Regulation of Renal Laminin in Mice with Type II Diabetes

TAE-SUN HA,* JEFFREY L. BARNES,* JENNIFER L. STEWART,* CHEOL W. KO,* JEFFREY H. MINER,‡ DALE R. ABRAHAMSON,† JOSHUA R. SANES,§ and BALAKUNTHALAM S. KASINATH*

*Department of Medicine, University of Texas Health Science Center and A. L. Murphy Veterans’ Administration Hospital, San Antonio, Texas; †Department of Cell Biology, University of Alabama, Birmingham, Alabama; and Departments of ‡Medicine and §Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, Missouri.

Abstract. This study examines the regulation of renal laminin in the db/db mouse, a model of type II diabetes characterized by extensive remodeling of extracellular matrix. Immunohistochemistry demonstrated an increase in the contents of laminin chains including β1 chain in the mesangium and tubular basement membranes at 1, 2, 3, and 4 mo of diabetes. Immunofluorescence with an antibody against the recently discovered laminin α5 chain showed that in the normal mouse, the protein had a restricted distribution to the glomerular and tubular basement membranes with scant expression in the mesangium of older mice. In the diabetic mouse, the laminin α5 chain content of the glomerular and tubular basement membranes was increased, with marked expression in the mesangium. Northern analysis revealed a significant decrease in the renal cortical contents of α5, β1, and γ1 chain mRNA in the diabetic mice compared to control, at each of the time points. In situ hybridization showed decreased abundance of α5 transcripts in the glomeruli of diabetic mice compared to nondiabetic controls. Analysis of mRNA changes by Northern and in situ hybridization studies demonstrated that the reduction in laminin transcripts involved both glomerular and tubular elements. These observations demonstrate that laminin accumulation in the db/db mice with type II diabetes is due to nontranscriptional mechanisms. Because previous investigations in rodents with type I diabetes have shown that the increase in renal laminin content was associated with a corresponding increment in laminin chain transcript levels, it appears that the mechanisms underlying augmentation in renal matrix laminin content may be distinct in the two types of diabetes.

Recent investigations have drastically changed the notion that the extracellular matrix is an inert entity, its importance being limited to providing mechanical support to cells. The scope of functions of matrix has been extended to include regulation of cell migration, cell division, extracellular fluid filtration, and storage of growth factors (1). Laminins, abundant and ubiquitous components of extracellular matrix, have been implicated in several of these functions (2).

Laminins are heterotrimers composed of α, β, and γ chains. Five α, three β, and two γ chains have been identified to date, as have at least 11 distinct heterotrimers (3,4). Distinct laminins are present in the glomerular and tubular basement membranes and the mesangial matrix. It is presently thought that the mature glomerular basement membrane contains laminin-11 composed of α5, β2, and γ1 chains, whereas the mesangium contains laminin-1 and/or laminin-2 consisting of α1/2, β1, and γ1 chains (4). The composition of laminin in tubular basement membrane appears to be more complex and variable, with α1/5, β1, and γ1 chains involved in the formation of laminin-1 and laminin-10 (4,5). Although laminin may potentially play many roles in renal function and development, the best-documented role is in the maintenance of structural integrity and selective permeability function of the glomerular basement membrane (6). Thus, mice deficient in β2 chain (formerly s-laminin) develop severe proteinuria despite normal contents of type IV collagen, agrin, perlecan, and entactin in the glomerular basement membrane. The structural abnormalities in the glomerulus are limited to occasional thickening and out-pocketing of the glomerular basement membrane, and fusion of foot processes of glomerular epithelial cells (6). Abnormalities in laminin deposition have been reported in a variety of renal diseases, including diabetic nephropathy, membranous glomerulonephritis, focal and segmental glomerulosclerosis, lupus nephritis, and polycystic kidney disease (7–11).

The status of laminin in renal parenchyma in type II diabetes has not been studied in detail, although previous studies have addressed changes in renal laminin in type I diabetes (7,8). In the present study, our aim was to investigate changes in renal parenchymal laminin content and its regulation in the db/db mouse, an animal model of type II diabetes.

