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ORIGINAL ARTICLE

**Hisashi Makino · Masashi Mukoyama · Akira Sugawara
Kiyoshi Mori · Takayoshi Suganami · Kensei Yahata
Yuriko Fujinaga · Hideki Yokoi · Issei Tanaka
Kazuwa Nakao**

Roles of connective tissue growth factor and prostanoids in early streptozotocin-induced diabetic rat kidney: the effect of aspirin treatment

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Abstract

Background. Connective tissue growth factor (CTGF) is a cysteine-rich growth factor induced by transforming growth factor-β (TGF-β) and is thought to play a critical role in TGF-β-stimulated extracellular matrix accumulation. To explore its involvement in early diabetic nephropathy, we investigated the time course of CTGF gene expression and its regulation in streptozotocin (STZ)-induced diabetic rat kidney.

Methods. Northern blot analysis for CTGF, TGF-β, and fibronectin expression was performed in the glomeruli of STZ-induced diabetic rats from 3 days to 12 weeks after the induction of diabetes, together with histological examination. To investigate the role of prostanoids in this process, aspirin was administered in one group of diabetic rats. Furthermore, CTGF expression was analyzed in rat mesangial cells cultured under high-glucose conditions.

Results. Glomerular expression of CTGF and TGF-β1 mRNA was coordinately upregulated as early as day 3, followed by fibronectin induction and mesangial matrix accumulation. Chronic aspirin treatment in diabetic rats significantly attenuated mesangial expansion, and effectively suppressed CTGF induction, as well as inhibiting the upregulation of TGF-β1 and fibronectin expression. In cultured mesangial cells, aspirin treatment abolished high glucose-stimulated CTGF upregulation.

Conclusions. These results indicate that CTGF expressed in the glomeruli is upregulated in the early stage of STZ-induced diabetic nephropathy in rats, and could be a critical mediator of the development of diabetic glomerulosclerosis. In addition, the modulatory effects of aspirin during this process suggest a role of the cyclooxygenase pathway in the progression of diabetic nephropathy.

Key words: Connective tissue growth factor · Transforming growth factor-β · Extracellular matrix · Diabetic nephropathy · Prostanoids · Cyclooxygenase inhibitor

H. Makino · M. Mukoyama (✉) · A. Sugawara · K. Mori · T. Suganami · K. Yahata · Y. Fujinaga · H. Yokoi · I. Tanaka · K. Nakao
Department of Medicine and Clinical Science, Kyoto University Graduate School of Medicine, 54 Shogoin Kawahara-cho, Sakyo-ku, Kyoto 606-8507, Japan
Tel. +81-75-751-4285; Fax +81-75-771-9452
e-mail: muko@kuhp.kyoto-u.ac.jp

Introduction

Diabetic nephropathy is the most important cause of endstage renal disease in the world that often leads to requirements for renal replacement therapy.[1] It is characterized by microalbuminuria, renal and glomerular hypertrophy, mesangial expansion with glomerular basement membrane thickening, arteriolar hyalinosis, and global glomerular sclerosis, which ultimately cause the progression of proteinuria and renal failure, with systemic hypertension.[1,2] Accumulation of the extracellular matrix (ECM) components in the mesangium and tubulointerstitium is postulated to be the basis for establishing early lesions of diabetic nephropathy.[2–4] Hyperglycemia is a necessary precondition for the development of the lesions.[5,6] Increasing lines of evidence have indicated the implication of transforming growth factor-β (TGF-β) in the pathogenesis of human and experimental diabetic glomerulosclerosis.[4,7] Indeed, hyperglycemia increases TGF-β production in mesangial cells, and TGF-β stimulates the synthesis of ECM proteins.[8,9] However, the molecular mechanisms by which TGF-β provokes downstream events leading to the accumulation of ECM components have not yet been fully elucidated.

Connective tissue growth factor (CTGF), originally isolated from conditioned media of human umbilical vein endothelial cells,[10] belongs to a new family of cysteine-rich growth factors (the CCN family) that consists of CTGF/fisp-12, cyr61/cef10, and nov.[11,12] In cultured fibroblasts, CTGF gene expression is strongly induced by TGF-β, but not by other growth factors, such as epidermal growth factor, platelet-derived growth factor, or basic fibroblast growth

factor.[13,14] The addition of CTGF, in turn, potently stimulates fibroblast proliferation and ECM protein synthesis.[14] Recently, it was shown that CTGF expression was upregulated in proliferative and fibrotic glomerular lesions of various human renal diseases, including glomerulonephritis and diabetic nephropathy.[15] Subsequent reports using in-vitro and animal models revealed that CTGF mRNA and protein were increased in cultured mesangial cells as well as in the renal cortex in the diabetic milieu, suggesting the involvement of CTGF in the pathogenesis of diabetic glomerulosclerosis.[16,17] Although these observations have led to the hypothesis that CTGF is a candidate factor mediating the fibrogenic properties of TGF-β during the progression of diabetic glomerular injury,[18,19] the regulation and time course of the upregulation of CTGF expression in the glomeruli has not been investigated.

