Effects of Mesangium Glycation on Matrix Metalloproteinase Activities
Possible Role in Diabetic Nephropathy

S.V. McLennan, S.K.Y. Martell, and D.K. Yue

High glucose concentrations can decrease degradation of mesangium by reducing the activities of matrix metalloproteinases (MMPs). The aim of this study was to investigate the effects of glycation of mesangium matrix on MMP-2, the principal MMP secreted by mesangial cells to degrade type IV collagen. Also examined were membrane type 1 MMP (MT1-MMP), tissue inhibitors of MMPs (TIMP)-1 and -2, and transforming growth factor-β (TGF-β), which together regulate MMP-2 activities in an interacting manner. Human fetal mesangial cells were grown on mesangium matrix glycated by incubation in 500 mmol/l ribose, with or without aminoguanidine. The activities and gene expression of the abovementioned enzymes/inhibitors were measured by degradation of radiolabeled mesangium matrix, RT-PCR, and zymography. Glycation of mesangium matrix resulted in a threefold increase in advance glycation end products and reduced by 45% the matrix-degrading activity of MMPs secreted by mesangial cells. Analogous to the direct effects of high glucose concentrations, glycation of matrix increased the gene expression of MMP-2 and TIMP-1 (control 100 ± 16.9 vs. glycated 197.3 ± 30.6%; control 100 ± 5.3 vs. glycated 152.1 ± 20.1%, respectively; P < 0.05) and decreased MT1-MMP (control 100 ± 1.17 vs. glycated 54.1 ± 15.2%; P < 0.05). However, unlike high glucose concentrations, glycation was not associated with decreased activation of MMP-2. Similarly, glycation but not high glucose increased expression of TIMP-2 (control 100 ± 5.9 vs. glycated 168.2 ± 31.4%; P < 0.05), and the effects of glycation on degradation can be abolished by anti-TIMP-2 antibody. Glycation of matrix decreased TGF-β mRNA by 38.2% and total and active TGF-β by 35.5 and 21.5%, respectively, opposite the effects of high glucose concentrations. Our results indicate that glycation of matrix affects the balance between MMP-2 and its activator and inhibitors, but this phenomenon is not due to TGF-β. The process of glycation may impart to the mesangium matrix a memory effect that contributes to the long-term toxicity of hyperglycemia. *Diabetes* 51:2612–2618, 2002

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The Diabetes Control and Complications Trial and the U.K. Prospective Diabetes Study have shown that hyperglycemia is an important factor in the development of diabetic complications (1–4). There are many biochemical pathways in which the effects of hyperglycemia could be mediated. One of the possible mechanisms is the accumulation of advanced glycation end products (AGEs), which have been shown to correlate with the degree of diabetic complications (3). It is widely known that diabetes is associated with an increase in extracellular matrix (ECM), which, because of its slow turnover, is particularly susceptible to AGE accumulation. ECM plays important roles in the regulation of cell function (5–10), and changes in its composition and structure as a result of AGEs therefore could have profound pathophysiological implications in the genesis of diabetic complications. In this context, mesangium matrix, which is the ECM found within the glomerulus, is of considerable interest because its accumulation correlates closely with renal impairment in diabetes (11–13).

High glucose concentrations have been shown to act on mesangial cells directly to decrease the activities of matrix metalloproteinases (MMPs), the group of enzymes responsible for mesangium matrix degradation (14–16). This phenomenon has been postulated to play a role in the enlargement of mesangium in diabetes (17–22). Whether high glucose can affect mesangial cell MMPs by the advanced glycation of matrix has not been investigated and is the aim of this study.

There are at least 24 different MMPs. The principal type secreted by mesangial cells, MMP-2, also known as 72-kDa gelatinase, is the main MMP responsible for degradation of type IV collagen (15,16,23). MMP-2 is secreted in an inactive form that becomes activated on the cell surface by a membrane type 1 MMP (MT1-MMP) (24–27). The activity of MMP-2 is also regulated by specific tissue inhibitors of MMPs (TIMP-1 and TIMP-2) (15,16,24,28). Our focus in this study was on MMP-2 and these associated enzymes that form a complex regulatory cascade. In addition, as transforming growth factor-β (TGF-β) can directly (29) and indirectly (via its effects on TIMPs) (20,30) modulate MMP-2 activities, the effect of matrix glycation on TGF-β was also examined.

