Overexpression of Transforming Growth Factor-β1 mRNA Is Associated With Up-Regulation of Glomerular Tenascin and Laminin Gene Expression in Nonobese Diabetic Mice

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ABSTRACT

Nonobese diabetic (NOD) mice spontaneously develop immune-mediated insulin-dependent diabetes mellitus and nephropathy, providing an opportunity to study the early molecular events in a model of diabetic glomerulosclerosis. The expression of several genes coding for growth factors and extracellular matrix was examined in microdissected glomeruli, by the use of reverse transcription-competitive polymerase chain reaction, in diabetic NOD mice (mean duration of diabetes, 28.5 ± 7 days) and agematched nondiabetic NOD mice with normal glucose tolerance. The levels of mRNA coding for transforming growth factor-β1, tenascin, and laminin B1 increased 1.9-, 2.0-, and 1.7-fold, respectively, whereas platelet-derived growth factor (PDGF)-B, α1(IV) collagen, 72-kd collagenase, α-smooth muscle actin, and β-actin mRNA remained stable in the diabetic mice. The kidney advanced glycosylation end-products levels increased 2.1-fold in the diabetic mice, and the diabetic glomeruli showed an accumulation of tenascin and laminin but not of type IV collagen by immunofluorescence microscopy. There was no increase in cell number per glomerulus after the onset of diabetes, a finding consistent with stable PDGF-B and α-smooth muscle actin mRNA levels. These findings provide evidence that increased glomerular transforming growth factor-β1, but not PDGF-B, mRNA is associated with the up-regulation of tenascin and laminin expression after advanced glycosylation endproduct accumulation, early after the onset of diabetes.

Key Words: Glomerulosclerosis, competitive polymerase chain reaction, extracellular matrix, growth factor, advanced glycosylation endproducts

The kidney disease of diabetes mellitus (KDDM) is a major cause of ESRD in the United States (1). Diabetes-induced alterations in the rate of the synthesis and/or degradation of extracellular matrix (ECM) and in growth factors are believed to play a role in the development of the glomerulosclerosis characterizing KDDM. Although alterations of collagen, laminin, fibronectin, heparan sulfate proteoglycan, and other matrix proteins have been extensively studied (2), there are conflicting data concerning the sequence of pathologic events in diabetic glomeruli in experimental animals.

Most animal models of diabetes use streptozotocin (STZ) to induce β cell destruction and islet inflammation (3). Given the fact that the glomerular lesions in STZ rats do not faithfully mimic those in humans, an animal model of spontaneous diabetes could be useful in the study of diabetic glomerulosclerosis (4).

Nonobese diabetic (NOD) mice spontaneously develop immune-mediated insulin-dependent diabetes mellitus (IDDM) (5). Autoimmune insulitis begins at 4 wk of age, and by 30 wk, 92% of the female and 66% of the male mice develop diabetes (6). This model mimics human IDDM in the genetic predisposition to the development of spontaneous diabetes, the absolute requirement of the insulin therapy, and the propensity to develop KDDM. We previously reported that NOD mice developed glomerular hypertrophy, as well as a mild glomerulosclerosis and an increased urinary albumin/creatinine excretion rate after a short period of diabetes mellitus (7). After 8 wk of diabetes, the peripheral glomerular basement membranes were thickened by quantitative electron microscopy. Thus, this inbred strain provides a unique opportunity to study the molecular events associated with glomeru-
In situ, were studied, and age-matched nondiabetic NOD mice TGF-β1 mRNA in association with the up-regulation of This was completed by light and immunofluorescence microscopy. We found an increase in glomerular TGF-β1 mRNA in association with the up-regulation of tenasin and laminin B1 expression in the glomeruli of NOD mice 4 wk after the spontaneous onset of diabetes, whereas the expression of the other mRNA tested remained stable.