In vitro, TGF-β-stimulated increase of CTGF expression is shown to be attenuated in fibroblasts treated with prostaglandin (PG) E2.[20] On the other hand, a number of reports have already suggested that prostanoids are involved in the pathogenesis of experimental as well as clinical diabetic nephropathy.[21] The synthesis of PGE2 and other prostanoids is increased in mesangial cells under high-glucose conditions and in the glomeruli of diabetic nephropathy,[21–24] and the inhibition of their synthesis by PG synthase or cyclooxygenase (COX) inhibitors appears to alleviate the progression of diabetic renal complications.[23–25] However, the role of the inhibition of PG synthesis in relation to CTGF regulation in vivo remains to be clarified.

In this study, to explore the involvement of CTGF in the glomerular injury in experimental diabetic nephropathy, we examined the time course of CTGF expression in the streptozotocin (STZ)-induced diabetic rat kidney. Furthermore, to examine the role of the prostanoid pathway in this process, we investigated the effect of chronic aspirin treatment.

Materials and methods

Experimental animals

All animal experiments were conducted in accordance with our institutional guidelines for animal research. Male Wistar rats weighing 200–250 g were assigned randomly to control (n = 6), diabetic (n = 12), and aspirin-treated diabetic (n = 8) groups. Diabetes was induced by a single intraperitoneal injection of STZ (60 mg/kg body weight in citrate buffer; pH 4.0), and all animals with a blood glucose level of more than 300 mg/dl after 24 h were included in this study. Control rats received citrate buffer only. One group of diabetic rats was given aspirin (Nacalai Tesque, Kyoto, Japan) added to the drinking water (1.5 mg/ml) throughout the study period.[24] Blood glucose levels and body weights were measured on days 3, 9, 14, 28, and 84 after the induction of diabetes. On day 84, blood urea nitrogen levels were also measured.[26] During the study period, two diabetic rats and one aspirin-treated diabetic rat dropped out because of death or insufficient blood glucose level. For urine measurements, each rat was separately housed in a metabolic cage (Natsume Manufacturing, Tokyo, Japan).[26] Urine PGE2 was assayed using a radioimmunoassay kit (NEN, Boston, MA, USA) after extraction with chloroform.[23] Urinary protein excretion was determined by the pyrogallol red-molybdenum method (SRL, Tokyo, Japan). Rats were killed on day 84 for histological analysis.

To analyze the time courses of gene expression, another set of rats (at each time point, n = 4 in controls and n = 4 to 6 in both diabetic and aspirin-treated diabetic groups) were killed at various time points, when the kidneys were harvested for tissue weight measurement and Northern blot analyses. For Northern blot analysis, glomeruli were isolated from each rat by the differential sieving method.[27]

Histology and morphometric analysis

For light microscopy, sagittal kidney sections were fixed in 4% buffered formaldehyde and embedded in paraffin. Two-micrometer-thick sections were stained with periodic acid-Schiff (PAS). The glomerular mesangial area was measured quantitatively, as the PAS-stained area, with a computer-aided manipulator (KS400; Carl Zeiss Vision, Munich, Germany) after tracing the outer margin of each glomerular tuft.[26] More than 20 consecutive glomerular sections randomly selected in each rat by scanning from the outer cortex to the cortico-medullary junction were examined by two investigators without knowledge of the origin of the slides, and the mean mesangial area was calculated.[26]

Cell culture

Cultured mesangial cells were established from the glomeruli isolated from 10-week-old male Sprague-Dawley rats by a differential sieving method,[27] and used at passages seven to ten. Cells were grown in DMEM (Gibco BRL, Grand Island, NY, USA) containing 20% fetal calf serum (FCS; Sanko Junyaku, Tokyo, Japan) with 100 units/ml of penicillin and 100 μg/ml of streptomycin. As the cells reached 80% confluence, cells were grown in DMEM containing 20% FCS supplemented with 5.6 mM glucose (normal glucose) or 25 mM glucose (high glucose) for 5 days. The medium was then changed to DMEM containing 0.2% FCS supplemented with 5.6 mM glucose or 25 mM glucose in the presence or absence of aspirin (0.1 mg/ml), and the cells were further incubated for 24 h.

