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Strain differences rather than hyperglycemia determine the severity of glomerulosclerosis in mice

FENG ZHENG, GARY E. STRIKER, CIRO ESPOSITO, ENRICO LUPIA, and LILIANE J. STRIKER

Renal Cell Biology Section, NIDDK, National Institutes of Health, Bethesda, Maryland; Ivax Research Institute, Ivax Corporation; and Laboratory of Renal Cell Biology, Department of Medicine, University of Miami School of Medicine, Miami, Florida, USA

Strain differences rather than hyperglycemia determine the severity of glomerulosclerosis in mice.

Background. We reported that ROP, but not C57, mice were prone to glomerulosclerosis (GS) after nephron reduction (*J Clin Invest* 97:1242, 1996).

Methods. In this study, we induced diabetes in ROP and C57 mice to determine if the glomerulosclerotic response was stimulus specific. We used the oligosyndactyly mutation (Os), to produce a congenital 50% reduction in nephron number. Stable hyperglycemia was induced by streptozotocin and mice were maintained for 12 weeks without insulin treatment.

Results. Glomerular hypertrophy occurred in diabetic ROP +/+ and C57 +/+ mice, but glomeruli of diabetic ROP +/+ mice had 1.92-fold higher laminin B1 and 1.5-fold higher tenascin mRNA levels than diabetic C57 +/+ mice. Diabetic ROP Os/+ mice had severe glomerulosclerosis with arteriolar and tubulointerstitial lesions while there was only moderate mesangial sclerosis in diabetic C57 Os/+ mice. Glomerular size was increased in all non-diabetic Os/+ mice. It was further increased in diabetic ROP Os/+ mice, but not in diabetic C57 Os/+ mice. Glomerular mRNA levels were higher in diabetic ROP OS/+ than in diabetic C57 OS/+ mice [α1 (IV) collagen 3.2-fold, laminin B1 2.1-fold, and tenascin 1.6-fold].

Conclusion. Overall, our data further support the hypothesis that the susceptibility to glomerulosclerosis is inherited, and suggest that hyperglycemia serves principally as a triggering event in the development of diabetic nephropathy. Since the acceleration of diabetic nephropathy by nephron reduction was also largely strain dependent, it appears that the propensity to glomerulosclerosis is a general renal response and is not stimulus specific.

Diabetic nephropathy is one of the major causes of morbidity and mortality among diabetics [1–3]. Hyperglycemia, early and advanced glycosylation end-products (AGEs), and elevated growth hormone levels (GH) have been implicated in the glomerular lesions of diabetic nephropathy [4, 5]. However, not all patients with these abnormalities develop glomerular lesions, and the severity and rate of progression of the nephropathy varies widely. Epidemiological studies reveal that there is family clustering of diabetic nephropathy and that ethnicity plays an important role in the risk of its development and progression [6–8]. The genesis of lesions in vascular beds other than the kidney, such as the eye, appears to be more closely related to the underlying metabolic abnormalities [6, 7].

The genetic make-up underlying the propensity to diabetic nephropathy has not been characterized. Further, it is not known whether the propensity to develop glomerulosclerosis is specific to the individual stimulus or represents a general response. One predisposing factor could be an inborn deficit in nephron number. The glomerular lesions due to this deficit may only become manifest in later life [9–11]. There is increasing evidence that the number of glomeruli at birth can be influenced by multiple factors, including placental weight, birth weight, and exposure to toxins *in utero* [12, 13]. Low birth weight is linked to an increased risk of hypertension, end-stage renal disease [12], and diabetic nephropathy [10]. While these data suggest that glomerulosclerosis is a general response, it has not been directly assessed.

A radiation induced mutation resulted in oligosyndactyly (Os) and a congenital reduction in the number of nephron [14]. Using mice carrying the Os mutation, we found that genetic susceptibility to glomerulosclerosis was independent of reduction in nephron number, and that a deficit in nephron number induced sclerosis only in susceptible strains [14]. In the current study, we tested the hypothesis that the severity of diabetic nephropathy is influenced by the genetic background and that hyperglycemia served principally as a triggering event. We found that the predisposition to the development of diabetic glomerulosclerosis depends largely on the mouse strain. The inborn reduction in nephron number served as a stimulus to promote the development of diabetic glomerulosclerosis in sclerosis-prone mice, but not in sclerosis-resistant mice.

Key words: sclerosis, diabetes, susceptibility to GS, insulin, nephropathy, glomerular lesions.

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METHODS

Experimental design

Female C57 +/+, ROP +/+, C57 Os/+, and ROP Os/+ mice were obtained from Jackson Laboratories (Bar Harbor, ME, USA) and diabetes was induced at 12 weeks of age using streptozocin (STZ). Seven to fourteen intraperitoneal injections of 50 μg/g body wt of STZ, or vehicle solution (citrate buffer) were administered within two weeks [5]. Mice were weighed and blood glucose levels were determined at baseline and weekly thereafter. Only mice that reached stable hyperglycemia between 240 to 400 mg/dl were included in the study. After a 12 weeks follow-up without insulin treatment, groups of six mice were sacrificed according to National Institutes of Health approved procedures. The left kidney was perfused with a buffer solution containing collagenase and RNAse inhibitors for glomerular microdissection as described previously [15]. The right kidney was used for microscopic studies.

Light microscopy

Coronal kidney sections were fixed in Carnoy's fixative, embedded in glycol methacrylate, cut at a thickness of 2 μm, and stained with periodic acid-Schiff. The sections were examined without knowledge of the experimental groups. The degree of glomerulosclerosis was assessed using a scale from 0 to 4+. Forty glomeruli per section were examined and the mean of scores for individual glomeruli was recorded [16, 17].

Morphometry

Morphometric analysis was performed on plastic-embedded sections using a digitizing tablet and a video camera. The mean glomerular volume was derived from the harmonic mean of the glomerular equatorial surface area [18].

Glomerular cell number and turnover

The nuclei of 40 to 50 successively encountered glomerular profiles were counted and the mean of glomerular cell number was calculated.

After ^{3}H thymidine administration, nuclei that contained >5 grains in 40 to 50 successive glomeruli were counted in each section. The labeling index was calculated as the number of labeled cells (excluding Bowman's capsular cells) divided by the total glomerular cell number $\times 100$ [18].