From the Department of Endocrinology, Royal Prince Alfred Hospital, and Department of Medicine, University of Sydney, Sydney, NSW, Australia.

Address correspondence and reprint requests to Dr. S. McLennan, Department of Medicine, University of Sydney, Sydney, NSW 2006, Australia. E-mail: sue@med.usyd.edu.au.

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AG, aminoguanidine; AGE, advanced glycation end product; AP, activator protein; APMA, aminophenylmercuric acetate; ECM, extracellular matrix; ELISA, enzyme-linked immunosorbent assay; MMP, matrix metalloproteinase; MT1-MMP, membrane type 1 MMP; TGF-β, transforming growth factor-β; TIMP, tissue inhibitor of MMPs.
**TABLE 1**

<table>
<thead>
<tr>
<th>Target gene</th>
<th>Oligonucleotide primer sequence</th>
<th>PCR product size (bp)</th>
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<tr>
<td>MMP-2</td>
<td>5’ CGC CGT CGC CCA TCA AGT 3’</td>
<td>400</td>
</tr>
<tr>
<td></td>
<td>5’ TGG ATT CGA GAA AAC CGC AGT GG 3’</td>
<td></td>
</tr>
<tr>
<td>MT1-MMP</td>
<td>5’ CCC TAT GCC TAC ATC CGT GA 3’</td>
<td>560</td>
</tr>
<tr>
<td></td>
<td>5’ TGG GCT GTG CAT ATT C</td>
<td></td>
</tr>
<tr>
<td>TIMP-1</td>
<td>5’ TAC TTC CCA AGG TCC CAC GCC C 3’</td>
<td>339</td>
</tr>
<tr>
<td></td>
<td>5’ GGC TAT CGT GGA CAG GGA CTG CCA 3’</td>
<td></td>
</tr>
<tr>
<td>TIMP-2</td>
<td>5’ CTC TGG AAA CAA CAT TTA TGG C 3’</td>
<td>332</td>
</tr>
<tr>
<td></td>
<td>5’ AGA TGT AGC ACG GGA TCA TGG G 3’</td>
<td></td>
</tr>
<tr>
<td>TGF-β</td>
<td>5’ AGG TCA CCC GCG TGC TAA T 3’</td>
<td>309</td>
</tr>
<tr>
<td>β-Actin</td>
<td>5’ TCA ACC ACT GGC GCA CAA CT 3’</td>
<td>164</td>
</tr>
<tr>
<td></td>
<td>5’ GAA TTC TGG CCA CCG CTG CTT CCA GCT 3’</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5’ AAG CTT TTT CGT GGA TGC CAC ACT AGT 3’</td>
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</table>

**RESEARCH DESIGN AND METHODS**

**Cell culture.** Human fetal mesangial cells were cultured and identified as previously described (31). Briefly, glomeruli were isolated by serial sieving from kidneys obtained from terminations of pregnancies in the second trimester. The mesangial cells were then cultured as outgrowths from the glomerular isolates in RPMI medium containing 10% FCS in 5% CO₂ and air. Cells grown in this manner were used to synthesize 1) matrix for glycation and 2) radiolabeled matrix as the substrate for studying matrix degradation. All experiments were carried out in triplicate and were repeated using cells derived from kidneys from at least three different sources. Cells were always used between the second and fourth passages.

**Glycation of mesangial matrix (as matrix to support mesangial cell culture).** Mesangial cells prepared as described above were trypsinized, plated onto six-well plates (Iwaki, Crown Scientific), and grown in RPMI medium containing 11 mmol/l glucose and 10% FCS. At confluence, the medium was decanted and the cells were washed with PBS and removed from the matrix by incubation in 0.25% NH₄OH at 37°C for 30 min. After washing extensively with distilled water, the matrix was fixed with 70% ethanol and sterilized by exposure to ultraviolet light for 10 min. The matrix was then glylated by incubation in PBS (pH 7.4) containing 500 mmol/l D-ribose at 37°C for 10 days as previously described (32,33). Matrix incubated in PBS alone was used as control. In some experiments, 500 mmol/l aminoguanidine (AG) (Sigma, Sydney, Australia) was added to the 500 mmol/l D-ribose solution to inhibit the formation of AGEs. The degree of matrix glycation was assessed by measurement of fluorescence at an ultraviolet wavelength of 370 nm and emission of 440 nm (Hitachi, Spectrophotometer model L3000). Mesangial cells were subsequently grown on glylated and control matrices produced in this manner, and the effects of glycation of matrix on MMP-2 activities were examined.