Northern blot analysis

Total RNA from isolated glomeruli and cultured mesangial cells was extracted by the acid guanidinium-phenol-chloroform method, using Trizol reagent (Gibco BRL).[26,27] Northern blot analysis was performed as described,[26,27] using 30 μg of total RNA electrophoresed in a 1% agarose gel and transferred to Biodyne nylon membranes (Pall

BioSupport, Port Washington, NY, USA). Hybridization was performed at 42°C overnight with [^{32}P]dCTP-labeled cDNA probes for rat CTGF (nucleotides [nt] 1043–1617),[19] TGF-β1 (nt 1142–1546),[28] and fibronectin (nt 619–1082),[29] which were prepared by reverse-transcription polymerase chain reaction (PCR) using rat kidney mRNA. The membranes were washed at 55°C in 1 × standard saline citrate (SSC)/0.1% sodium dodecyl sulfate (SDS), and autoradiography was performed with a BAS-2500 (Fuji Photo Film, Tokyo, Japan) for 6 h. The filters were rehybridized with a human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA probe (Clontech, Palo Alto, CA, USA) for normalization.

Statistical analysis

Data values were expressed as means ± SE. Statistical analysis was performed using analysis of variance followed by Scheffe's test. A P value of less than 0.05 was considered statistically significant.

Results

General characteristics of experimental animals

Most rats injected with STZ developed diabetes, with blood glucose levels ranging from 350 mg/dl to 500 mg/dl, and exhibited a constantly high blood glucose level during the study period. The parameters measured during the induction of diabetes are summarized in Table 1. Body weight was lower in diabetic rats compared with nondiabetic control rats throughout the course. Diabetic rats exhibited a tendency to show renal hypertrophy compared with control rats, as demonstrated by a significantly increased kidney weight/body weight ratio from day 9 through day 84. Aspirin treatment did not significantly affect blood glucose or body weight changes, but attenuated the increase of the kidney weight/body weight ratio on day 84.

Urinary PGE2 excretion was increased by 3.5- to 4.5-fold in diabetic rats compared with nondiabetic controls (10.2 ± 0.6 and 24.9 ± 2.7 ng/day on days 28 and 84, respectively, in diabetic rats, versus 2.9 ± 0.5 and 5.5 ± 2.2 ng/day in controls; $n = 6$; $P < 0.01$), as reported in previous investigations.[21–24] Aspirin treatment abolished this increase (2.4 ± 0.3 and 4.6 ± 1.2 ng/day on days 28 and 84, respectively), suggesting that the dose used in this study was sufficient to correct the overproduction of PGE2 in diabetic rats.

Time courses of CTGF, TGF-β, and fibronectin expression in diabetic rat glomeruli

The upregulation of TGF-β expression in glomeruli is postulated to be critical in establishing the early lesions of diabetic nephropathy, leading to glomerulosclerosis.[4] To investigate the involvement of CTGF in the process of TGF-β-stimulated ECM accumulation in diabetic glomeruli, we analyzed the time-dependent expression of CTGF mRNA in the glomeruli isolated from control and diabetic rats, together with the expression of the TGF-β1 and fibronectin genes (Fig. 1). In diabetic rats, the glomerular expression of all three genes was significantly upregulated (Fig. 1B) compared with the expression in control rats (Fig. 1A). Quantitative analysis revealed that there was a difference in the time courses of induction among these three genes (Fig. 1C). TGF-β1 gene expression was significantly induced as early as 3 days after the onset of diabetes (1.5-fold of control; $n = 4$; $P < 0.05$) and reached a plateau on day 14. CTGF mRNA expression was also significantly upregulated from day 3 (1.4-fold of control; $n = 4$; $P < 0.05$) and peaked on day 28. On the other hand, fibronectin expression was not apparently increased on day 3, but was significantly upregulated on day 9 and peaked on day 28.