METHODS

Mice

Female NOD mice, bred in the Joslin Diabetic Center, that developed spontaneous diabetes (N = 6; mean age, 24 ± 2.8 wk) were studied, and age-matched nondiabetic NOD mice (N = 5; mean age, 25.3 ± 0.5 wk) served as controls. Mice were fed ad libitum. No marked difference of food consumption was noted between diabetic and nondiabetic mice. The duration of diabetes was 28.5 ± 7 days. Nondiabetic NOD mice were screened by glucose tolerance testing to exclude animals with impaired glucose tolerance (7). The urine of all mice was checked twice per week, and overt diabetes was defined as the first appearance of glucosuria and persistent hyperglycemia of 1-wk duration (blood glucose of more than 14 mmol/L). Regular insulin and NPH (1:1) were administered sc in the morning and evening, according to the level of glucosuria (7). The average insulin dosage was 0.7 to 1.5 U/day, and blood glucose levels were maintained at 14 to 22 mmol/L. Hemoglobin A1c levels, measured in a series of mice receiving an identical treatment regimen, were 6.0 ± 0.3% in diabetic mice (N = 8; mean age, 21.4 ± 1.7 wk; duration of diabetes, 27.9 ± 1.1 days) and 3.7 ± 0.2% in nondiabetic mice (N = 5; mean age, 31.4 ± 1.1 wk).

Glomerular Microdissection and Reverse Transcription In Situ

After anesthesia with sodium pentobarbital (50 μg/g body wt, ip), mice were euthanized by decapitation. The left kidneys were perfused with saline containing collagenase and RNAse inhibitors. Glomeruli were isolated by microdissection, permeabilized, and sonicated, and cDNA was obtained after reverse transcription in situ as described previously (8). The right kidneys were saved for histologic examination.

Primer Construction and Competitive PCR

Primers. The mouse α1(IV) collagen primers were as described previously (8). The size of the corresponding amplified product was 484 base pairs (bp). The 72-kd collagenase primer pair was sense 5'-CTT TGC AGGA CAA GTAC TCG G-3' and antisense 5'-TTA AAG TGG TGC AGT TAT CCG G-3' to yield an expected PCR product of 701 bp. The tenasin primer pair was sense 5'-TAC GAG AGA TTC AGT GGT GG-3' and antisense 5'-TTG TAG ACT GTT GTC TTT GG-3' and yielded a 548-bp PCR product. The primer pair for laminin B1 was sense 5'-CAA ATC TGT GTA CGA AAG CTG GG-3' and antisense 5'-TTA TCT TGG TCA CCG AGC-3' and yielded a 443-bp PCR product. The TGF-β1 primer pair was sense 5'-ATA CAG GCC GTC TGA TTC AGC-3' and antisense 5'-GTC CAG CGT CCA AAT ATA GG-3' and yielded a 360-bp PCR product. The PDGF-B primer pair was sense 5'-TGG GAG ACA GTA ATG ACC-3' and antisense 5'-TTG GAG TCA AGA GAA GAC-3' and yielded a 435-bp PCR product. The β-actin primer pair was sense 5'-TCT AGG CAC CAA GGT GTG-3' and antisense 5'-TCA TGA GTG ATG CCG TCA GG-3' and yielded a 460-bp PCR product. The α1(II) collagen primer pair was sense 5'-TGG TCC CTC TGG AAA TGC TGG ACC-3' and antisense 5'-CAG GAG AAC CAG GAG AAC CAG G-3' to yield a 297-bp PCR product.

These primers were chosen so that only specific mRNA-derived cDNA sequences but not genomic DNA sequences would be amplified. Restriction enzyme analysis was performed on each of the PCR products to confirm the amplification of specific mRNA sequences.

Competitive PCR Assay. The mutated cDNA templates competed with test cDNA on an equimolar basis, as previously described (8-10). For α1(IV) collagen, a point mutation constructed to yield a new BclI restriction site resulted in two fragments of 219 and 265 bp. For 72-kd collagenase, a new EcoRI restriction site resulted in two fragments of 342 and 359 bp. For laminin, laminin B1, TGF-β1, PDGF-B, α-SMA, and β-actins, deletion cDNA mutant templates were developed to create 62-, 57-, 58-, 55-, 84-, and 103-bp deletions in the middle of the molecules, resulting in mutant cDNAs of 486, 386, 282, 380, 350, and 357 bp respectively.

