature (Lond)* 346: 371–374, 1990
14. Border WA, Noble NA, Yamamoto T, Harper JR, Yamaguchi Y, Pierschbacher MD, Ruoslahti E: Natural inhibitor of transforming growth factor-β protects against scarring in experimental kidney disease. *Nature (Lond)* 360: 361–364, 1992
15. Isaka Y, Fujiwara Y, Ueda N, Kaneda Y, Kamada T, Imai E: Glomerulosclerosis induced by *in vivo* transfection of transforming growth factor-β or platelet-derived growth factor gene into the rat kidney. *J Clin Invest* 92: 2597–2601, 1993
16. Akagi Y, Isaka Y, Arai M, Kaneko T, Takenaka M, Moriyama T, Kaneda Y, Ando A, Orita Y, Kamada T, Ueda N, Imai E: Inhibition of TGF-β1 expression by antisense oligonucleotides suppressed extracellular matrix accumulation in experimental glomerulonephritis. *Kidney Int* 50: 148–155, 1996
17. Kanai H, Mitushashi N, Ono R, Yano M, Naruse N: Increased excretion of urinary transforming growth factor beta in patients with focal glomerular sclerosis. *Nephron* 66: 391–395, 1994
18. Kopp JB, Factor VM, Mozes M, Nagy P, Sanderson N, Bottinger EP, Klotman PE, Thorgeirsson SS: Transgenic mice with increased plasma levels of TGF-β1 develop progressive renal disease. *Lab Invest* 74: 991–1003, 1996
19. Clouthier DE, Comerford SA, Hammer RE: Hepatic fibrosis, glomerulosclerosis, and lipodystrophy-like syndrome in PEPCK-TGFβ transgenic mice. *J Clin Invest* 100: 2697–2713, 1997
20. Border WA, Okuda S, Languino R, Ruoslahti E: Transforming growth factor β regulates production of proteoglycans by mesangial cells. *Kidney Int* 37: 689–695, 1990
21. Nakamura T, Okuda S, Ruoslahti E, Border WA: Production of extracellular matrix by glomerular epithelial cells is regulated by transforming growth factor-β1. *Kidney Int* 41: 1213–1221, 1992
22. MacKay K, Stricker L, Stauffer JW, Doi T, Agoda LY, Stricker GE: Transforming growth factor β: Murine glomerular receptors and responses of isolated glomerular cells. *J Clin Invest* 83: 160–167, 1989
23. Ziyadeh FN, Sharma K, Ericksen M, Wolf G: Stimulation of collagen gene expression and protein synthesis in murine mesangial cells by high glucose is mediated by activation of transforming growth factor-β. *J Clin Invest* 93: 536–542, 1994
24. Sharma K, Jin Y, Ziyadeh FN: Neutralization of TGF-β by anti-TGFβ antibody attenuates kidney hypertrophy and the enhanced extracellular matrix gene expression in STZ-induced diabetic mice. *Diabetes* 45: 522–530, 1996
25. Baricos WH, Cortez SL, El-Dahr SS, Schnaper HW: ECM degradation by cultured human mesangial cells is mediated by a plasminogen activator/plasmin/MMP-2 cascade. *Kidney Int* 47: 1039–1047, 1995
26. Wong AP, Cortez SL, Baricos WH: Role of plasmin and gelatinase in extracellular matrix degradation by cultured rat mesangial cells. *Am J Physiol* 263: F1112–F1118, 1992
27. Feng L, Tang WW, Loskutoff DJ, Wilson CB: Dysfunction of glomerular fibrinolysis in experimental antiglomerular basement membrane antibody glomerulonephritis. *J Am Soc Nephrol* 3: 1753–1764, 1993
28. Malliaros J, Holdsworth SR, Wojta J, Erlich J, Tipping PG: Glomerular fibrinolytic activity in anti-GBM glomerulonephritis in rabbits. *Kidney Int* 44: 557–564, 1993
29. Tomooka S, Border WA, Marshall BC, Noble NA: Glomerular matrix accumulation is linked to inhibition of the plasmin protease system. *Kidney Int* 42: 1462–1469, 1992
30. Loskutoff DJ, Sawdey M, Keeton M, Schneiderman J: Regulation of PAI-1 gene expression *in vivo*. *Thromb Haemostasis* 70: 135–137, 1993
31. Kitching AR, Holdsworth SR, Ploplis VA, Plow EF, Collen D, Carmeliet P, Tipping PG: Plasminogen and plasminogen activators protect against renal injury in crescentic glomerulonephritis. *J Exp Med* 185: 963–968, 1997
32. Kanalas JJ, Hopfer U: Effect of TGF-β1 and TNF-α on the plasminogen system of rat proximal tubular epithelial cells. *J Am Soc Nephrol* 8: 184–192, 1997
33. Shultz PJ, Knauss TC, Men P, Abboud HE: Mitogenic signals for thrombin in mesangial cells: Regulation of phospholipase C and PDGF genes. *Am J Physiol* 257: F366–F374, 1989
34. Zucker S, Conner C, DiMassmo BI, Ende H, Drews M, Seiki M, Bahou WF: Thrombin induces the activation of progelatinase A in vascular endothelial cells. *J Biol Chem* 270: 23730–23738, 1995
35. Abboud HE: Growth factors in glomerulonephritis. *Kidney Int* 43: 252–267, 1993
36. Martin J, Davies M, Thomas G, Lovett DH: Human mesangial cells secrete a GBM-degrading neutral proteinase and a specific inhibitor. *Kidney Int* 36: 790–801, 1989
37. Glass WF, Kreisberg JI, Troyer DA: Two-chain urokinase, receptor, and type 1 inhibitor in cultured human mesangial cells. *Am J Physiol* 264: F532–F539, 1992
38. Wilson HM, Haites NE, Reid FJ, Booth NA: Interleukin-1-beta up-regulates the plasminogen activator/plasmin system in human mesangial cells. *Kidney Int* 49: 1097–1104, 1996
39. Okumura Y, Sato H, Seiki M, Kido H: Proteolytic activation of the precursor of membrane type 1 matrix metalloproteinase by human plasmin: A possible cell surface activator. *FEBS Lett* 402: 181–184, 1997