Table 1. Changes in the blood glucose level, body weight, and kidney weight in control nondiabetic and streptozotocin-induced diabetic rats

	Day 3	Day 9	Day 14	Day 28	Day 84
Blood glucose (mg/dl)					
Control	142 ± 35	151 ± 21	182 ± 3	200 ± 15	176 ± 17
Diabetes	403 ± 36**	430 ± 40**	438 ± 46**	475 ± 50**	556 ± 18**
Diabetes + aspirin	415 ± 30**	499 ± 11**	398 ± 60**	436 ± 37**	577 ± 20**
Body weight (g)					
Control	255 ± 5	310 ± 10	320 ± 10	462 ± 18	530 ± 36
Diabetes	234 ± 4	232 ± 11*	265 ± 16*	302 ± 10*	352 ± 19*
Diabetes + aspirin	–	–	242 ± 15*	279 ± 23*	388 ± 54*
Kidney weight (mg)					
Control	1.10 ± 0.03	1.20 ± 0.14	1.29 ± 0.06	1.46 ± 0.03	1.75 ± 0.05
Diabetes	1.03 ± 0.03	1.16 ± 0.09	1.42 ± 0.13*	1.58 ± 0.08	1.82 ± 0.08
Diabetes + aspirin	–	–	–	1.56 ± 0.18	1.97 ± 0.19
Kidney weight/body weight (mg/g)					
Control	4.29 ± 0.17	3.85 ± 0.31	4.01 ± 0.04	3.16 ± 0.11	3.34 ± 0.23
Diabetes	4.40 ± 0.14	5.08 ± 0.17*	5.55 ± 0.29*	5.81 ± 0.33*	5.21 ± 0.16*
Diabetes + aspirin	–	–	–	5.96 ± 0.74*	5.01 ± 0.56

* $P < 0.05$; ** $P < 0.01$ compared with nondiabetic controls

Values for blood glucose levels and the measurement on day 84 are expressed as means ± SE for control ($n = 6$), diabetes ($n = 10$), and diabetes plus aspirin ($n = 7$). For kidney and body weight measurement, each group contains $n = 4$, except for data on day 84

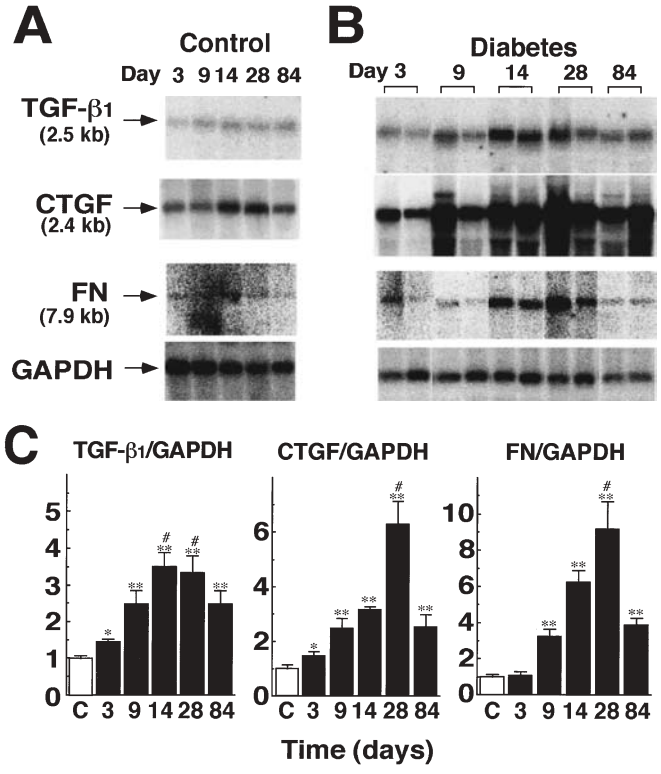

Fig. 1. A, B Representative Northern blots for transforming growth factor-β1 (*TGF-β1*), connective tissue growth factor (*CTGF*), and fibronectin (*FN*) mRNA expression in glomeruli isolated from control and streptozotocin-induced diabetic rats. **A** Each lane contains 30 μg of total RNA prepared from glomeruli of control rats. **B** Each lane contains 30 μg of total RNA from glomeruli of diabetic rats on day 3 to day 84 after induction of diabetes. **C** Quantitative analysis of glomerular expression of *TGF-β1*, *CTGF*, and fibronectin mRNA in diabetic rats normalized with glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*). Control levels (*C*, *open columns*) are averaged from day 3 to day 84 ($n = 10$). Each time point in diabetic rats (*closed columns*) has $n = 4$. Values are expressed as means ± SE. $^*P < 0.05$; $^{**}P < 0.01$ versus control; $\#P < 0.05$ versus days 3, 9, and 84

The degrees of augmentation above baseline levels were 3.5-, 6.2-, and 9.1-fold of the control levels for TGF-β1, CTGF, and fibronectin gene expression, respectively (Fig. 1C). The expression of all three genes appeared to be decreased on day 84, but was still significantly higher than the expression in control rats.