**Effects of glycation of matrices on MMP and TGF-β**

**Effects on conditioned media.** Mesangial cells were grown in RPMI medium on a glycated or nonglycated matrix in the presence of normal glucose concentration (5 mmol/l glucose and 10% FCS). At confluence (6 days later), the medium was removed and replaced with FCS-free RPMI containing 5 mmol/l glucose and 0.1% albumin. After an additional 48 h, the conditioned medium was collected, centrifuged at 2000 rpm for 10 min, and stored at −20°C before determination of 1) degradative capacity of the medium (see below); 2) protein concentration of MMP-2, TIMP-1, and TIMP-2 by Western blot and TGF-β enzyme-linked immunosorbent assay (ELISA) (Promega, Sydney, Australia); and 3) activation of MMP-2 by zymography.

**Effects on mesangial cells.** Cells grown on a glycated or nonglycated matrix were washed with PBS and scraped from the wells. The RNA from an aliquot of the cells was extracted for measurement of MMP-2, MT1-MMP, TIMP-1, and TIMP-2 by Western blot and TGF-β gene expression. Another aliquot was resuspended in PBS for 1 min, followed by 28 cycles of 94°C for 15 s, 58°C for 30 s, and 72°C for 30 s. For each gene product, a final extension of 72°C for 7 min was used. The expression of the housekeeping gene β-actin was also measured as control for loading and reverse transcription efficiency. Negative control RT-PCR reactions were performed by omitting either the reverse transcriptase enzyme or the RNA from the reaction mixture. All negative controls failed to produce PCR amplifications (data not shown). The number of cycles for each product was selected to be within the linear range. The resulting PCR products were electrophoretically fractionated on agarose gels (1% containing 0.2 μg/ml ethidium bromide. The gels were photographed using the GelDoc system, and the images were directly digitized using GrabIt software (UVP, Cambridge, Massachusetts) for analysis.

**Measurement of matrix degrading capacity of conditioned media.** Two types of substrates were used for these experiments. The [³⁵S]methionine-labeled mesangial matrix was used to assess overall matrix degradative capacity, and the [³¹H]gelatin was used for specific assessment of gelatinase activity.

**³⁵S]methionine mesangial matrix substrate.** A biosynthetically labeled matrix was synthesized by mesangial cells as described above but in the presence of [³⁵S]methionine. This resulted in the production of a biosynthetic radiolabeled matrix that is used as the substrate for degradation studies. Details of the methods have been described previously (31,34).

For determining matrix degrading capacity of the various conditioned media collected as described above, an aliquot of the media (500 μl) was added to the radiolabeled matrix and incubated at 37°C. After 24 h, the conditioned medium was removed and its matrix degrading capacity was determined by counting the amount of radioactivity released from the matrix to the culture medium. Results were expressed as a percentage of the total amount of radioactivity contained in the radiolabeled matrix. Each experimental condition was studied at least three times, using three replicates per experiment. The matrix degrading capacity of the medium was also determined after activation of secreted enzymes with the organonemural aminophenylmercuric acetate (APMA) (1 mmol/l for 2 h at 37°C). The nonspecific release of radioactivity was measured by incubation of the radiolabeled matrix substrate in PBS (50 mmol/l containing CaCl₂ and 0.1% albumin). This was found to be 7% of the total count incorporated.

**[^H]gelatin substrate.** Gelatin was prepared by denaturation of type I collagen from rat tails at 60°C for 20 min. It was then labeled with [³¹H]acetic anhydride according to the method of Cawston and Barrett (35). Each degradation experiment was performed in triplicate using 100 μl [³¹H]gelatin (1 ng/ml), 50 μl of 200 mmol/l Tris (pH 7.6), 60 mmol/l CaCl₂, and 100 μl conditioned medium. Incubation of the labeled gelatin substrate with 10 μg trypsin in 100 μl H₂O served as a positive control. Samples were incubated at 37°C for 20 h, then placed on ice for 30 min before the addition of 50 μl cold TCA (90%). After an additional 30 min, the samples were centrifuged at 14,000 rpm for 15 min at 4°C to pellet undigested gelatin. The supernatant (200 μl) was then added to 4 ml liquid scintillation fluid (Ultima Gold, Canberra, Packard, Sydney, Australia) and counted in a β counter (2500TR; Packard Instruments, Meriden, CT). Results were again expressed as a percentage of the total radioactivity contained within the gelatin.