To quantitate cDNA, competitive PCR assays were performed by the addition of decreasing amounts of mutant templates to glomerular cDNA. The test template for all PCR was an aliquot of cDNA collected from a pool of 50 glomeruli. Each aliquot corresponded to 1/50 of a glomerulus. After agarose gel electrophoresis, amplification bands stained by ethidium bromide were quantitated from the film negative by scanning laser densitometry (PDI Imageware Systems, Huntington Station, NY). As reported, the ratio of mutant to wild-type band density was calculated for each lane and plotted as a function of the amount of initial mutant template added to the reaction. The amount of glomerular cDNA was derived from linear regression analysis (8-10) with duplicate or triplicate assays. The mean values for diabetic mice are expressed as a percentage of those for nondiabetic mice.

Light Microscopy

Coronal kidney sections were fixed in Carney's fixative and embedded in glycol methacrylate. Four-micrometer sections were stained with hematoxylin/eosin, periodic acid–Schiff, and periodic acid–Schiff silver methenamine and were examined without knowledge of the experimental groups.

Immunofluorescence Microscopy

Frozen or paraffin-embedded kidney sections were used. Paraffin sections were deparaffinized and lightly trypsinized at 37°C; sections were coated with fluorescein isothiocyanate–conjugated monoclonal anti-MAC-1 (Boehringer Mannheim, Indianapolis, IN) or biotin-conjugated goat anti-rat immunoglobulin (IgG), anti-mouse IgM (Tago Inc. Burlinger, Indiana). Glomerular and tubular fluorescence is observed (Fig. 1). Control reactions included negative controls and avidin–biotin complex, with similar glomerular and tubular staining to the primary antibody.
ingame, CA), or rabbit anti-TGF-β1 (a generous gift from Dr. K. Flanders, NIH, Bethesda, MD); rabbit anti-mouse type IV collagen (Biosdesign, Kennebunkport, ME); rabbit anti-rat laminin (Chemicon International Inc, Temecula, CA); rabbit anti-human tenascin (Gibco BRL, Gaithersburg, MD) followed by biotin conjugated goat anti-rabbit IgG (Tago Inc., Burlingame, CA); and streptavidin-conjugated fluorescein isothiocyanate (Zymed Laboratories, Inc., San Francisco, CA). The sections were coded and examined, and the fluorescence intensity was graded on a 0 to 4+ scale as described previously (11).

Measurement of Advanced Glycosylation Endproducts in the Kidney

Total kidney advanced glycosylation endproduct (AGE) levels were measured in one series of mice. Three 16-wk-old female diabetic NOD mice (duration of diabetes, 14 ± 1 days) and three female age-matched nondiabetic mice were examined. The kidneys from each group were pooled, homogenized, delipidated, and digested with collagenase as previously described (12). Total kidney AGE was measured by enzyme-linked immunosorbent assay (ELISA), and collagen content was determined as described previously (12). Kidney AGE levels were expressed as units per milligram of hydroxyproline.

Glomerular Volume

The profile areas of 100 glomeruli were measured with a computer-assisted planimeter for morphometric analysis as described previously (11). The mean glomerular volume was derived from the harmonic mean of the glomerular equatorial surface area (11).

Mean Glomerular Cell Number

The mean glomerular cell number was determined as described previously (13). Briefly, the nuclei of 50 successive glomerular profiles were counted by the scanning of hematoxylin/eosin-stained tissue sections in a serpentine fashion. Taking into account the mean cell number per glomerular profile (C), the simple mean of the glomerular equatorial area (A), and glomerular volume (GV), the mean glomerular cell number (N) was derived by the following equation.

\[ N = \frac{C}{A} \times GV \]

The means of these values in diabetic NOD mice were expressed as a percentage of the mean values in nondiabetic NOD mice.