Renal histology and function in diabetic rats, and effect of aspirin

Histologically, clinical and experimental diabetic nephropathy is characterized by hypertrophic changes in all renal compartments, including an increase of both glomerular and renal size with mesangial matrix accumulation.[2–4] Examination by light microscopy on day 3 and on day 28 after the induction of diabetes showed no apparent changes in untreated diabetic or aspirin-treated diabetic rats as compared with controls (data not shown). Microscopic examination 12 weeks after the induction of diabetes, however, revealed a significant difference in glomerular histology among the three groups (Fig. 2). Untreated diabetic rats exhibited marked mesangial expansion with glomerular hypertrophy (Fig. 2B), compared with nondiabetic controls (Fig. 2A). Interestingly, chronic administration of aspirin in diabetic rats appeared to attenuate the mesangial matrix accumulation (Fig. 2C). Morphometric analysis revealed a significant decrease in the mesangial area at 12 weeks in aspirin-treated rats (Fig. 2D). The data clearly demonstrate that aspirin treatment alleviates the mesangial expansion.

There was a significant increase in blood urea nitrogen in diabetic rats compared with nondiabetic controls at 12 weeks (30.1 ± 2.3 versus 19.9 ± 1.0 mg/dl; $n = 6$; $P < 0.05$). Aspirin treatment significantly inhibited this increase (22.6 ± 1.4 mg/dl; $n = 6$; $P < 0.05$ versus untreated diabetic rats), suggesting prevention of renal impairment. Urinary protein excretion increased significantly in untreated diabetic rats compared with nondiabetic controls at 12 weeks (38.2 ± 5.1 vs 13.4 ± 1.4 mg/day; $n = 6$; $P < 0.01$); aspirin treatment tended to attenuate the proteinuria (30.0 ± 6.5 mg/day at 12 weeks; $n = 6$), but the level was still significantly higher than that in controls ($P < 0.05$).

Effect of aspirin on glomerular CTGF expression

Because the chronic aspirin administration ameliorated mesangial expansion in diabetic rats, we next investigated the effect of aspirin on glomerular expression of the CTGF gene, together with that of TGF-β1 and fibronectin. Treatment with aspirin for 4 weeks almost completely suppressed the induction of CTGF mRNA expression, as well as inhibiting the upregulation of TGF-β1 and fibronectin expression (Fig. 3A,B). Such inhibitory effects of aspirin were still significant at 12 weeks (Fig. 3).

Effects of high glucose and aspirin on CTGF expression in cultured mesangial cells

Previous reports have shown that high glucose induces TGF-β and CTGF expression in mesangial cells.[8,16,17] To explore the mechanisms underlying the beneficial effects of aspirin on glomerular histology and gene expression, we examined its effects on high glucose-induced gene activation in cultured mesangial cells. High-glucose conditions significantly augmented CTGF mRNA expression, together with TGF-β1 and fibronectin expression (1.7-, 1.8-, and 1.8-fold of each control; $n = 4$; $P < 0.02$) (Fig. 4A,B). Such upregulation of all three genes was effectively abolished by the addition of aspirin ($P < 0.05$; Fig. 4).

Discussion

Accumulating evidence has indicated a close relationship between the increased expression of TGF-β and the development and progression of diabetic glomerulosclerosis.[3,4,7–9] Indeed, the blocking of TGF-β1 with neutralizing antiserum or antisense oligonucleotide effectively suppresses the ma-