Materials and Methods

Type II diabetes was studied in the db/db mice (Jackson Laboratories, Bar Harbor, ME). Lean littermate mice served as nondiabetic controls. Three separate groups of mice were used in this study. At each time point, i.e., 1, 2, 3, and 4 mo, eight to 10 controls and an equal number of diabetic animals were studied. Body weight, plasma
glucose concentration, and 24-h urine excretion of albumin and creatinine were monitored in both the control and diabetic mice at 1-mo intervals for up to 4 mo. The animals were kept in individual metabolic cages and urine was collected for 24 h. Plasma glucose levels were measured with a Beckman glucose analyzer-2. Urine albumin levels were determined in 24-h urine samples from control and db/db mice by a sandwich enzyme-linked immunosorbent assay that used sheep anti-mouse albumin antibody as the detecting antibody, and goat anti-mouse albumin antibody as the capturing antibody. The latter was conjugated to peroxidase and the amount of antibody binding was estimated in a peroxidase reaction. Urine albumin excretion was normalized to urine creatinine; the latter was measured using a commercially available creatinine assay kit (Sigma Chemical Co., St. Louis, MO). Animal studies had the prior approval of the Institutional Animal Care Committee.

**Immunohistochemistry**

Kidney sections from the control and diabetic animals from each experiment were processed together under identical conditions. Small pieces of renal cortex were snap-frozen on aluminum foil strips in liquid nitrogen and stored in cryovials at −70°C. Frozen sections (6 μm thick) were cut and processed for subsequent immunoperoxidase detection (12). A rabbit polyclonal antibody with reactivity against mouse laminin-1 (α1, β1, and γ1) (Collaborative Research/Becton Dickinson, Bedford, MA) was used in an immunoperoxidase reaction on kidney sections from control and diabetic mice. Although the antibody is raised against laminin-1, it can, in theory, recognize component chains of other laminins, e.g., γ1 chain in laminin-2 and laminin-11. Therefore, changes in staining with this antibody will be referred to as changes in the laminin chains. Additionally, a rat monoclonal antibody against mouse laminin β1 chain (5A2) was used in an immunoperoxidase reaction to evaluate the status of β1 chain of laminin in murine kidney (13,14). The status of α5 chain of laminin was examined by immunofluorescence, using a polyclonal rabbit antibody (4) and FITC-linked donkey anti-rabbit IgG as the second antibody (Chemicon International, Temecula, CA). The intensity of immunostaining was evaluated in a minimum of 30 glomeruli in each tissue section from two to six mice at each time point in each group. The data were compared on an arbitrary scale of 1+ to 4+. Data were obtained from two individual batches of mice at each time point.

**RNA Extraction and Northern Analysis**

Because glomerular isolation procedures result in low yield in mice, renal cortices were dissected from the kidneys and homogenized using precautions to prevent degradation of RNA. Total RNA was extracted by RNAzol method (Tel-Test, Friendswood, TX) and analyzed for content using standard 260/280 optical density spectrophotometric readings (15,16). RNA integrity was confirmed by visualizing ethidium bromide-stained 28S and 18S ribosomal bands on 1% agarose gels. Northern analysis was performed, as described previously, on total RNA from renal cortices of individual mice in separate experiments using three distinct batches of animals (15,16).

Forty micrograms of total RNA extracted from renal cortices of control and diabetic mice was electrophoresed on a 1% agarose, 2.2 M formaldehyde gel with 1× 4-morpholinepropanesulfonic acid buffer, transferred to a nylon membrane using TurboBlotter System (Schleicher & Schuell, Keene, NH), and cross-linked by exposure to ultraviolet light. The membrane was prehybridized in 50% formamide, 5× SSC, 150 μg/ml denatured salmon sperm DNA, sodium pyrophosphate-ethylenediaminetetra-acetic acid buffer for 1 h at 42°C. Hybridization was performed in the same buffer with 3 to 5×10^5 cpm/ml

---

**Table 1. Parameters of type II diabetes in the db/db mice**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>1 mo (n = 21 to 35)</th>
<th>2 mo (n = 21 to 35)</th>
<th>3 mo (n = 21 to 35)</th>
<th>4 mo (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Body weight (g)</strong></td>
<td>23.2 ± 1.2</td>
<td>38.2 ± 0.8</td>
<td>47.1 ± 0.8</td>
<td>23.0 ± 0.7</td>
</tr>
<tr>
<td><strong>Plasma glucose (mM)</strong></td>
<td>11.1 ± 0.2</td>
<td>30.0 ± 1.8</td>
<td>41.3 ± 0.4</td>
<td>12.7 ± 0.2</td>
</tr>
<tr>
<td><strong>U_{alb}/U_{creat} (mg/g)</strong></td>
<td>0.075 ± 0.003</td>
<td>0.070 ± 0.003</td>
<td>0.069 ± 0.003</td>
<td>0.054 ± 0.002</td>
</tr>
</tbody>
</table>

* U_{alb}/U_{creat}, ratio of urine albumin excretion to urine creatinine excretion.