Isolation of glomeruli, reverse transcription *in situ*, and competitive PCR

Glomeruli were isolated by microdissection and glomerular cDNA was obtained by *in situ* reverse transcription [15]. The primers for mouse α1 (IV) collagen, laminin B1, tenascin, and β-actin were previously described [19]. The corresponding polymerase chain reaction (PCR) products were 484 bp for α1 type (IV) collagen, 443 bp for laminin B1, 548 bp for tenascin, and 460 bp for β-actin.

Competitive PCR assays were performed by adding decreasing amounts of mutant templates to glomerular cDNA. After PCR amplification, the ratio of mutant to glomerular cDNA band density was calculated by laser densitometry and plotted as a function of the amount of initial mutant template added to the reaction [15]. The amount of glomerular cDNA was derived from linear regression analysis and expressed as mRNA levels. Duplicate assays were performed. A representative competitive PCR assay is shown in Figure 1. Glomerular α1 type (IV) collagen, laminin B1 and tenascin mRNAs levels were corrected for β-actin mRNA levels and expressed as ratios.

Statistical analysis

All values were expressed as mean $\pm$ SD. The two-tailed unpaired Student's test was used to evaluate differences between means for corresponding sets of data obtained from the diabetic and control mice. The level of significance ($P < 0.05$) was chosen *a priori* for all the analyses performed in the present study. The two-tailed unpaired Mann-Whitney nonparametric test was used to analyze differences in mRNA levels and sclerosis score between strains.

RESULTS

Body weight, heart weight and blood glucose levels

There were no significant differences in body or heart weight between diabetic C57 +/+, ROP +/+, C57 Os/+, ROP Os/+, and non-diabetic controls (Table 1). Blood glucose remained at the same levels in all groups of diabetic mice during the three months follow-up, and none received insulin.

Glomerular volume

Diabetic ROP +/+ and C57 +/+ mice had a significant increase in glomerular volume (Fig. 2A), but the increment was higher in the diabetic ROP +/+ mice. As previously described, glomerular volume was significantly increased in C57 Os/+ and ROP Os/+ mice as compared to their respective controls (Table 1) [14].

Glomerular volume was further increased in ROP Os/+ mice after the induction of diabetes, while there was no increase in glomerular volume in diabetic C57 Os/+ mice (Fig. 2B).

Glomerular cell number and turnover

No significant increase in glomerular cell number or labeling index was found in the four groups of diabetic mice compared to their respective controls. We previously found that both mean glomerular cell number and labeling index were increased significantly in C57 Os/+ and ROP Os/+ mice, compared to non-Os controls [14].

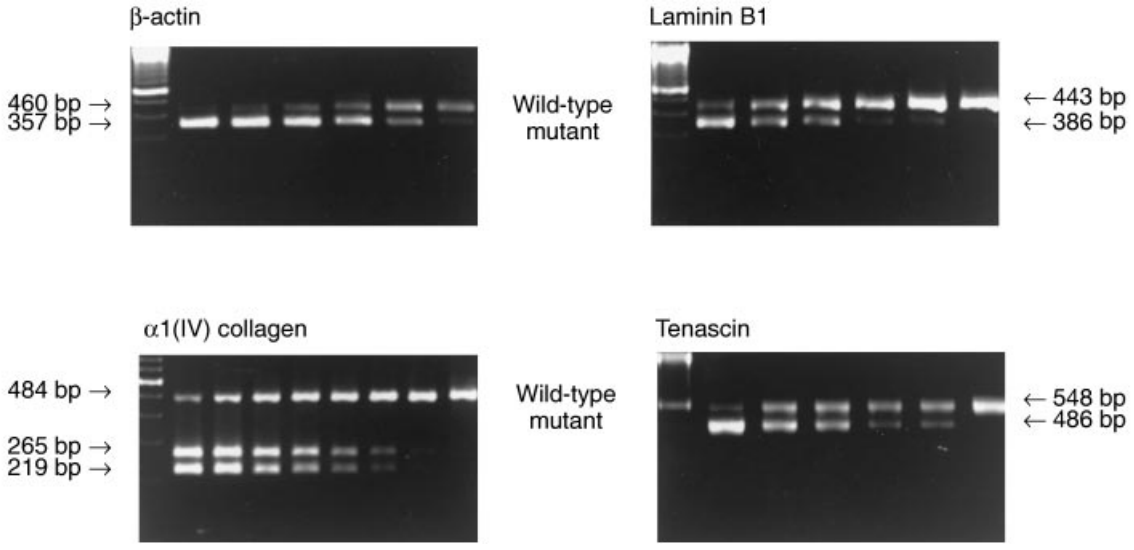

Fig. 1. Representative experiments for competitive polymerase chain reaction (PCR) for glomerular β-actin, laminin B1, α1 (IV) collagen and tenascin. Competitive PCR was performed as described. cDNA from 1/10th glomerulus (wild-type) competed with decreasing amount of mutant cDNA. In these experiments, the mutant range was 0.02-0.001 amol (lane 1 to 6) for β-actin, 0.004-0.0001 amol (lanes 1 to 6) for laminin B1, 0.04-0.0002 amol (lanes 1 to 8) for α1 (IV) collagen, and 0.01-0.002 amol (lanes 1 to 6) for tenascin. Glomerular cDNAs were measured at least in duplicate. PCR products were separated on 4% agarose gel.

Table 1. Comparison of body weight, heart weight, heart weight/body weight ratio, and blood glucose levels

Strain:	C57 +/+		ROP +/+		C57 Os/+		ROP Os/+	
Group:	Control	Diabetic	Control	Diabetic	Control	Diabetic	Control	Diabetic
Body weight *g*	21.3 ± 0.9	20.78 ± 0.77	21.88 ± 1.07	21.5 ± 1.41	24.32 ± 1.67	21.93 ± 1.36	23.42 ± 2.36	19.32 ± 2.21
Heart wt *mg*	112 ± 14	107.33 ± 4.52	90.63 ± 3.26	105.71 ± 18	109.9 ± 7.46	126.13 ± 26.45	120.42 ± 13.57	94.28 ± 18.91
Heart wt/body wt	0.005 ± 0.0001	0.005 ± 0.001	0.004 ± 0.0003	0.005 ± 0.001	0.005 ± 0.0001	0.006 ± 0.001	0.005 ± 0.001	0.005 ± 0.001
Blood glucose *mg/dl*	86.67 ± 20.66	340 ± 59.28	78.33 ± 13.29	314 ± 66	87.5 ± 11.72	333 ± 32.66	78.33 ± 13.29	340 ± 54

There were six mice in each group.