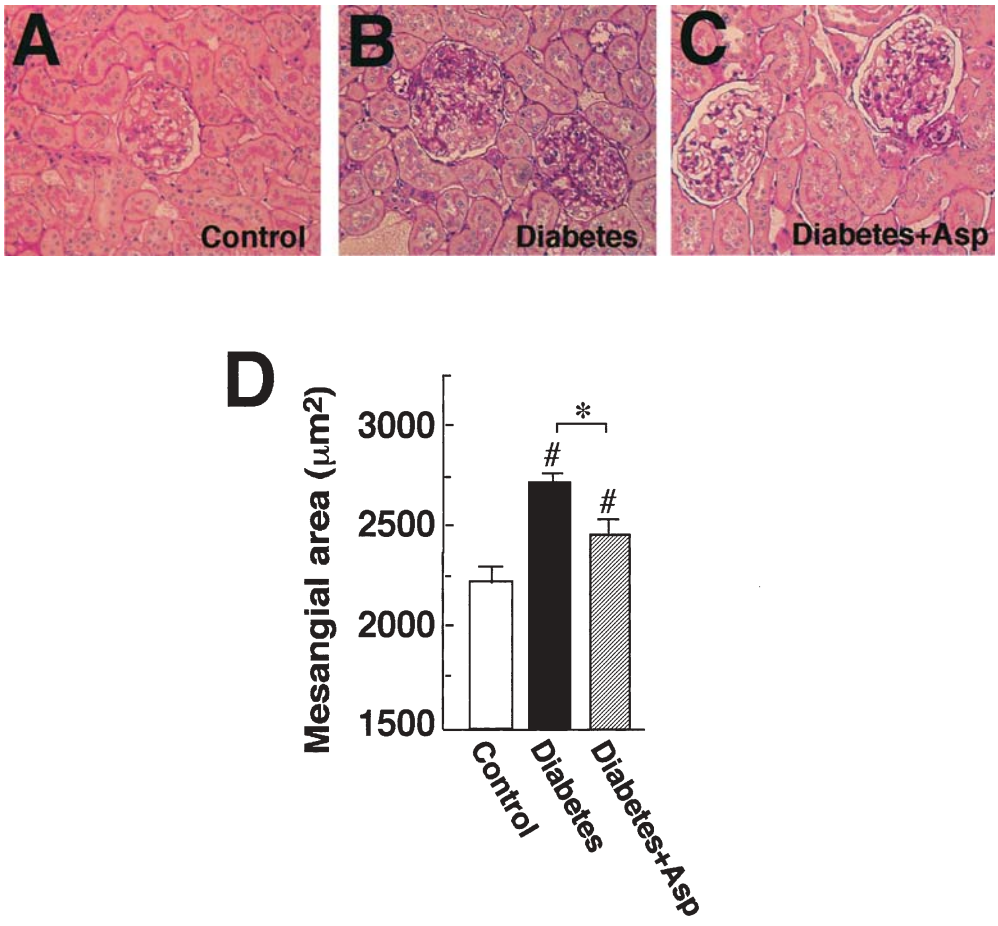

Fig. 2. **A–C** Glomerular histology of control and diabetic rats at 12 weeks after induction of diabetes. **A** Control; **B** diabetes; **C** diabetes plus aspirin (*Asp*). **D** Glomerular mesangial area in control ($n = 6$), diabetic ($n = 10$), and aspirin-treated diabetic rats ($n = 7$) at 12 weeks. Values are expressed as means ± SE. #$P < 0.05$ versus control; *$P < 0.05$. **A–C** Periodic acid Schiff (PAS), ×400

trix protein accumulation and mesangial expansion in experimental diabetic nephropathy models.[30–32] These studies have provided plausible evidence for TGF-β as a potential therapeutic target against diabetic nephropathy. An important caveat for this strategy, however, is that the systemic longterm suppression of TGF-β, a multifunctional cytokine, might be detrimental because of its antiproliferative and anti-inflammatory effects. In fact, TGF-β1-null mice exhibit excessive inflammation with tissue necrosis in specific organs, finally leading to organ failure and death.[33] Therefore, to design anti-fibrotic strategies in diabetic nephropathy, it is important to elucidate the mechanisms of the local upregulation of TGF-β, as well as the downstream pathways specific to the profibrotic actions of TGF-β.

In the present study, we have shown that CTGF may be a likely mediator of TGF-β-stimulated fibronectin induction in a rat model of diabetic nephropathy and cultured mesangial cells. CTGF expression was coordinately upregulated with TGF-β1 in diabetic rat glomeruli from an early stage of diabetic nephropathy, followed by a marked increase of fibronectin expression (Fig. 1). These induction patterns suggest that CTGF may be located downstream of TGF-β1, but upstream of fibronectin in the process of TGF-β-stimulated ECM accumulation during the progression of diabetic nephropathy. This observation is compatible with the different time courses for the induction of the TGF-β1, CTGF, and fibronectin genes noted in obstructive nephropathy in rats.[19] In addition, there was a parallel induction of TGF-β1, CTGF, and fibronectin genes by high glucose in vitro (Fig. 4). Moreover, we and others recently demonstrated that TGF-β1-stimulated induction of fibronectin and collagen genes in cultured renal fibroblasts was markedly inhibited by treatment with antisense oligonucleotides or antibodies against CTGF.[19,34] Together with the potent profibrotic property of CTGF, it is conceivable that CTGF mediates TGF-β-dependent ECM induction in diabetic glomeruli as well.