P < 0.001, P < 0.005,
32P-labeled partial cDNA probes for mouse laminin \( \beta_1 \) and \( \gamma_1 \) chains (kindly provided by Y. Yamada, National Institute of Dental Research, Bethesda, MD) and \( \alpha_5 \) chain of laminin (17). The cDNA probes were labeled by the random priming method (Amersham, Arlington Heights, IL). RNA loading was corrected by hybridization with 32P-labeled cDNA probe for GAPDH. The blot was washed in 2\( \times \) SSC, 0.1\% SDS for 15 min at 45°C twice, followed by a wash in 0.2\( \times \) SSC, 0.1\% SDS for 15 min at 50°C and autoradiographed. The intensity of hybridization bands was measured by laser densitometry (Hoefer Scientific Instruments, San Francisco, CA).

**In Situ Expression of Laminin \( \alpha_5 \) Chain**

Two *in situ* hybridization experiments were performed on separate batches of mice. In each experiment, investigations involved four mice with diabetes with four respective controls, at 1 mo and 3 mo of the disease (eight control and eight diabetic mice in all). In each mouse, we examined at least 10 to 15 glomeruli and the surrounding tubules. Synthesis of riboprobe, tissue preparation, *in situ* hybridization, and autoradiography has been described previously (12,18,19). A 392-bp laminin \( \alpha_5 \) cDNA fragment (bases 724-1115) subcloned into BlueScript II SK+ (clone DI5) was used for the generation of 35S-labeled antisense riboprobe; sense riboprobe served as a negative control. Linearized cDNA was transcribed *in vitro* using a Riboprobe System II kit (Promega, Madison, WI). Either T3 or T7 RNA polymerase and [35 S]-uridine-5'- (a-thio)-triphosphate (1300 Ci/mmol; New England Nuclear, Boston, MA) were included in the reaction mixture to generate [35S]-labeled antisense and sense riboprobes. The reaction mixture was incubated for 60 min at 40°C, then the DNA template was removed by digestion with 0.5 U of RNase-free DNase, followed by removal of unincorporated nucleotides by phenol/chloroform extraction and ethanol precipitation. RNA probes (activity approximately \( 4 \times 10^6 \) cpm/\( \mu l \)) were stored at \(-70^\circ\)C and used within 3 d. Frozen sections (6 \( \mu m \)) were cut and collected onto aminosilane-glutaraldehyde-treated slides, and fixed for 20 min in 4%
paraformaldehyde in 0.01 M phosphate-buffered saline, pH 7.4. The sections were washed twice in phosphate-buffered saline, dehydrated through a graded series of ethanols, air dried, and immediately used for in situ hybridization. In situ hybridization procedures were performed as described previously, involving prehybridization, hybridization, and removal of nonspecifically bound probe (18,19). Prehybridization steps included treatment with 0.2N HCl, proteinase K (1 µg/ml), and acetic anhydride to block background and enhance probe penetration. Twenty-five microliters of hybridization mixture containing 50% formamide, 10% dextran sulfate, 10 mM dithiothreitol, 0.1 M Tris-HCl, pH 7.5, 0.1 M sodium phosphate, 0.3 M sodium chloride, 50 mM ethylenediaminetetra-acetic acid, 1× Denhardt’s solution, 0.2 mg/ml yeast tRNA, and 2× 106 cpm of [35S]-labeled riboprobe was applied to each section and covered with a siliconized coverslip. Hybridizations were performed in a sealed humid chamber for 18 h at 50°C. Excess probe was removed by washing in 10 mM Tris-HCl, pH 7.5, 1 mM ethylenediamine tetra-acetic buffer, and the sections were treated with RNase A to decrease nonspecific background activity and rinsed in 2× SSC. Sections were dehydrated in graded ethanols, air-dried, and immersed, in the dark, in Kodak NTB-2 photographic emulsion (Eastman-Kodak, Rochester, NY). After air drying, the sections were exposed for 4 wk at 4°C. The emulsion was developed and sections were stained with hematoxylin and eosin for subsequent bright- and dark-field light microscopic analysis.