Histology

There were no glomerular lesions by light microscopy in control C57 +/+ and ROP +/+ mice (Fig. 3A and 3C). Diabetic C57 +/+ mice exhibited a minimal, but diffuse, increase in PAS positive material in the mesangial areas without any increase in peripheral basement membrane thickness (Fig. 3B). The increase in mesangial matrix was more pronounced in ROP +/+ than in C57 +/+ diabetic mice and was not associated with an increase in the number of nuclei (Fig. 3D). This was further documented using a semiquantitative scoring method that showed that the mean mesangial sclerosis was 1.33 ± 0.26 in diabetic ROP +/+ mice compared to 0.83 ± 0.26 in diabetic C57 +/+ mice ($P < 0.05$). As described previously [14], glomerular cell number was increased in both C57 Os/+ and ROP Os/+ mice and C57 Os/+ mice had minimal glomerular lesions, while ROP Os/+ mice had moderate to severe and diffuse glomerulosclerosis consisting of an increase in mesangial matrix but not in peripheral basement membrane thickness. No area of segmental sclerosis with synechiae was observed. Most Bowman's capsules appeared normal or minimally thickened (Fig. 4 A, C). Diabetic C57 Os/+ mice had moderate mesangial sclerosis that did not affect all spaces and was not associated with any aneurysmal dilation of peripheral loops. The interstitial compartment did not show any lesions. Tubular basement membrane were not thickened. Arteries and arterioles were normal (Fig. 4B). In contrast, all diabetic ROP Os/+ mice had diffuse and severe glomerulosclerosis with Kimmelstiel-Wilson nodules. All mesangial spaces were markedly enlarged. Occasional aneurysmal dilations of the vascular loops were also present. In many glomeruli there was a significant decrease in the vascular lumina. Large synechiae were present. Occasional obsolescent glomeruli were found. Bowman's capsules were massively thickened. The interstitial tissue was markedly increased and contained areas of cellular infiltration. The majority of tubules exhibited severe tubular basement thickening. There were arteriolar lesions consisting in subintimal deposits. (Fig. 4D). Accordingly, the mean mesangial sclerosis score was 2.83 ±

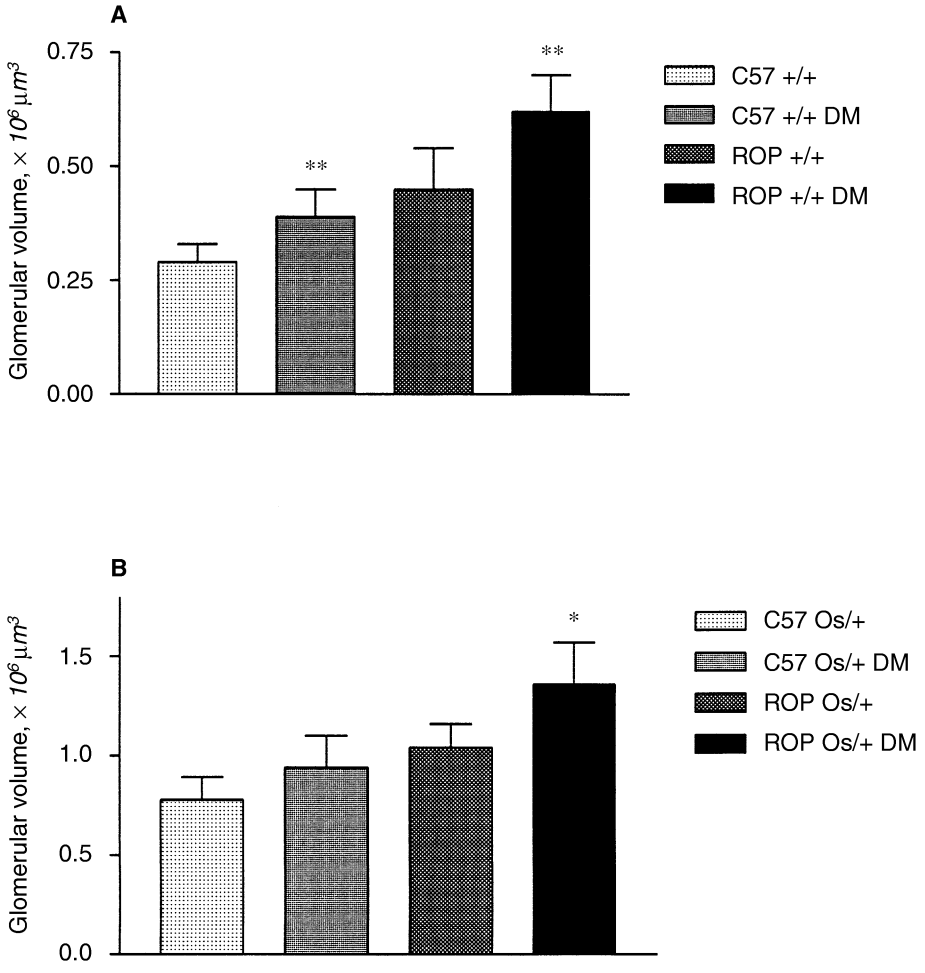

Fig. 2. Glomerular volume in diabetic and non-diabetic mice. (*A*) Glomerular volume in C57 +/+ and ROP +/+ mice. $^{**}P < 0.01$, diabetic ROP +/+ and C57 +/+, compared to non-diabetic controls. (*B*) Glomerular volume in C57 Os/+ and ROP Os/+ mice. $^{*}P < 0.01$, diabetic ROP Os/+ mice, compared to non-diabetic controls.

0.4 in the diabetic C57 Os/+ mice and 3.75 ± 0.27 in diabetic ROP Os/+ mice ($P < 0.01$).

mRNA levels

The values of α1(IV) collagen, laminin B1 and tenascin obtained by competitive PCR were normalized to β-actin (Fig. 5). There was a comparable increase in glomerular α1(IV) collagen mRNA levels in diabetic C57 +/+ and ROP +/+ mice, compared to controls (Fig. 5A). As reported previously, glomerular α1(IV) collagen mRNA levels were higher in ROP Os/+ mice than in C57 Os/+ mice. Diabetic ROP OS/+ but not C57Os/+ mice, had increased α1(IV) collagen mRNA levels, compared to non-diabetic controls (Fig. 5 B).