Statistical Methods

Differences of cDNA levels between groups were analyzed with the unpaired t test. The Mann-Whitney nonparametric test was used to analyze differences of immunofluorescence scores between groups. All values are expressed as means ± SE.

RESULTS

Kidney Weight and Mean Glomerular Volume

Kidney weight and mean glomerular volume were significantly increased in the diabetic as compared with the nondiabetic mice (kidney weight, 209 ± 9 versus 175 ± 6 mg; P < 0.05; mean glomerular volume, 3.47 ± 0.18 versus 2.66 ± 0.14 \times 10^5 \mu m^3; P < 0.01).

Quantification of Glomerular mRNA

The mean glomerular tenascin mRNA level was increased 2.0-fold in diabetic mice (200.0 ± 25.5 versus 100.0 ± 6.9%; P < 0.01) (Figure 1A), and the laminin B1 mRNA level was increased 1.7-fold in diabetic mice (168.8 ± 21.8 versus 100.0 ± 8.4%; P < 0.05) (Figure 1B). The glomerular TGF-β1 mRNA level was increased 1.9-fold in diabetic mice (190.8 ± 28.9 versus 100.0 ± 16.6%; P < 0.05) (Figure 2A). In contrast, there were no statistically significant differences in the glomerular levels of PDGF-B (104.0 ± 17.2 versus 100.0 ± 16.58%; P = 0.900) (Figure 2B), α1(IV) collagen (93.8 ± 7.8 versus 100.0 ± 8.7%; P = 0.375) (Figure 3A), 72-kd collagenase (109.9 ± 23.6 versus 100.0 ± 17.8%; P = 0.706) (Figure 3B), α-SMA (143.6 ± 18 versus 100.0 ± 13.4%; P = 0.096) (Figure 4A), and β-actin mRNA (123.9 ± 30.2 versus 100.0 ± 25.8%; P = 0.567) (Figure 4B) between diabetic and nondiabetic mice. α1(I) collagen mRNA was not detected in the glomeruli from any of the groups, despite increasing the number of PCR cycles to 40.

Light and Immunofluorescence Microscopy

Tenasin accumulated in large amounts in the mesangium of diabetic, but not in nondiabetic, mouse glomeruli (mean score, 3.4 ± 0.2 versus 0.9 ± 0.0; P < 0.005). An increased amount of laminin was found in the mesangium and peripheral basement membranes in the diabetic mouse glomeruli compared with the nondiabetic (mean score, 3.3 ± 0.4 versus 2.6 ± 0.1; P < 0.01). The type IV collagen staining pattern and intensity were similar in the diabetic and nondiabetic mice (mean score, 2.3 ± 0.1 versus 2.4 ± 0.1; P = 0.561) (Figure 5). There was faint TGF-β1 immunostaining in the mesangium, which did not appear to differ in the two groups. Only a few MAC-1-positive cells were found in either diabetic or nondiabetic glomeruli (zero to one MAC-1-positive cells in 20 glomeruli of each section examined). We confirmed our previous findings of an increase in IgG and IgM in the diabetic glomeruli (7). Apart from the increase in

\[ \frac{C}{A} \times GV \]
size, no obvious glomerular changes were detected by light microscopy in the diabetic mice (data not shown).

Mean Glomerular Cell Number

There were no statistically significant differences in mean glomerular cell number found between diabetic and nondiabetic mice (104.7 ± 6.4 versus 100.0 ± 4.2%; \(P = 0.58\)).

Measurement of Kidney Age Levels

Total kidney AGE levels measured by enzyme-linked immunosorbent assay in pooled extracts of kidneys showed a 2.1-fold increase in the diabetic mice (diabetic, 38.9 U/mg of hydroxyproline; nondiabetic, 18.5 U/mg of hydroxyproline).