Statistical Analyses
Data are expressed as mean ± SEM and analyzed for group differences using the two-sample t test. P < 0.05 was considered statistically significant.

Results
Type II Diabetes
The parameters of type II diabetes are shown in Table 1. Plasma glucose concentrations in the db/db mice ranged between 30 and 45 mM for up to 4 mo of diabetes, significantly higher than those in the control mice (Table 1). Urine albumin excretion corrected to urine creatinine was elevated 13- to 44-fold in diabetic mice compared with control mice for the duration of the study (Table 1).

Changes in Renal Laminin Content
Mice with type II diabetes showed an increase in mesangial matrix at both 1 and 4 mo of diabetes, as demonstrated by periodic acid-Schiff and methenamine silver stains (data not shown). Changes in renal matrix correlated with the increment in the renal content of laminin. At 4 mo of diabetes, immunoperoxidase reaction with the polyclonal anti-laminin trimer antibody revealed a significant increase in laminin chain content of the diabetic renal cortex (Figure 1B) relative to corresponding control (Figure 1A). Staining with a specific rat monoclonal antibody against mouse β1 chain of laminin also revealed enhanced deposition of the laminin chain in the mesangial matrix and the tubular basement membranes of mice diabetic for 4 mo compared to control mice of the same age (Figure 1, C and D).

Immunofluorescence with antibody against α5 laminin chain revealed a restricted distribution in normal mice. It was present throughout the glomerular basement membrane, but only some segments of tubular basement membrane and Bowman’s capsule expressed the protein. In addition, small amounts of the peptide could be seen in the mesangium of the older mice (Figure 1E). In diabetic mice, an increase in α5 chain content was seen in the glomerular and tubular basement membranes, and with longer duration of diabetes there was a prominent increase in mesangial expression of laminin α5 chain (Figure 1F).

Serial changes in the staining intensities of the antibodies against laminin (α1, β1, and γ1), laminin β1, and α5 chains were evaluated and the results are shown in Table 2. In the control mice, there was a mild increase in the content of these proteins over 4 mo. In the diabetic mice, there was a clear and easily detectable increment in the laminin chains, including the β1 and α5 chains, at every time point studied compared to control mice of the same age. Additionally, within the diabetic group there was also a progressive accumulation of laminin trimer, and the β1 and α5 chains with time.

To examine whether the increase in laminin content in murine diabetic kidney was due to enhanced gene expression of laminin, we performed Northern analysis (data from four experiments). At 1, 2, 3, and 4 mo of diabetes, the mRNA abundance of laminin β1 chain in total RNA from renal cortex was decreased by 27 ± 9% (P < 0.05), 56 ± 4.7% (P < 0.001), 67 ± 13.6% (P < 0.005), and 58 ± 11.6% (P < 0.005), respectively (Figure 2). The course of changes in γ1 chain mRNA abundance in diabetic renal cortex was similar (data from six experiments). At 1, 2, and 3 mo of diabetes, there was a 29 ± 7.1% (P < 0.01), 46 ± 7% (P < 0.001), and 41 ± 4% (P < 0.001) reduction, respectively; however, at 4 mo, there was almost a full recovery of laminin γ1 chain mRNA abundance relative to control (Figure 2). Evaluation of α5 chain mRNA levels showed that it was unchanged at 1 mo, but was reduced by 54 ± 20.5% (P < 0.05) and 55 ± 12.7% (P < 0.001) at 3 and 4 mo, respectively.

Table 2. Staining intensities of antibodies against laminin chains

<table>
<thead>
<tr>
<th>Category</th>
<th>1 mo</th>
<th>2 mo</th>
<th>3 mo</th>
<th>4 mo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laminin (α1, β1, γ1)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>db</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Laminin β1</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>db</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Laminin α5</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>db</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

The staining intensities of the antibodies against laminin (α1, β1, γ1), laminin β1, and laminin α5 chains in renal sections of lean litter control (LL) and diabetic (db) mice were evaluated on a 1+ to 4+ scale. At each time point shown, renal sections containing eight to 10 glomeruli and generous areas of renal cortex from two to six mice belonging to at least two individual batches were evaluated. The data represent composite averages of intensities of staining with each antibody in the renal sections from mice studied at each time point.
0.01) at 2 and 3 mo of diabetes, respectively, in the \textit{db/db} mouse (data from three experiments) (Figure 3).