Glomerular laminin B1 mRNA levels were increased in both diabetic C57 +/+ and ROP +/+ mice, compared to controls (Fig. 5C). However diabetic ROP +/+ mice had 1.92-fold higher laminin B1 mRNA levels than diabetic C57 +/+ mice ($P < 0.05$). Furthermore, diabetic ROP Os/+ had significantly increased laminin B1 mRNA levels, compared to non-diabetic controls (Fig. 5 D), while there were no significant differences between diabetic and non-diabetic C57 Os/+ mice.

Glomerular tenascin mRNA levels were increased in diabetic C57 +/+ and ROP +/+ mice, compared to controls (Fig. 5E). However, diabetic ROP +/+ mice had 1.5-fold higher tenascin mRNA levels than diabetic C57 +/+ mice ($P < 0.01$). Diabetic ROP Os/+ but not C57 Os/+ mice had significantly increased tenascin mRNA levels (Fig. 5F) compared to non-diabetic Os/+ mice.

DISCUSSION

Multiple data suggest the existence of a genetic propensity for renal lesions in patients with diabetes mellitus. For instance, it is well established that only a fraction of IDDM and NIDDM patients develop diabetic nephropathy [6, 7]. Pima Indians and African Americans with diabetes have a much higher risk of development of diabetic nephropathy than Caucasians [8]. In family studies Seaquist et al found that the incidence of nephropathy was 83% in siblings of

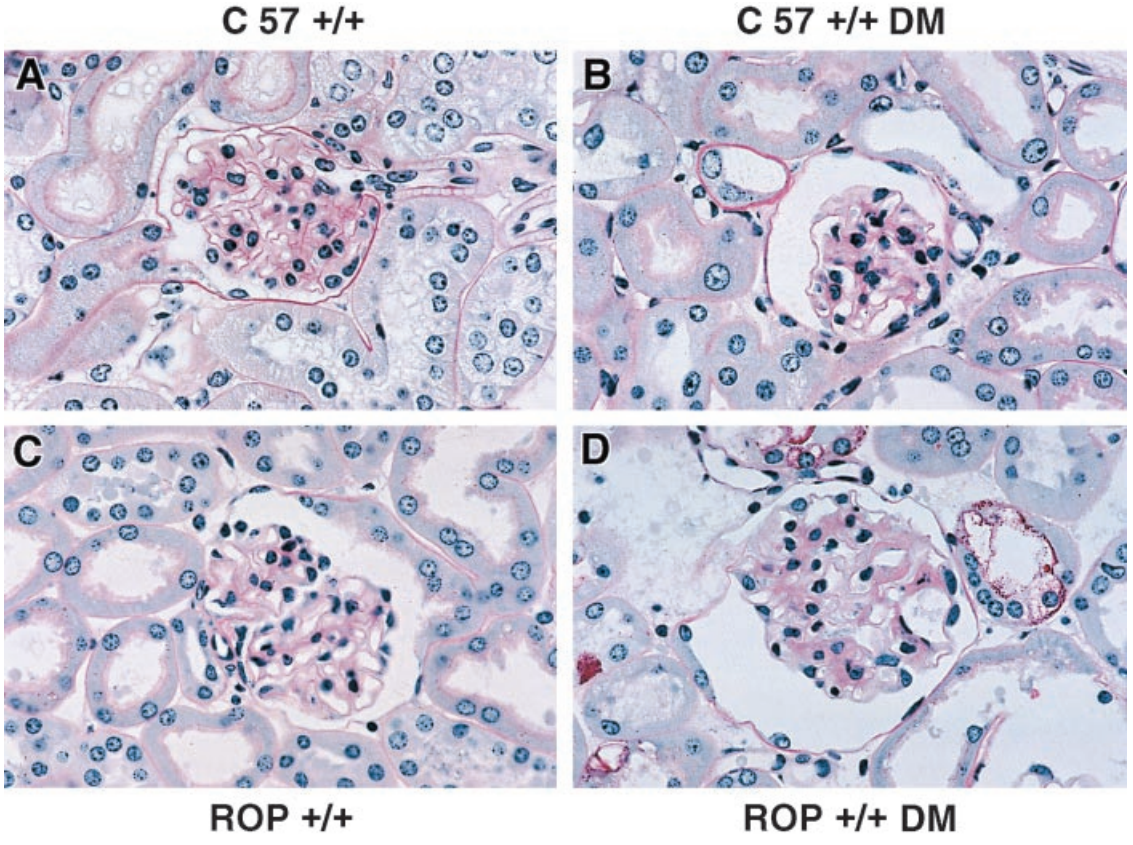

Fig. 3. Light microscopy. Periodic acid-Schiff methenamine-stained plastic embedded sections, ×400. (*A*) C57 +/+, (*B*) C57 +/+ diabetic, (*C*) ROP +/+, and (*D*) ROP +/+ diabetic.