**DISCUSSION**

We took advantage of the increased sensitivity and reproducibility of competitive PCR in microdissected glomeruli to examine growth factors and ECM gene expression in NOD mice early after the onset of diabetes. There were a 1.9-, 2.0-, and 1.7-fold increases of TGF-\(\beta\)1, tenascin, and laminin B1 mRNA in the glomeruli of diabetic NOD mice. This up-regulation was not due to an increased cell number in the glomeruli or to infiltrating macrophages. PDGF-B, \(\alpha\)1(IV) collagen, and 72-kd collagenase mRNA were unchanged, and \(\alpha\)1(1) collagen mRNA remained undetectable. Although there was no obvious difference by light microscopy, except for the enlarged glomeruli in the diabetic mice, tenascin accumulated in the glomerular mesangium and laminin was found increased both in the mesangium and in the peripheral glomerular basement membranes in diabetic mice by immunofluorescence microscopy. We did not perform a long-term study because of the difficulties in maintaining dia-
abetic NOD mice because of their absolute requirement for insulin and the presence of a brittle diabetes.

The changes in ECM components have been the subject of multiple studies in both human and experimental diabetes. Alterations of ECM components may reflect structural and/or functional changes of the glomerulus induced by hyperglycemia or AGE (14) in vitro. Among others, tenasin, a recently described matrix component, has been found to be increased in glomerulosclerosis (15). Tenasin is a large, multisubunit glycoprotein containing domains with fibronectin type III repeats that have RGD-like sequences and domains with epidermal growth factor-like sequences. Because it also has antiadhesive activity (16), it has been implicated in the modulation of cell-cell interaction, cell-matrix adhesion, migration, and growth (17–20) and as an autocrine growth factor (21). Although it is expressed at very low levels in some normal adult tissues, tenasin expression is increased in actively dividing mesenchymal cells during morphogenesis, wound healing, and tumorigenesis (15,22–24). In the kidney, tenasin is expressed in the mesenchymal/epithelial interface during organogenesis (25) and is a component of normal mesangial matrix. It is upregulated in expanded ECM, regardless of the underlying pathologic process (15). Although tenasin accumulation was observed in experimental diabetic animals (26) and in advanced human diabetic kidneys (15), its expression relative to the expression of growth factors or ECM components in early diabetes has not been examined. In this study, we found that tenasin and laminin were up-regulated both at the message and at the protein levels, whereas some ECM components were unchanged. This increase suggests that tenasin is one of the major components of diabetic glomerulosclerosis in NOD mice.

On the basis of our findings that tenasin expression was restricted to the glomerular mesangium and vessel walls, the smooth muscle mesangial cells could be the main source of this product in the glomerulus. Given the fact that vascular smooth muscle cells synthesize an excess of tenasin in hypertensive state in response to angiotensin II (27,28), part of the observed changes in diabetic NOD mice may represent a response of smooth muscle–like mesangial cells to hemodynamic and/or metabolic alterations of diabetes mellitus.

Laminin is a major component of noncollagenous glycoproteins in the glomerular mesangium and the peripheral basement membrane. We found that the glomerular levels of laminin mRNA increased shortly after the onset of diabetes in NOD mice and that the protein accumulated in both the mesangium and the peripheral glomerular basement membrane. This result is consistent with data reported in experimental diabetes models. For instance, in STZ-induced diabetes in rats, laminin mRNA was found to be increased in total kidney preparations 2 wk after the onset of diabetes in one study (29) and between 11 and 28 wk after the onset of diabetes in another (30). In the same model, laminin mRNA was increased in glomeruli 4 wk after the onset of diabetes (31). In contrast, however, in obese KKAY mice, a model of spontaneous non-insulin-dependent diabetes, total kidney laminin B1 mRNA levels did not differ from those of age-matched nondiabetic mice at 4 and 6 months (32).