CTGF expression is upregulated in various renal diseases characterized by fibrosis.[15–19,35] Increased CTGF expression has also been shown in a variety of human and experimental fibrotic diseases, including those of the skin, blood vessels, lung, and liver.[18] Whether CTGF plays a role in vivo in the progression of fibrosis in these disease states still remains to be elucidated and obviously requires further clarification. Whether CTGF upregulation in these conditions is TGF-β-dependent is another issue to be clarified. Of note, it has been reported that dexamethasone potently induces CTGF while suppressing TGF-β, suggesting the presence of a TGF-β-independent pathway of CTGF activation and also the possible involvement of CTGF in the profibrotic actions of glucocorticoids.[36] Besides stimulating fibrogenesis, CTGF plays a role in various biological actions, including endothelial cell migration and proliferation, angiogenesis, and vascular smooth muscle cell apoptosis, thereby potentially participating in tissue remodeling in various disease states.[18]

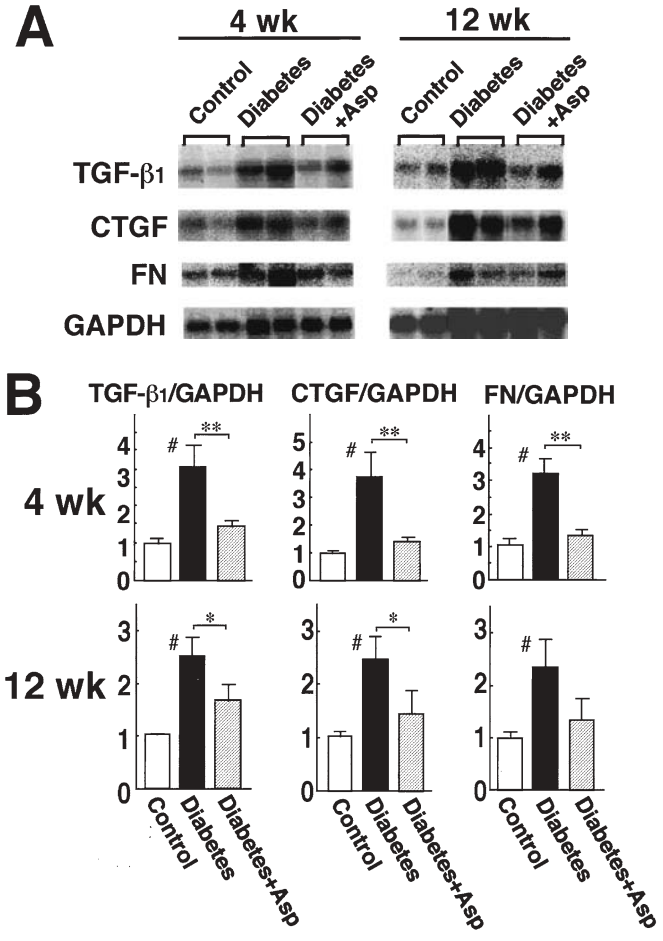

Fig. 3. **A** Representative Northern blots for *TGF-β1*, *CTGF*, and fibronectin (*FN*) mRNA expression and **B** their quantitative analysis in glomeruli isolated from control and diabetic rats at 4 and 12 weeks after induction of diabetes. Values are expressed as means ± SE for control, diabetic, and aspirin-treated diabetic rats. $n = 6$; #$P < 0.01$ versus control; *$P < 0.05$, **$P < 0.01$. *wk*, weeks

CTGF expression has been shown to be attenuated by treatment with PGE2 as well as cyclic adenosine monophosphate (cAMP) in cultured lung and kidney fibroblasts.[20,34] On the other hand, a number of previous studies have indicated that PGE2 overproduced in diabetic glomeruli has a role in enhancing mesangial proliferation and matrix accumulation in vitro,[21–24,27,37] suggesting a potential contribution to glomerular injury in diabetic states. In order to clarify the involvement of prostanoids in the development of glomerular injury and the regulation of TGF-β and CTGF expression in vivo, we performed chronic aspirin treatment in diabetic rats. We found a significant attenuation of mesangial expansion (Fig. 2D), as well as the inhibited upregulation of the glomerular expression of the CTGF, TGF-β1, and fibronectin genes by aspirin administration (Fig. 3). These results suggest that the ameliorated mesangial expansion at 12 weeks in diabetic rats treated with aspirin was caused by the persistent inhibition of TGF-β1 and CTGF upregulation in diabetic glomeruli. Furthermore, aspirin treatment also effectively abolished the high glucose-induced upregulation of these genes in cultured mesangial cells (Fig. 4). Therefore, these findings suggest that the high glucose-induced increase of prostanoid production in mesangial cells activates the TGF-β1/CTGF/fibronectin cascade in an autocrine/paracrine manner, and that aspirin suppresses this cascade, probably by inhibiting TGF-β1 upregulation. Taken together, these results strongly suggest that prostanoids overproduced in vitro and in vivo have a potential role in mesangial matrix accumulation by activating the TGF-β1/CTGF/ECM cascade, and that the mesangium is an important site of the production and action of prostanoids in this aggravation mechanism. Although aspirin treatment clearly inhibited CTGF, TGF-β1, and fibronectin upregulation from 4 weeks, it exerted only marginal effects on renal hypertrophy (Table 1) or proteinuria at 12 weeks. The reason for this is not clear at present, but it might be due to aspirin having less efficient hemodynamic corrective effects than its effects in matrix gene activation. Interestingly, such effects of aspirin may be similar to those exerted by anti-TGF-β1 antibody treat-