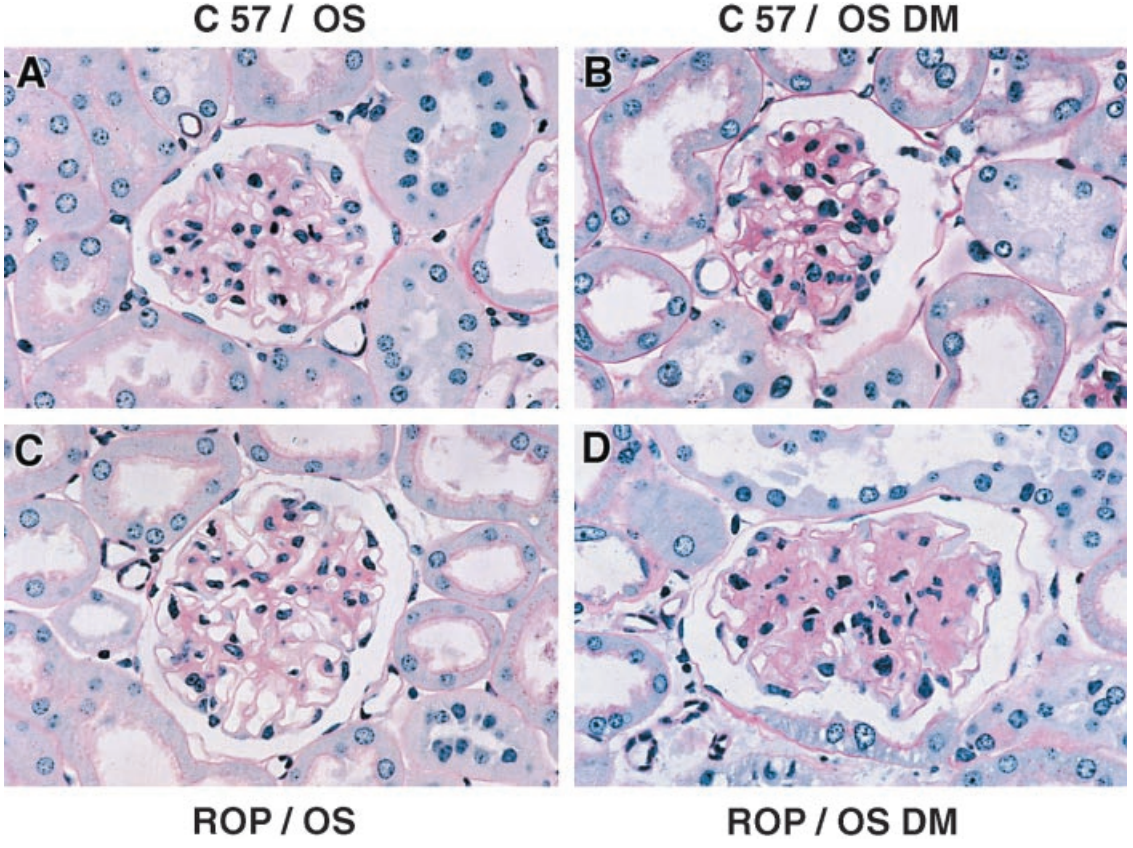

Fig. 4. Light microscopy. Periodic acid-Schiff methenamine-stained plastic embedded sections, ×400. (*A*) C57 Os/+, (*B*) C57 Os/+ diabetic, (*C*) ROP Os/+, and (*D*) ROP Os/+ diabetic.

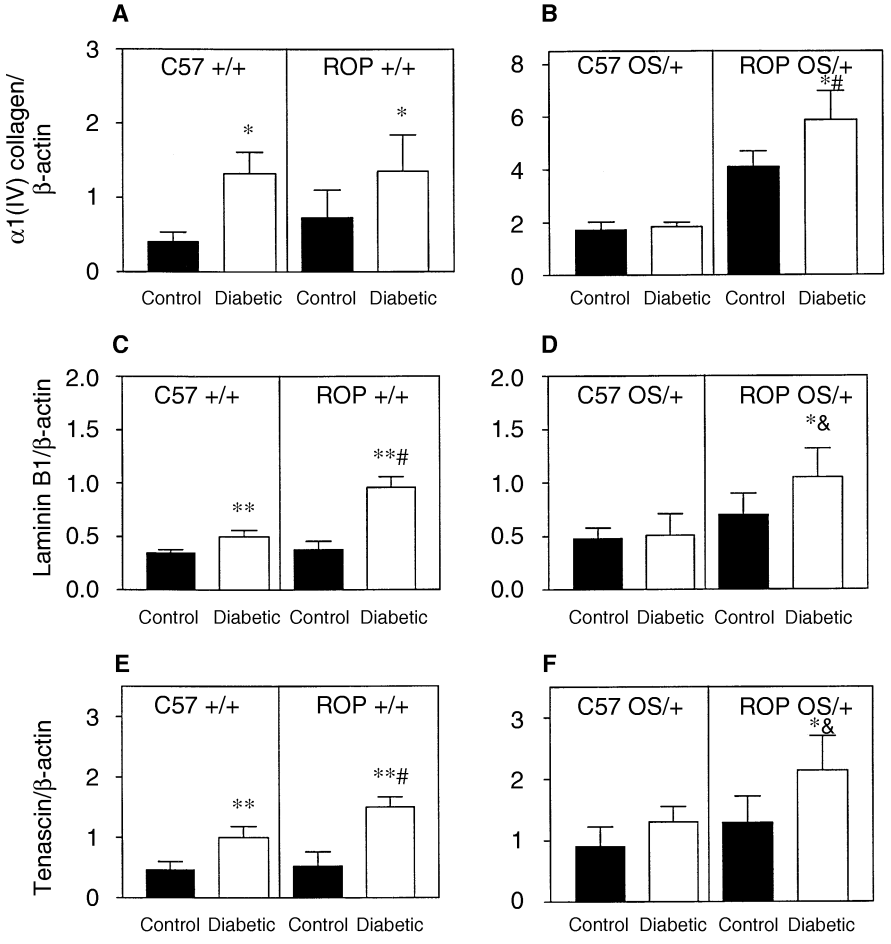

Fig. 5. Glomerular mRNA levels in C57 +/+, ROP +/+, C57 Os/+, and ROP Os/+ mice corrected for β-actin. (*A and B*) α1 (IV) mRNA levels. $^{*}P < 0.05$, diabetic C57 +/+ and ROP +/+ mice compared to controls. C57 Os/+ and ROP Os/+ mice. $^{*}P < 0.05$, diabetic ROP Os/+ compared to ROP Os/+ controls. $^{\#}P < 0.01$, diabetic ROP Os/+ compared to diabetic C57 Os/+ mice. (*C and D*) Laminin mRNA levels. $^{**}P < 0.05$, diabetic C57 +/+ and ROP +/+ mice compared to controls. $^{\#}P < 0.01$, diabetic ROP +/+ mice compared to diabetic C57 +/+ mice. $^{*}P < 0.05$, diabetic ROP Os/+ compared to control ROP Os/+. $^{\&}P < 0.01$, diabetic ROP Os/+ mice compared to diabetic C57 Os/+. (*E and F*) Tenascin mRNA levels. $^{**}P < 0.01$, diabetic C57 +/+ and ROP +/+ mice compared to non-diabetic controls. $^{\#}P < 0.01$, diabetic ROP +/+ mice compared to diabetic C57 +/+ mice. $^{*}P < 0.05$, diabetic ROP Os/+ compared to non-diabetic ROP Os/+ controls. $^{\&}P < 0.01$, diabetic ROP Os/+ mice compared to diabetic C57 Os/+ mice.

probands with diabetic nephropathy but only 17% in siblings of probands without diabetic nephropathy [20]. These observations suggest that the predisposition to diabetic nephropathy is hereditary. Although several models have been proposed on the basis of human studies, the exact contribution of genetic factors to the pathogenesis of diabetic glomerulosclerosis is not clear [21, 22].