**Measurement of mesangial cell gene expression by RT-PCR**

**RNA extraction and reverse transcription.** Mesangial cells were grown to confluence on glycated and nonglycated matrices as described. The cells were washed twice with PBS; RNA was extracted using TRI-reagent (Sigma, Sydney, Australia), and the RNA concentration was determined with the use of the SYBR green RNA assay (Molecular Probes, Eugene, OR). Each reaction mixture contained 1 μg RNA and was reverse-transcribed into cDNA using oligo dT (10 pmol; Gibco-BRL, Sydney, Australia) and Superscript II RNAse H⁻ (Gibco-BRL) according to the manufacturer’s instructions.

**PCR.** MMP-2, MT1-MMP, TIMP-1, TIMP-2, and TGF-β cDNA were amplified using 2 units of Taq polymerase (PE Biosystems, Foster City, CA), 10 pmol of dNTPs, and 20 pmol of each forward and reverse primer (Table 1) and the PCR conditions as follows: MMP-2 (1 μl first-strand cDNA) and TIMP-2 (2 μl first-strand cDNA), 94°C for 2 min, followed by 30 cycles of 94°C for 15 s, 58°C for 30 s, and 72°C for 30 s; MT1-MMP (2 μl first-strand cDNA), 94°C for 2 min, followed by 32 cycles of 94°C for 15 s, 55°C for 30 s, and 72°C for 30 s; and TIMP-1 and TGF-β (1 μl first-strand cDNA), 94°C for 2 min, followed by 28 cycles of 94°C for 15 s, 60°C for 30 s, and 72°C for 30 s. For each gene product, a final extension of 72°C for 7 min was used. The expression of the housekeeping gene β-actin was also measured as control for loading and reverse transcription efficiency. Negative control RT-PCR reactions were performed by omitting either the reverse transcriptase enzyme or the RNA from the reaction mixture. All negative controls failed to produce PCR amplifications (data not shown). The number of cycles for each product was selected to be within the linear range. The resulting PCR products were electrophoretically fractionated on agarose gels (1% containing 0.2 μg/ml ethidium bromide. The gels were photographed using the GelDoc system, and the images were directly digitized using GrabIt software (UVP, Cambridge, Massachusetts).
Measurement of MMPs and TIMPs by Western blot analysis

**Cell/matrix.** The cellular layer was washed in PBS and solubilized in 10% SDS, and total protein concentration was determined using the BioRad DC method (BioRad, Sydney, Australia). For analysis of MT1-MMP and MMP-2, 20-μl aliquots of protein were reduced and run on 10% SDS-PAGE and transferred to polyvinylidene difluoride membranes (Amersham, Sydney, Australia). The membranes were then probed with anti-human MT1-MMP (1:500 dilution; Oncogene, Cambridge, MA) or MMP-2 antibodies (1:1000 dilution; ICN, Sydney, Australia), incubated in the appropriate horseradish peroxidase–conjugated secondary antibodies (Santa Cruz Biotechnology, Santa Cruz, CA), and detected using the ECL detection system (Amersham).

**Conditioned media.** Conditioned media were concentrated using Minicon10 (Millipore, Sydney, Australia), and protein concentration was determined. TIMP-1 and TIMP-2 were electrophoresed on 12.5% SDS-PAGE and MMP-2 was electrophoresed on 10% SDS-PAGE and transferred as above. The membranes were then incubated with anti-human TIMP-1 (1:200; ICN), TIMP-2 (1:200; ICN), or MMP-2 (1:100; ICN) antibodies and detected using appropriate secondary antibodies (Santa Cruz Biotechnology) and ECL.