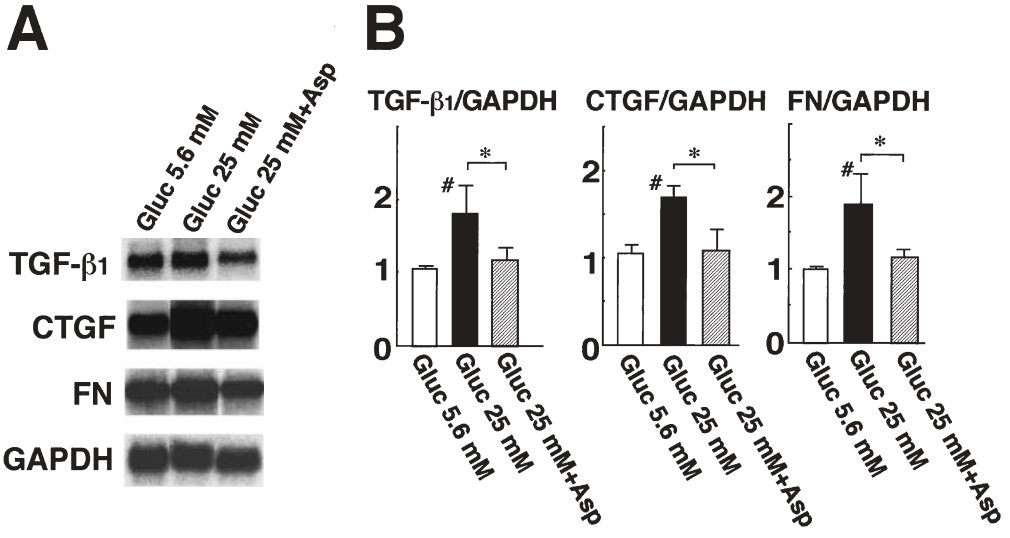

Fig. 4. **A** Representative Northern blots for *TGF-β1*, *CTGF*, and fibronectin (*FN*) mRNA expression and **B** their quantitative analysis in cultured rat mesangial cells. Values are expressed as means ± SE for low glucose (*gluc*; 5.6 mM), high glucose (25 mM), and high glucose plus 0.1 mg/ml aspirin. $n = 4$; #$P < 0.02$ versus low-glucose control; *$P < 0.05$

ment, in which antibody-treated mice showed significantly ameliorated mesangial expansion and renal dysfunction, but still exhibited glomerular hypertrophy and proteinuria.[31]

We have already demonstrated that increased production of PGE2, acting on the E-type prostanoid (EP)1 receptor in an autocrine/paracrine fashion, is responsible for enhanced mesangial proliferation by high glucose in vitro.[27] We further revealed that such growth-promoting action of PGE2 under high-glucose conditions was, at least in part, caused by a marked attenuation of cAMP production via the EP4 receptor, occurring in a protein kinase C (PKC)-dependent manner.[27] If this were true in vivo, enhanced PGE2 in diabetic glomeruli might stimulate CTGF expression, because cAMP signaling that mediates CTGF inhibition is greatly blunted. Koya et al.[38] reported the importance of PKC β-mediated PGE2 production in stimulating matrix accumulation in diabetic glomeruli. The pathogenic role of PKC in matrix gene activation, hemodynamic abnormalities, and proteinuria has been shown in diabetic nephropathy models using a PKC β inhibitor.[39,40] Although the precise mechanisms by which prostanoids aggravate matrix gene activation in diabetic states should be further investigated, these findings suggest the therapeutic potential of agents that inhibit the prostanoid pathway against the progression of diabetic nephropathy.

In summary, these results indicate that CTGF expressed in the glomeruli is upregulated in the early stage of STZ-induced diabetic nephropathy in rats, and could be a critical mediator of the development of diabetic glomerulosclerosis. In addition, the modulatory effects of aspirin during this process suggest a role of the cyclooxygenase pathway in the progression of diabetic nephropathy. Whether treatment that modulates this pathway represents a new therapeutic option for patients with diabetic nephropathy remains to be established in further studies.

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