The experimental models in this study allows the assessment of the relative contributions of genetic factors and hyperglycemia in the development of diabetic glomerulosclerosis. We compared the glomerular responses to hyperglycemia in sclerosis-resistant (C57 +/+) and sclerosis-prone (ROP +/+) mice after three months of stable STZ-induced hyperglycemia. Although C57 +/+ and ROP +/+ mice had identical glycemic levels, the renal lesions were more severe in ROP +/+ than in C57 +/+ mice. To determine whether the renal lesions were stimulus specific, we studied animals with a congenital deficit in nephron number. This stimulus was chosen since it has been shown that an inborn nephron deficit is a risk factor for the development of progressive renal disease [10, 22]. The model chosen was the oligosyndactyly defect, a radiation induced mutation localized on mouse chromosome 8 that results in an inborn deficit in nephron number. Although ROP +/+ and C57 +/+ mice both have a 50% decrease of nephron number when they carry the Os gene, we reported that ROP Os/+ mice developed severe spontaneous glomerulosclerosis while C57 Os/+ mice had only minimal glomerular lesions [14]. The mice were maintained with similar level of hyperglycemia for three months without insulin treatment. At sacrifice the renal lesions appeared quite severe in the diabetic ROP Os/+, contrasting with a moderate increase in mesangial sclerosis in the C57 Os/+ mice. Thus, the influence of nephron number on the severity of diabetic glomerulosclerosis appeared to be strain dependent. These data are consistent with that obtained in BB rats, which have been shown to be resistant to glomerulosclerosis after 5/6 nephrectomy [23]. Feld et al also reported that diabetic BB rats developed only minimal glomerular lesions including a slight increase in mesangial volume and in basement membrane thickness despite severe, prolonged hyperglycemia [24]. These results further support the role of an inherited susceptibility for the development of glomerulosclerosis. However, in their study, sclerosis-susceptible rats were not examined.

Other studies in rats have suggested a genetic basis for

Table 2. Glomerular cell turnover, glomerular volume, and glomerular sclerosis score

Strain:	C57 +/+		ROP +/+		C57 Os/+		ROP Os/+	
Group:	Control	Diabetic	Control	Diabetic	Control	Diabetic	Control	Diabetic
GC	31.48 ± 1.24	30.77 ± 2.64	33.91 ± 4.25	33.83 ± 2.04	36.93 ± 3.51[a]	36.47 ± 1.63	39.09 ± 1.52	39.47 ± 5.23[b]
LI %	0.101 ± 0.06	0.23 ± 0.21	0.11 ± 0.06	0.17 ± 0.15	0.32 ± 0.1[a]	0.43 ± 0.16	0.31 ± 0.14[a]	0.46 ± 0.35
GV 10^6 μm^3	0.29 ± 0.04	0.39 ± 0.06[c]	0.45 ± 0.09	0.62 ± 0.08[c]	0.78 ± 0.11	0.94 ± 0.16	1.04 ± 0.12	1.36 ± 0.21[c,f]
SS	0	0.83 ± 0.26[d]	0	1.33 ± 0.26[d,e]	1.2 ± 0.27	2.01 ± 0.66[d]	2.3 ± 0.27	3.75 ± 0.27[d,f]

There were six mice in each group. Abbreviations are: GC, glomerular cell number; LI, labeling index; GV, glomerular volume; and SS, sclerosis score.
[a] $P < 0.05$,
[b] $P < 0.01$, when C57/OS+ and ROP/OS+ mice were compared to C57 +/+ and ROP +/+ mice, respectively.
[c] $P < 0.05$
[d] $P < 0.01$, diabetic C57 +/+, ROP +/+, C57/OS+, and ROP OS/+ mice were compared to their respective non diabetic controls.
[e] $P < 0.01$, diabetic ROP +/+ mice compared to diabetic C57 +/+ mice
[f] $P < 0.01$, diabetic ROP OS/+ mice compared to diabetic C57 OS/+ mice

both the development and resistance to glomerulosclerosis [25–27]. Brandis et al described an age-dependent glomerulosclerosis in the Milan normotensive strain (MNS), although rats remain normotensive, while no glomerular lesions were found in the Milan hypertensive strains (MHS) rats, despite the presence of hypertension [28].

The component(s) of genetic susceptibility to sclerosis have been recently examined. In an elegant study utilizing Fawn-hooded rats, Brown et al have identified two loci located on chromosome 1 linked to the development of renal impairment irrespective of hypertension [29]. Genes involved in IDDM etiology, glucose metabolism, as well as blood pressure regulation, such as the insertion/deletion polymorphism of angiotensin-converting enzyme and the molecular variant of angiotensinogen have also been investigated [21, 30]. Our previous data demonstrated that the expression of α1 type (IV) collagen, laminin B1 and tenascin was selectively up-regulated in several models of glomerulosclerosis [31]. Since the thickening of the glomerular basal membrane and the accumulation of mesangial matrix are hallmarks of glomerular lesions in diabetic nephropathy [3], we investigated the differences in the glomerular extracellular matrix responses to diabetes by studying the expression of α1 type (IV) collagen, laminin B1 and tenascin in microdissected glomeruli. We found that the mRNA levels of laminin B1 and tenascin were more elevated in diabetic ROP +/+ than in diabetic C57 +/+. Similarly, extracellular matrix (ECM) mRNAs were increased in diabetic ROP Os/+ but not in C57 Os/+ mice. Previously we had found that hyperglycemia increased glomerular expression of α1(IV) and laminin B1 mRNAs in C57/SJL mice [5]. Fukui et al reported that when rats with STZ-induced diabetes were treated with sufficient insulin to maintain blood glucose close to normal, the overexpression of glomerular α1 type (IV) collagen and laminin B1 was reduced [32]. These data suggested that hyperglycemia *per se* stimulated the glomerular expression of α1(IV) collagen and laminin. However, there are results that do not agree with this conclusion. For instance, in diabetic NOD mice, glomerular laminin B1 and tenascin, but not type (IV) collagen mRNAs were found to be increased [19]. In KKAy mice, mRNA cortical levels for type (IV) collagen were significantly increased, while laminin B1 mRNA levels remained unchanged [33]. These diverse data may reflect differences between cortex and isolated glomeruli, mice strains, the type of diabetic model, the level of hyperglycemia, as well as insulin treatment. In the present work, the significant differences in laminin B1 and tenascin mRNA levels between diabetic C57 +/+ and ROP +/+ mice, and between diabetic C57 Os/+ and ROP Os/+ mice seem to indicate that genetic factors may be involved in the regulation of extracellular matrix gene expression. The cause for the different response to hyperglycemia of glomerular type (IV) collagen, laminin B1 and tenascin mRNA expression in C57 +/+ and C57 Os/+ mice is not clear.