**Measurement of gelatinase activity by zymography.** Gelatinase activity in the conditioned media or cell/matrix was determined by zymography as previously described (34). Briefly, samples (20 μg protein) were electrophoresed in 10% SDS polyacrylamide gels containing 1 mg/ml gelatin under nonreducing conditions (125 mA/gel). The gels were washed in 50 mmol/l Tris buffer (pH 7.6) containing 2.5% Triton X-100 and subsequently incubated overnight in 50 mmol/l Tris buffer (pH 7.6) containing 5 mmol/l CaCl2. Gels were stained with Coomassie brilliant blue G250 and destained. Gelatinases appear as clear bands on a blue background and are identified by their molecular weight. Band area was determined after photography using the GelDoc system (UVP, Uppland, CA) and quantified using the Phoretix image analysis program. As in previous reports, the predominant band was the 72-kDa proenzyme form of MMP-2, together with a less intense band at 57 kDa being the activated form of MMP-2. The MMP-2 activities were determined by densitometry, and for each sample the total MMP-2 activity (i.e., area 72 kDa + area 57 kDa bands) and percentage activation of MMP-2 (i.e., area 57 kDa band ÷ [area 72 kDa band + area 57 kDa] × 100) band were calculated.

**Effect of addition of anti-TIMP antibodies on degradative capacity of mesangial cells.** For evaluating the effect of TIMP-1 and TIMP-2 on the degradative capacity of mesangial cells, monoclonal anti–TIMP-1 (ICN) or anti–TIMP-2 (clone 67-4H11; ICN) antibody was added in separate experiments to mesangial cells cultured on glycated or nonglycated matrices in 96-well plates. After 72 h, the medium was removed and its degradative capacity was measured. Each experiment was performed in triplicate, and the results were expressed as a percentage of the total count incorporated into the matrix substrate as before.

**Measurement of TGF-β protein by ELISA.** The total and active forms of TGF-β in the conditioned medium were determined by ELISA (Promega) before (active) and after (total) acidification. Results were expressed as a percentage of the values obtained for the nonglycated matrices.

**Statistical analysis.** Each experiment was performed in triplicate at least three times, and the results were expressed as mean ± SD. Data were compared using analysis of variance followed by post hoc comparison using Duncan’s multiple range test.

**RESULTS**

**Glycation of matrix.** After 10 days, there was a threefold increase in AGE-associated fluorescence of the glycated matrix when compared with controls. This increase was prevented by co-incubation in aminoguanidine (Table 2). Growth of mesangial cells on glycated or nonglycated matrices did not show a difference in the cell number or the media protein concentration (Table 2).

**The effects of glycation on matrix degradative capacity of conditioned media from mesangial cells.** The degradative capacity of medium obtained from cells grown on a glycated matrix was significantly lower than that of medium obtained from cells grown on a control nonglycated matrix. This decrease was abolished when advanced glycation was prevented by aminoguanidine (Fig. 1A). Addition of APMA (which activates most MMPs) to the conditioned media uniformly increased by two- to threefold the ability of all samples to degrade matrix without changing the overall pattern (results not shown). To determine whether this decrease in degradation was caused by alterations in gelatinase activity, we used [3H]gelatin as a substrate for the degradation assay. As shown in Fig. 1B, glycation of matrix significantly decreased the gelatinolytic activity. This decrease was again prevented by aminoguanidine.

**Effects of glycation on the MMPs and their inhibitors.** Mesangial cells grown on a glycated matrix showed a significant increase in the gene expression of MMP-2 (Fig. 2A) but a decrease in its cell membrane bound activator MT1-MMP (Fig. 2B). There was a trend that aminoguanidine reversed these changes, although reaching statistical significance only for the former. These

**TABLE 2**

<table>
<thead>
<tr>
<th>Incubation condition</th>
<th>AGE (fluorescence)</th>
<th>Cell number (×10^4 cells/well)</th>
<th>Media protein (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.01 ± 0.82</td>
<td>14.18 ± 2.28</td>
<td>2.38 ± 0.11</td>
</tr>
<tr>
<td>500 mmol/l ribose</td>
<td>15.45 ± 2.42</td>
<td>12.46 ± 3.40</td>
<td>2.44 ± 0.15</td>
</tr>
<tr>
<td>+ AG</td>
<td>6.62 ± 0.34</td>
<td>14.35 ± 2.84</td>
<td>2.43 ± 0.20</td>
</tr>
</tbody>
</table>

Data are means ± SD. *P < 0.05, significantly different from control and 500 mmol/l ribose + AG.

