High Glucose Concentration Causes a Decrease in Mesangium Degradation

A Factor in the Pathogenesis of Diabetic Nephropathy

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Mesangium enlargement is a constant feature of diabetic nephropathy and is likely to be important in the pathogenesis of this diabetic complication. Whether decreased degradation of mesangium plays any role in causing the enlargement is uncertain. We developed a system of preparing radioactively labeled mesangium matrix from mesangial cell cultures to be used as substrates for studies of mesangium degradation. Degradation is commenced by growing mesangial cells on the labeled matrix and monitored by the release of radioactivity into the culture medium. High glucose concentration (30 mM), whether present *1*) when the matrix is being made or *2*) when the degradation is taking place, reduces the rate of mesangium degradation. The second but not the first of these two phenomena was abolished by aminoguanidine. Phorbol 12-myristate 13-acetate, added in a manner to antagonize the action of protein kinase C, inhibited mesangium degradation and was not able to nullify the effect of high glucose. Thus it appears unlikely that a high glucose concentration inhibits mesangium degradation by increasing mesangial cell protein kinase C activity. We conclude that decreased degradation of mesangium as a result of hyperglycemia may play a role in causing the mesangium enlargment that occurs in diabetic nephropathy. *Diabetes* 43:1041–1045, 1994

Diabetic nephropathy is a serious complication of diabetes and accounts for much of its morbidity and mortality. The pathogenesis of this condition is not well understood, although it is known that development of the overt clinical phase is preceded for many years by the presence of microalbuminuria and can be delayed by treatment of hypertension (1,2). It is now also established that strict metabolic control can prevent or delay the onset of nephropathy (3). Nevertheless, given the technology currently available, diabetic nephropathy will still occur in a significant number of patients. It therefore remains essential to explore further the mechanism underlying the development of diabetic nephropathy.

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FCS, fetal calf serum; PBS, phosphate-buffered saline; TCA, trichloroacetic acid; PDBU, phorbol 12,13 dibutyrate; PMA, phorbol 12-myristate 13-acetate; SDS, sodium dodecyl sulfate.

Many pathogenetic mechanisms have been studied intensively to explain the occurrence of diabetic nephropathy. One area that has received much attention is enlargement of the mesangium, which is always present in clinically significant nephropathy. The synthesis of various mesangium components has been shown to be increased by a high glucose concentration and likely contributes to mesangium enlargement (4–6). However, regulation of mesangium size is a dynamic process involving degradation as well as synthesis. The phenomenon of mesangium degradation has not been evaluated extensively in diabetes. In this study, we developed a system in which the degradation process can be measured by release of radioactivity from labeled mesangium matrix biosynthetically produced from monolayer culture of mesangial cells. The effects of a high glucose concentration and the possible role of protein kinase C activity in the degradation of mesangium matrix were investigated. Results indicate that a high glucose concentration can indeed reduce the degradation of mesangium matrix and that this process is unlikely to be modulated by changes in protein kinase C activity.

RESEARCH DESIGN AND METHODS

Isolation and culture of glomerular mesangial cells. Mesangial cell cultures were established from glomeruli obtained from human fetal kidneys of between 14- and 20-weeks gestation. Briefly, glomeruli were isolated under sterile conditions by differential sieving of diced renal cortexes. Glomeruli were then incubated in Hanks' buffered saline containing 750 U/ml collagenase (CLS4, Worthington, NJ) for 30 min at 37°C followed by a wash in Hanks' buffered saline to remove epithelial cells (7). The remaining glomerular fragments were plated in RPMI-1640 tissue culture medium (Cytosystems, New South Wales, Australia) containing 10% fetal calf serum (FCS), penicillin (100 U/ml), and streptomycin (100 μg/ml) at 37°C in 5% CO_2 and 95% air. The media were changed every 3 days, and mesangial cell outgrowths appeared after 1–2 days in culture, reaching confluence after 1 week. The mesangial cells were characterized by their morphology, sensitivity to mitomycin C, and negative staining with factor VIII antibody (Dakopatts, Glostrup, Denmark). For these studies, cells were used between passage 3–6.