Another component of the glomerular response to hyperglycemia is glomerular hypertrophy. There is considerable experimental evidence that increased glomerular size is a marker of a propensity to develop glomerulosclerosis [34], and there are also data suggesting that a similar relationship exists in humans [35]. In this study, we found that the correlation between glomerular hypertrophy and the severity of diabetic glomerulosclerosis depended on mouse strain. Diabetic ROP +/+ mice developed more severe lesions than C57 +/+ mice, though their glomerular sizes were equal. Interestingly, glomerular size and lesions were both markedly accentuated in diabetic ROP Os/+ mice, whereas the glomerular size was not changed in diabetic C57 Os/+ mice and the lesions were much less severe. These results suggest that in sclerosis-prone strains glomerular hypertrophy may be unregulated. In contrast, glomerular size reaches a plateau in sclerosis-resistant strains, and additional stimuli do not result in further hypertrophy.

The role of an increased cell number in the glomerular hypertrophy was also investigated. There was no change in total glomerular cell number, but there was a small increase in the labeling index in all diabetic mice. This data suggest the possibility of apoptosis in diabetic glomeruli, but we were unable to detect a difference in glomerular apoptotic

Table 3. Quantitation of glomerular MRNA levels by competitive polymerase chain reaction (PCR)

Strain:	C57 +/+		ROP +/+		C57 Os/+		ROP Os/+	
Group:	Control	Diabetic	Control	Diabetic	Control	Diabetic	Control	Diabetic
α1 (IV)	0.41 ± 0.13	1.32 ± 0.29[a]	0.73 ± 0.37	1.35 ± 0.49[a]	1.72 ± 0.3	1.84 ± 0.17	4.1 ± 0.59	5.88 ± 1.11[a,d]
Laminin B1	0.35 ± 0.03	0.5 ± 0.06[b]	0.38 ± 0.08	0.96 ± 0.1[b,c]	0.48 ± 0.1	0.51 ± 0.2	0.7 ± 0.2	1.05 ± 0.27[b,d]
Tenascin	0.46 ± 0.14	1.0 ± 0.18[b]	0.52 ± 0.24	1.5 ± 0.17[b,c]	0.9 ± 0.32	1.3 ± 0.25	1.29 ± 0.43	2.14 ± 0.56[b,d]

Competitive PCR was performed in duplicate or triplicate. Means of six mice per group are shown. The values were expressed as the ratios after being corrected for β-actin mRNA levels.

[a] $P < 0.05$,

[b] $P < 0.01$, diabetic C57 +/+, ROP +/+, ROP OS/+ mice compared to their respective non diabetic controls

[c] $P < 0.01$, diabetic ROP +/+ mice compared to diabetic C57 +/+ mice

[d] $P < 0.01$, diabetic ROP OS/+ mice compared to diabetic C57 OS/+ mice

cells between diabetic and controls mice by immunostaining (data not shown). Young et al found an early increase in mesangial cell turnover after the onset of STZ-induced diabetes in rats, but the total glomerular cell number did not increase significantly [36].

It should be noted that sclerosis-resistant mice respond to both nephron reduction and hyperglycemia, but that the responses appear to be self-limited. In contrast, the response to these stimuli in sclerosis-prone strains appears to be progressive. This may be representative of the case in the spectrum in diabetic patients, where many have an elevated albumin excretion rate, but a limited number develop end-stage renal disease.

Overall, our data support the hypothesis that hyperglycemia is a necessary, but not sufficient stimulus for the development of diabetic glomerulosclerosis. In combination with our previous data, it appears that susceptibility to glomerulosclerosis is not stimulus-specific, in that both diabetes mellitus and nephron reduction may serve as initiators of sclerosis in a susceptible mouse strain. Finally, the resistance to glomerulosclerosis is also independent of the stimuli, since both diabetes mellitus and nephron reduction resulted in much less severe lesions in a sclerosis-resistant mouse strain.

Reprint requests to Liliane J. Striker, M.D., Laboratory of Renal Cell Biology, Department of Medicine, Division of Nephrology, University of Miami School of Medicine, P.O. Box 016910 (R126), Miami, Florida 33101, USA.
E-mail: lstriker@newssun.med.miami.edu