Although interstitial collagen (Type I) has been observed in advanced human diabetic glomerular lesions (33), it was not found in this study, possibly because of the early phase of the lesions. Type IV collagen has been shown to accumulate in the glomerular basement membrane and mesangium of human diabetes with advanced nephropathy (34) and in diabetic animals (35) by light or electron microscopic immunohistochemistry. The data established concerning Type I and Type IV collagen expression in experimental animals are contradictory. Ihm et al. (36) found that glomerular α1(IV) and α1(I) collagen mRNA levels remained unchanged in the STZ-diabetic rat at 7 and 28 days. In contrast, Fukui et al. (31) reported an increase in glomerular α1(IV) as well as in α1(I) collagen in a study of STZ-diabetic rats 4 wk after the onset of diabetes. When total kidney was studied, Ihm et al. (36) observed an increase of α1(IV) collagen mRNA at 7 days, but not at 28 days, in STZ diabetic rats. This change was localized to proximal tubules by in situ hybridization. In another study of whole-kidney extracts, Kilen et al. (29) found an increase in α1(IV) collagen mRNA in the STZ diabetic rat 2 wk after the onset of diabetes. In their study of the kidney of obese KKAY mice, Ledbetter et al. (32) showed an increased cortical level of α1(IV) collagen mRNA at 4 and 6 months of age (twofold and fourfold, respectively). In contrast, Poulsom et al. (30) found that whole-kidney α1(IV) collagen mRNA levels were lower in STZ diabetic rats than in control animals between 2 and 11 wk of diabetes, and reached the control levels thereafter.

These diverse and conflicting results in the literature may reflect variations among diabetic models, between species, or between IDDM and non-insulin-dependent diabetes, as well as the level of glycemic control attained. Insulin has been reported to induce (37) or decrease collagen synthesis (31) in vitro and in vivo. Strict glycemic control with insulin can prevent long-term diabetic complications. For instance, it has been shown that intensive insulin treatment reduces the risk of diabetic nephropathy in humans (38) and decreases the accumulation of Type IV collagen in the glomerular mesangium in STZ diabetic rats (35). Furthermore, insulin treatment prevented the increase of glomerular α1(IV) collagen, laminin B1, and α1(I) collagen mRNA in STZ diabetic rats (31). Interestingly, the increase in α1(IV) collagen mRNA in the kidney of STZ diabetic rats could be completely abrogated by low doses of insulin that were insufficient to normalize renal size and serum glucose (36). Those findings suggested that insulin could play a specific role in preventing Type IV collagen accumulation. The glomerular changes noted herein occurred in mice treated with an insulin regimen that did not normalize
the blood glucose. Because NOD mice have brittle diabetes and require insulin therapy for survival, we were not able to examine the mice without insulin treatment.

The 72-kd collagenase mRNA, coding for the major Type IV collagen degradative enzyme, was identical in nondiabetic and diabetic mice. We have not explored other components of the degradative enzyme or the enzyme activity.

Both TGF-β1 and PDGF have been implicated in the pathogenesis of glomerulosclerosis. TGF-β1 is a peptide with multiple functions and a broad spectrum of cellular targets and actions, including the regulation of ECM homeostasis. Disturbances of ECM regulation characterize all sclerotic diseases, and TGF-β1 has been shown to be increased when there is an accumulation of ECM (39). The induction of TGF-β1 mRNA found here suggests that this peptide may participate locally in the early response of the glomerulus to the diabetic milieu. As reported earlier, TGF-β1 inhibited mesangial cell proliferation when they were not confluent but stimulated overall protein and collagen production. Increased cell size was found in response to chronic TGF-β1 treatment (40). Furthermore, the addition of TGF-β1 to glomerular epithelial cells in vitro induces ECM synthesis (41) and induces tenascin expression in fibroblasts (42). The concomitant increase of TGF-β1 mRNA and of tenascin and laminin induced by diabetes suggests that the increase in TGF-β1 mRNA may mediate the changes in ECM gene expression as well as the glomerular hypertrophy.