**FIG. 1.** Degradation of [35S]methionine radiolabeled mesangium matrix (A) or [3H]gelatin labeled matrix (B) by conditioned media obtained from mesangial cells grown on control, glycated (500 mmol/l ribose), or glycated + AG (500 mmol/l ribose + aminoguanidine) matrices. Results are disintegrations per minute released to the culture media expressed as percentage of the total amount of count in the matrix. *P < 0.05, significantly different from control and glycated + AG.
changes in MMP-2 and MT1-MMP expression were also seen at the protein level and were again prevented by aminoguanidine (Fig. 2C). An opposite pattern was observed when the gene expression of TIMP-1 and TIMP-2 was studied. Glycated matrix caused an increase in the expression of these two inhibitors that was partially reversed by aminoguanidine (Fig. 3A and 3B, respectively). The pattern of changes in gene expression of MMP-2, TIMP-1, and TIMP-2 was mimicked by their protein concentrations in the media (Fig. 3C). Growth of the cells on glycated or nonglycated matrices did not alter the expression of β-actin (results not shown).

Analysis by zymography of these samples and the corresponding media revealed no effects of glycation of matrix or aminoguanidine on the total amount of MMP-2 as determined by the sum of area under the curve for both proMMP-2 and MMP-2. In addition, glycation of matrix did not affect the amount of activated MMP-2 (i.e., small band preceding the larger band of proMMP-2 in conditioned media [Fig. 4A]). This activated form of MMP-2 was not seen when the cell/matrix layer was analyzed by zymography (Fig. 4B).

**Effects of neutralizing TIMPs on degradative capacity.** The addition of anti–TIMP-1 antibody significantly increased the degradative capacity of media from cells grown on control nonglycated matrices but did not prevent the decrease in degradative capacity caused by glycation of matrix (Fig. 5A). Addition of anti–TIMP-2 antibody again significantly increased the degradative capacity of cells grown on control nonglycated matrices but also abolished the effect of glycation of matrix (Fig. 5B). These actions of anti–TIMP-2 antibodies occurred without apparent changes to the low-molecular-weight MMP-2 band that represents the active form.

**Effects of glycation of matrix on the gene expression of TGF-β.** As shown in Fig. 6A, glycation of matrix led to a reduction in TGF-β mRNA when compared with cells grown on a control nonglycated matrix. The media concentrations of both total and active TGF-β were also decreased (Fig. 6B). These changes in gene and protein

**FIG. 2.** RT-PCR analysis of MMP-2 (A) and MT1-MMP (B) gene expression from samples grown on control, glycated, and glycated + AG matrices. The mean values (± SD) of four experiments expressed as a percentage of control are shown with representative gels. C: Representative results of Western blot analysis of MMP-2 and MT1-MMP obtained from corresponding samples. *P < 0.05, significantly different from control and glycated + AG; **P < 0.05, significantly different from control.

**FIG. 3.** RT-PCR analysis of TIMP-1 (A) and TIMP-2 (B) gene expression from samples grown on control, glycated, and glycated + AG matrices. The mean values (±SD) of four experiments expressed as a percentage of control are shown with representative gels. C: Representative results of Western blot analysis of TIMP-1 and TIMP-2 obtained from media of the corresponding samples. *P < 0.05, significantly different from control.

**FIG. 4.** Zymographic analysis of mesangial cell MMP-2 activity. A: Representative zymogram of results obtained from conditioned media from mesangial cells grown on control, glycated, or glycated + AG matrices. B: Representative zymogram of results obtained from solubilized cell/matrix of mesangial cells grown on control, glycated, or glycated + AG matrices.
were partially blocked by prevention of glycation with aminoguanidine.

DISCUSSION

Diabetic nephropathy is characterized by accumulation of mesangium matrix and thickening of basement membrane within the glomeruli. These changes are the result of an imbalance in the synthesis and degradation of ECM components, in particular collagen, fibronectin, and laminin. How diabetes leads to these changes has been the subject of considerable research. In mesangial cell cultures, high glucose concentrations increase the synthesis (36–40) and decrease the degradation (17,21,31) of ECM. In a previous study, we demonstrated that multiple factors explain the latter effect, one of which is a decrease in the activities of mesangial cell MMPs (21,31). Although this decrease can result from direct actions of a high glucose concentration, there may also be a contribution by indirect effects of glucose on mesangium matrix that is in intimate contact with mesangial cells. There are many examples of how changes in matrix composition can affect cellular differentiation, migration, and proliferation (5–10). One of the major changes to mesangium matrix in diabetes is accumulation of AGEs as a result of chronic hyperglycemia. Recent studies have suggested that this can play an important role in the pathophysiology of cell–matrix interactions in diabetes. For example, glycation of basement membrane from Engelbreth-Holm-Swarm tumor inhibited angiogenesis (33) and decreased tyrosine phosphorylation as well as activation of mitogen-activated protein kinase of endothelial cells (41). Glycation of matrix has also been shown to perturb the stretch receptors of fibroblasts, impairing their ability to contract collagen (42). However, there is a paucity of information about the effects of glycation of matrix in the kidney. Bearing these factors in mind, we therefore sought to determine in the present study whether glycation of matrix can affect the activities of the MMPs and thereby mediate indirectly some of the effects of hyperglycemia.