REFERENCES

1. Krolewski AS, Warram JH, Christlieb AR, Busick EJ, Kahn CN: The changing natural history of nephropathy in Type I diabetes. *Am J Med* 78:785–794, 1985
2. Borch-Johnsen K, Kreiner S: Proteinuria: Value as a predictor of cardiovascular mortality in insulin-dependent diabetes. *Br Med J* 294:1651–1654, 1987
3. Østerby R, Parving HH, Hommel E, Jørgensen HE, Lokkegaard H: Glomerular structure and function in diabetic nephropathy. Early to advanced stages. *Diabetes* 39:1057–1063, 1990
4. Yang CW, Vlassara H, Peten EP, He CJ, Striker GE, Striker LJ: Advanced glycosylation end-products upregulate gene expression found in diabetic glomerular disease. *Proc Nat Acad Sci USA* 91:9436–9440, 1994
5. Esposito C, Liu ZH, Striker GE, Phillips C, Chen NY, Chen WY, Kopchick JJ, Striker LJ: Inhibition of diabetic nephropathy by a GH antagonist: A molecular analysis. *Kidney Int* 50:506–514, 1996
6. Borch-Johnsen K, Nøgaard K, Hommel E, Mathiesen ER, Jensen JS, Deckert T, Parving HH: Is diabetic nephropathy an inherited complication? *Kidney Int* 41:719–722, 1992
7. Ballard DJ, Humphrey LL, Melton LJ III, Frohnart PP, Chu PC, O'Fallen WM, Palumbo PJ: Epidemiology of persistent proteinuria in type II diabetes mellitus. Population-based study in Rochester, Minnesota. *Diabetes* 37:405–412, 1988
8. Cowie CC, Port FK, Wolfe RA, Savage PJ, Moll PP, Hawthorne M: Disparities in incidence of diabetic end-stage renal disease according to race and type of diabetes. *N Engl J Med* 321:1074–1079, 1989
9. Kiprov DD, Colvin RB, McCluskey RT: Focal and segmental glomerulosclerosis and proteinuria associated with unilateral renal agenesis. *Lab Invest* 46:275–281, 1982
10. Rossing P, Tarnow L, Nielsen FS, Hansen BV, Brenner BM, Parving HH: Low birth weight: A risk factor for development of diabetic nephropathy? *Diabetes* 44:1405–1407, 1995
11. Leroy B, Josset P, Morgan G, Costil J, Merlet-Benichou C: Intrauterine growth retardation (IUGR) and nephron deficit: Preliminary study in man. (abstract) *Pediatr Nephrol* 5:C21, 1991
12. Brenner BM, Chertow GM: Congenital oligonephropathy and the etiology of adult hypertension and progressive renal injury. *Am J Kidney Dis* 23:171–175, 1994
13. Gilbert T, Lelievre-Pegorier M, Merlet-Benichou C: Long-term effects of mild oligonephronia induced in utero by gentamicin in the rat. *Pediatr Res* 30:450–456, 1991
14. He CJ, Esposito C, Phillips C, Zalups RK, Henderson DA, Striker GE, Striker LJ: Dissociation of glomerular hypertrophy, cell proliferation, and glomerulosclerosis in mouse strains heterozygous for a mutation (Os) which induces a 50% reduction in nephron number. *J Clin Invest* 97:1242–1249, 1996
15. Peten EP, Garcia-Perez A, Terada Y, Woodrow D, Martin BM, Striker GE, Striker LJ: Age-related changes in a1- and a2-chain type IV collagen mRNAs in adult mouse glomeruli: Competitive PCR. *Am J Physiol* 263:F951–F957, 1992
16. Mackay K, Striker LJ, Stauffer JW, Agodoa LY, Striker GE: Relationship of glomerular hypertrophy and sclerosis: Studies in SV40 transgenic mice. *Kidney Int* 37:741–748, 1990
17. Doi T, Hattori M, Agodoa LYC, Sato T, Yoshida H, Striker LJ, Striker GE: Glomerular lesions of the non-obese diabetic mouse: Before and after the onset of hyperglycemia. *Lab Invest* 63:204–211, 1990
18. Pesce CM, Striker LJ, Peten EP, Elliot SJ, Striker GE: Glomerulosclerosis at both early and late stages is associated with increased cell turnover in mice transgenic for growth hormone. *Lab Invest* 65:601–605, 1991
19. Yang CW, Hattori M, Vlassara H, He CJ, Carome MA, Yamato E, Elliot S, Striker GE, Striker LJ: Overexpression of TGF-β1 mRNA is associated with up-regulation of glomerular tenascin and laminin gene expression in non-obese diabetic mice. *J Am Soc Nephrol* 5:1610–1617, 1995
20. Seaquist ER, Goetz FE, Rice S, Barbosa J: Familial clustering of

diabetic kidney disease: Evidence for genetic susceptibility to diabetic nephropathy. *N Engl J Med* 320:1161–1165, 1989
21. Doria A, Warram JH, Krolewski AS: Genetic susceptibility to nephropathy in insulin-dependent diabetes: From epidemiology to molecular genetics. *Diabetes Metabol Rev* 11:287–314, 1995
22. Parving HH, Tarnow L, Rossing P: Genetics of diabetic nephropathy. *J Am Soc Nephrol* 7:2501–2517, 1996
23. Van Liew JB, Yang CW, Striker LJ, Feld LG: Resistance to glomerulosclerosis in biobreeding rats. (abstract) *J Am Soc Nephrol* 5:973, 1994
24. Feld LG, Cachero S, Ellis EN, Dedeoglu O, Ueda Y, Leonard C, Letizia R, Mallon V, Van Liew JB: Resistance to glomerular injury in the diabetic biobreeding rat. *Exper Physiol* 80:991–1000, 1995
25. Striker GE, Nagle RB, Kohnen PW, Smuckler EA: Response to unilateral nephrectomy in old rats. *Am Assoc Arch Pathol* 87:439–442, 1969
26. Kreisberg JI, Karnovsky MJ: Focal segmental sclerosis in the Fawn-hooded rat. *Am J Pathol* 92:637–652, 1978
27. Matsuyama M, Ogiu T, Kontani K, Yamada C, Kawai M, Hiai H, Nakamura Y, Shimuzu F, Toyokawa T, Kinoshita Y: Genetic regulation of the development of glomerular sclerotic lesions in the BUF/Mna rat. *Nephron* 54:334–337, 1990
28. Brandis A, Bianchi G, Reale E, Helmchen U, Kuhn K: Age-dependent glomerulosclerosis and proteinuria occurring in rats of the Milan normotensive strain and not in rats of the Milan hypertensive strain. *Lab Invest* 55:234–243, 1986
29. Brown DM, Provoost AP, Daly MJ, Landers EJ, Jacob HJ: Renal disease susceptibility and hypertension are under independent genetic control in the fawn-hooded rat. *Nature Med* 12:44–51, 1996
30. Fogarty DG, Krolewski AS: Genetic susceptibility and the role of hypertension in diabetic nephropathy. *Curr Opin Nephrol Hypertens* 6:184–191, 1997
31. Striker GE, He CJ, Liu ZH, Yang DCW, Zalups RK, Esposito C, Striker LJ: Pathogenesis of non-immune glomerulosclerosis: Studies in animals and potential applications to humans. *Lab Invest* 73:596–605, 1995
32. Fukui M, Nakamura T, Ebihara I, Shirato I, Tomino Y, Koide H: ECM gene expression and its modulation by insulin in diabetic rats. *Diabetes* 41:1520–1527, 1992
33. Ledbetter S, Copeland EJ, Noonan D, Vogeli G, Hassell JR: Altered steady-state mRNA levels of basement membrane proteins in diabetic mouse kidneys and thromboxane synthesis inhibition. *Diabetes* 39:196–203, 1990
34. Zatz R, Fujihara K: Glomerular hypertrophy and progressive glomerulopathy. Is there a definite pathogenetic correlation? *Kidney Int* 45(Suppl 45):S27–S29, 1994
35. Pesce CM, Schmidt K, Fogo A, Okoye ML, Kim R, Striker LJ, Striker GE: Glomerular size and the incidence of renal disease in African Americans and Caucasians. *J Nephrol* 7:355–359, 1994
36. Young BA, Johnson RJ, Alpers CE, Eng E, Gordon K, Floege J, Couser WG: Cellular events in the evolution of experimental diabetic nephropathy. *Kidney Int* 47:935–944, 1995