In the Northern analysis experiments, we used total RNA from renal cortex, which is derived mostly from tubular elements. From these data, it was not possible to distinguish whether the changes in laminin chain transcripts were limited to the tubules or also involved the glomerulus. To resolve this issue, we performed in situ hybridization using antisense ribo-probe to detect laminin \(\alpha_5\) chain mRNA in two separate experiments. Each experiment was performed on four mice with diabetes with four respective controls, at 1 and 3 mo of the disease (eight control and eight diabetic mice in all). In each mouse, at least 10 to 15 glomeruli and the surrounding tubules were examined. At both 1 and 3 mo, the diabetic mouse kidneys displayed consistent and easily detectable reductions in the grain density over the glomeruli and a suggestion of a

Figure 2. Regulation of renal laminin \(\beta_1\) and \(\gamma_1\) chains in mice with type II diabetes. (A) Northern analysis was performed on 40 \(\mu\)g of total RNA from the renal cortex of control (Ctrl.) and diabetic (Diab.) mice at 1, 2, 3, and 4 mo of diabetes. Each lane represents data from an individual animal. A representative blot from six experiments is shown. Note the decrease in laminin \(\beta_1\) chain mRNA abundance in the renal cortex at each time point. The laminin \(\gamma_1\) chain mRNA levels were also reduced at 1, 2, and 3 mo of diabetes; at 4 mo there was full recovery. (B) Composite data from four to six experiments are shown in graph form: \(\beta_1\) chain and \(\gamma_1\) chain mRNA in control (C), \(\beta_1\) chain mRNA in diabetic mice (B1), and \(\gamma_1\) chain mRNA in diabetic mice (G1). \(*P < 0.05; **P < 0.01; \#P < 0.001; ***P < 0.005\) compared to respective control.
similar change over the tubules (Figure 4). When the data from Northern analysis and in situ hybridization are considered together, it is evident that reduction in laminin transcripts involves both the glomerulus and the tubules in nephropathy complicating type II diabetes.

In a separate experiment, we evaluated the specificity of mRNA regulation by estimating the abundance of \( \alpha_1 \) chain of type IV collagen and laminin \( \beta_1 \) chain transcripts in the same samples. Previous studies have shown that type IV collagen gene expression is increased in renal cortex of \( db/db \) mouse at 4 mo of age (20). As shown in Figure 5, a 60% reduction in mRNA abundance of laminin \( \beta_1 \) chain was associated with 62% increase in \( \alpha_1(IV) \) collagen chain mRNA levels, indicating that the regulation of renal laminin is distinct from \( \alpha_1(IV) \) collagen in type II diabetes.

Discussion

Our data show that renal laminin metabolism is altered in previously unrecognized ways in mice with type II diabetes. First, the increase in renal laminin content appears to be due to mechanisms other than enhanced gene expression. In the \( db/db \) mouse, augmented laminin deposition in renal parenchyma was associated with a decrease in the expression of \( \alpha_5, \beta_1 \), and \( \gamma_1 \) chain genes. Second, noncoordinate regulation is observed in the metabolism of individual chains of laminin in the diabetic mouse. For instance, the \( \gamma_1 \) chain transcript levels normalize between the third and fourth months of diabetes, but not those of \( \beta_1 \) chains. Third, the distribution of \( \alpha_5 \) laminin chain is altered. This protein is normally restricted to the glomerular and tubular basement membranes (Figure 1E) (4), but is prominently expressed in the mesangium of the diabetic kidney (Figure 1F).

The changes in renal laminin metabolism have been studied in greater detail in type I diabetes than in type II diabetes. Fukui et al. reported a progressive increment in mRNA levels of laminin \( \beta_1 \) and \( \gamma_1 \) chains in glomeruli of rats at 1, 3, and 6 mo of streptozotocin-induced diabetes (7). In a model of spontaneous type I diabetes in mice (nonobese diabetic [NOD] mice), the increment in laminin \( \beta_1 \) chain transcript was observed at 1 mo of diabetes in association with increase in laminin protein deposition in the glomerulus (8), similar to rats with type I diabetes. In contrast to the NOD mice with type I diabetes and our data in the \( db/db \) mice with type II diabetes suggest that the differences in renal laminin gene expression between the two types of diabetes are most likely related to metabolic factors that are unique to type II diabetes. Ledbetter et al. have reported that laminin \( \beta_1 \) chain transcripts were essentially unchanged in the renal cortex of KKAy mouse, a model of type II diabetes; however, the status of laminin protein was not examined (21).