Results of this study demonstrate that glycated mesangium matrix can decrease the overall ability of MMPs secreted by mesangial cells into conditioned media to degrade ECM. This phenomenon persisted in the presence of APMA, which activates all MMPs, suggesting that glycation causes a decrease in the total amount of MMPs secreted by mesangial cells. These effects of glycated matrix on matrix degrading capacity are similar to those
observed when mesangial cells are cultured in a high glucose milieu (34,43). This is consistent with the hypothesis that some of the actions of high glucose concentrations on matrix degradation are mediated indirectly via glycation of matrix. This notion is further confirmed by the findings that aminoguanidine, an inhibitor of advanced glycation, was able to prevent the changes. Studies in our laboratory show that identical effects of matrix glycation on metalloproteinases are also seen with adult baboon mesangial cells, indicating that the changes observed are not restricted to cells of fetal origin.

Compared with the previous findings of ourselves and others, the effects of glycated matrix on the gene expression of MMP-2, MT1-MMP, and TIMP-1 were similar to those due to high glucose alone (17,34,43). In summary, the expression of MMP-2 and TIMP-1 was increased, whereas that of MT1-MMP was decreased. However, there were some subtle differences. First, TIMP-2 expression was increased only by glycation of matrix. Second, despite the lower MT1-MMP expression when mesangial cells were grown on a glycated matrix, there was not a concomitant decrease in the activation of MMP-2 (i.e., the smaller molecular weight band preceding the main MMP-2 band) that could be observed when mesangial cells were exposed to high glucose alone. The ability of anti–TIMP-2 (but not anti–TIMP-1) antibody to abolish the effects of glycation on overall matrix degradative capacity suggests that TIMP-2 plays a role in mediating the effects of glycation. From previous studies, it is known that TIMP-2 can bind to MMP-2 and MT1-MMP (24,28,44). These two interactions have the opposite effects of enhancing and inhibiting the activation of MMP-2, respectively (44). However, we were not able to show any changes in the activation of MMP-2 by anti–TIMP-2 antibody, suggesting that in this system the main role of TIMP-2 was on direct inhibition of MMP-2 action rather than on activation.

How glycated matrix induces cellular changes is of considerable interest. Among the mechanisms postulated are decrease in tyrosine phosphorylation and decrease in mitogen-activated protein kinase phosphorylation (8,32, 33,41,45). Alterations in these integrin-mediated signaling pathways may affect transcriptional activators (e.g., activator protein [AP]-1). It is noteworthy that many MMPs and TIMPs but not MMP-2 have AP-1 binding sites on their promoters. However, there are other possibilities: both high glucose concentration and glycated albumin have been well documented to increase TGF-β activity (39,46–50). Theoretically, some of our observed changes of MMPs and TIMPs therefore could be due to increased mesangial cell TGF-β when exposed to glycated matrix. However, this was not supported by our experimental findings. In our hand, both active and total TGF-β were actually decreased by glycated matrix.

In summary, the impact of glycation of matrix on mesangial cell behavior is complex and not always identical to that of high glucose alone. What the composite effects of glycation and high glucose are remain to be investigated. Our findings indicate that glycated mesangium matrix could affect one important aspect of mesangial cell behavior: its ability to degrade ECM. This is of importance not only from a mechanistic point of view but also in the clinical context. Clinicians are familiar and frustrated by the all-too-common problem that moderate and advanced diabetic nephropathy cannot be readily reversed by meticulous glycemic control. If the changes observed in our study were applicable in the in vivo situation, then they may serve as a mechanism to explain how a memory effect of high glucose is installed in the matrix, perpetuating the damaging effects of hyperglycemia.

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REFERENCES

43. McLennan SV, Martell SY, Yue DK: High glucose concentration inhibits the expression of membrane type metalloproteinase by mesangial cells: possible role in mesangium accumulation. Diabetologia 43:642–648, 2000