In this study, because of the low intensity in the immunostaining of TGF-β1 in glomeruli, we could not distinguish diabetic from nondiabetic mice, even though mRNA levels were increased in the former. We believe that this is not only because of the semiquantitative, less sensitive nature of the immunohistochemistry but also because of the low amount of growth factor detectable in the glomeruli when sclerosis is mild. This might be different for glomerular ECM proteins, which are present in large amounts. On the other hand, the staining of TGF-β1 in diabetic glomeruli was intense when glomerulosclerosis was conspicuous (26). Because the inhibition of TGF-β1 action in animal models ameliorates matrix accumulation (43), the regulation of TGF-β1 in early diabetes may be a fruitful area of future investigation for the prevention of glomerulosclerosis in human diabetic nephropathy.

PDGF-B, a growth factor implicated in glomerular cell proliferation, was not increased in diabetic NOD mice. Increased PDGF-B has been observed in proliferative glomerulonephritis (44) and in the remnant kidney model before glomerulosclerosis occurs (45). In contrast to our own observations in NOD mice, both TGF-β1 and PDGF-B mRNA were found to be increased after 4 wk of diabetes in the STZ diabetic rat (46). Our data show that the autocrine synthesis of PDGF-B is not increased in diabetic NOD glomeruli, but this does not exclude a role for exogenous PDGF in the diabetic glomerulus.

AGE accumulates in diabetic patients because hyperglycemia induces the nonenzymatic glycosylation of proteins and has been implicated in diabetic complications, including nephropathy (47). AGE induced increased expression of ECM genes in mesangial cells (14), and the injection of AGE to normal mice up-regulated the expression of glomerular TGF-β1 and several ECM mRNA (48). In this study, there was a 2.1-fold increase in AGE in the kidney of the diabetic mice. Therefore, there may be a causal link between the increase of kidney AGE and the up-regulation of mRNA encoding for TGF-β1 and ECM observed in this model.

Dietary protein restriction attenuates the increased expression of TGF-β1 both in puromycin aminonucleoside–induced glomerulosclerosis (49) and in mesangial proliferative glomerulonephritis (50) in rats. In this study, we did not measure protein or caloric intake; therefore, it is not clear whether the increased TGF-β1 mRNA was the result of possible increased protein intake in the diabetic state or the result of the metabolic consequences of hyperglycemia, i.e., AGE accumulation. It would be of considerable interest to study the effect of dietary protein restriction on TGF-β1 regulation in this model.

As we reported earlier, IgG and IgM accumulated in the glomeruli of diabetic NOD mice (7), which was confirmed in this study. The deposition of immunoglobulins is a component of diabetic microangiopathy (51). The presence of immunoglobulins in diabetic glomeruli in vivo (52) and the increased binding of immunoglobulin to glycosylated human glomerular basement membrane in vitro (53) may suggest a role for increased AGEs as one of the mechanisms accounting for the deposition of immunoglobulins in the glomeruli.

The level of α-SMA mRNA, a marker of mesangial cell activation (54), was not significantly elevated. The stable levels of PDGF-B and α-SMA mRNA and the unchanged glomerular cell number suggest that mesangial cell activation is not a major phenomenon at the early phase of diabetes in this model. Finally, we confirmed our previous findings that the kidney weight and mean glomerular volume increased 20 and 30%, respectively, in the diabetic compared with the nondiabetic NOD mice, as previously observed (7).

In summary, we found that glomeruli isolated from diabetic NOD mice exhibited an increased expression of TGF-β1, tenascin, and laminin B1 4 wk after the onset of diabetes. The glomerular level of PDGF-B, α1(IV) collagen, and 72-kd collagenase mRNA did not increase, and α1(I) collagen mRNA remained undetectable. Although a single-point study does not allow the generalization to the progression of diabetic glomerulosclerosis, the selective up-regulation of certain genes in the glomeruli suggest that they may be targets of the metabolic events characterizing the early phase of diabetic glomerulosclerosis.
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