The combination of increased renal laminin immunoreactivity and decrease in its mRNA levels in type II diabetes suggests decreased turnover of laminin as a potential mechanism of laminin accumulation. This could occur by decreased degradation of laminin by matrix metalloproteinases (MMP) and/or resistance of laminin to breakdown. For example, MMP-3 has been implicated in the degradation of laminin (22), and diminished MMP-3 transcript expression has been observed in the kidneys of humans with type II diabetes (23). In type I diabetes, both the gene expressions and activities of renal MMP have been reported to be reduced (24–27). At the level of tubular basement membrane, diminished degradation of laminin could be due to altered activity of meprin-A, a neutral protease that is richly present in the proximal tubules (28). Preliminary observations indicate that activity of meprin-A is diminished in a time-dependent manner in tubules of rats with
streptozotocin-induced diabetes (29), although its activity in type II diabetes has not been evaluated.

An additional mechanism underlying accumulation of laminin in kidneys of db/db mice could include resistance of laminin to catabolism in the setting of diabetes. Laminin has been shown to be susceptible to nonenzymatic glycation (30). Glomerular basement membranes glycated in vitro or isolated from diabetic kidneys have been shown to resist degradation (31,32).

A third possible mechanism is that the efficiency of translation of the laminin transcripts may be increased in the diabetic kidney. This is plausible because insulin, a potent stimulus for protein translation, is increased in the plasma of db/db mice with type II diabetes. Insulin is known to activate the eukaryotic initiation factor 4E system leading to increased efficiency of translation (33). Addition of insulin to rat mesangial cells grown in routine culture medium increases the synthesis of β1 and γ1 chains (34). The role of insulin in regulation of laminin metabolism in the kidney in type II diabetes remains to be investigated.

The decrease in α5, β1, and γ1 chain transcripts below the control level in association with an increase in laminin protein content raises the possibility of a negative feedback mechanism in which accumulating laminin affects its own gene expression. We have no evidence to refute or confirm the hypothesis of negative feedback regulation, but such a feedback mechanism in laminin regulation has been postulated previously (6). During normal renal development, β1 chain of laminin appears in the glomerular basement membrane in the initial stages but is lost during maturation, replaced by β2 chain (5). In the β2 laminin chain-deficient mice, β1 chain remains associated with the mature glomerular basement membrane (6). These data suggest that β2 chain deficiency may be detected by the embryonic renal elements in some way, resulting in retention of β1 chain in a compensatory effort, and that in the normal embryo, a feedback mechanism may regulate the switch from β1 to β2 chain in the maturing glomerular basement membrane (5).

In summary, the regulation of laminin differs between the two types of diabetic nephropathy in animal models. Whereas augmented gene expression contributes to laminin accumulation in the renal parenchyma in type I diabetes (7,8), the increase in laminin content in the renal tissue of mice with type II diabetes is due to mechanisms other than enhanced gene expression. Wolf et al. have reported that in the db/db mice, mRNA levels of a cyclin kinase inhibitor, p27Kip1, are unchanged despite an increase in its protein expression (35). We anticipate this paradigm to be revisited in the metabolism of a variety of proteins in type II diabetes. Recent observations indicate that there are other differences in the pathogenesis of matrix changes between the rodent models of two types of diabetes. Whereas the transcripts of TGF-β1 are increased in the glomeruli of rats with type I diabetes (36), there is no change in steady-state mRNA levels in the renal cortex of db/db mice with type II diabetes, with the expression of type II receptor for the growth factor increased instead (37). Thus, the
pathogenesis of renal matrix expansion in rodents with the two types of diabetes can no longer be considered identical.

Acknowledgments

These studies were supported in part by the Veterans Administration (Drs. Kasinath and Barnes), the American Diabetes Association (Dr. Kasinath), and the National Kidney Foundation of South Texas (Dr. Kasinath). We thank Dr. Hanna E. Abboud for thoughtful comments on the manuscript and Dr. Lynda F. Bonewald for technical assistance. Dr. Y. Yamada (National Institutes of Health, Bethesda, MD) kindly provided the cDNA probes for β1 and γ1 chains of laminin.

References

29. Kaushal GP, Xiong X, Shah S: Alterations in matrix-degrading