Preparation of radioactively labeled mesangium matrix. Mesangial cells were cultured in 12-well plates under culture conditions described above but in the absence of antibiotics. When the cells reached ~60% confluence and were laying down matrix rapidly, [^{35}S]methionine (Tran[^{35}]S-label, ICN, Irvine, CA) was added to reach a final concentration of 10 μCi/ml. After 5 days of labeling, the media was removed and the monolayer culture washed once with phosphate buffered saline (PBS) (pH 7.2 containing 2.68 mM KCl, 1.47 mM KH_2PO_4, 0.145 M NaCl, 7.69 mM Na_2HPO_4). The mesangial cells were lyzed by incubation in 0.25 M NH_4OH at 37°C for 30 min and removed from the matrix by washing with water followed by 70% ethanol. The matrix was then dried and exposed to ultraviolet light for 10 min before being washed once again with PBS and used as substrate for the study of mesangium degradation. Control experiments showed that the intraplate variability in the label-

ing of matrix was <6%. In addition, 67 ± 9% of the ^{35}S-label incorporated into the matrix was precipitable with 10% trichloroacetic acid (TCA).

Study of mesangium degradation. Using matrix prepared in this manner, the degradation of mesangium can be studied either by *1*) growing mesangial cells on the radioactively labeled matrix or *2*) adding proteolytic enzymes extracted from mesangial cells or leukocytes to the labeled matrix. Only the findings related to the degradation of matrix by growing mesangial cells will be presented here. Using this system, the degradation of the matrix is commenced by seeding mesangial cells at a density of 4.0–5.0 × 10^4 cells/well into wells containing labeled matrix. The cells are grown to confluence in antibiotic-free RPMI-1640 medium containing 10% heat-inactivated FCS (60°C for 1 h). Degradation was assessed by monitoring the release of ^{35}S radioactivity from the matrix and was measured by counting an aliquot of the culture media on days 1, 3, and 5. The addition of 10% TCA precipitated 15 ± 5% of the count released into the media by degradation, and this was not affected by glucose concentration or the presence of modulators of protein kinase C activity, such as phorbol 12,13 dibutyrate (PDBU) or phorbol 12-myristate 13-acetate (PMA). The nonspecific release of ^{35}S radioactivity from the matrix in the absence of cells was 3 ± 2% of the total incorporation. This was always monitored and subtracted from the count released from the matrix in the presence of cells. Results are expressed as ^{35}S released as a percentage of the total radioactivity incorporated into the matrix. To prevent cellular re-uptake of [^{35}S]methionine released from the mesangium matrix, all degradation experiments were carried out in the presence of 2 mM unlabeled methionine. Each degradation experiment was performed at least twice, and each incubation condition was represented by 6–8 wells. Cell growth over the corresponding period was monitored by trypsinization and counting in a hemocytometer.

Effects of glucose concentration on mesangium degradation. The effects of a high glucose concentration on mesangium degradation were tested in two ways. In the first, high glucose (30 mM) was present during synthesis of the radioactively labeled matrix. Mesangial cells were then plated and grown on the matrix in a normal glucose concentration (9 mM). Thus, degradation of the matrix takes place at a normal glucose concentration. In the second system, radioactively labeled matrix is synthesized by mesangial cells in a normal glucose concentration. Mesangial cells are then plated and grown in a high glucose concentration on this matrix. Thus, degradation occurs in a high-glucose milieu.

The effect of aminoguanidine on the degradation of mesangium matrix was also studied in both systems by adding this compound at a concentration of 12.5 mM to the high-glucose media. To test the effect of osmolarity on the degradation of mesangium matrix, mannitol, sorbitol, and ribose were added to the culture media at a concentration of 21 mM (making a final sugar concentration of 30 mM, including glucose).

Effects of modulating protein kinase C activity on mesangium degradation. The role of protein kinase C in mesangium degradation was studied by adding PMA (1 μM) and PDBU (0.1 μM) to the culture media. To avoid interference with cell plating, PMA or PDBU were added to the culture media 4–6 h after the plating of mesangial cells. Chronic exposure of mesangial cells to PMA at this concentration has been shown to inhibit protein kinase C activity, and PDBU (0.1 μM) has been shown to stimulate protein kinase C activity (7). Degradation of matrix was measured by counting an aliquot of the media as described above. Control experiments showed that DMSO used as the vehicle for both PMA and PDBU had no effect on the degradation of mesangium matrix (results not shown).

Effects of glucose concentration on mesangium matrix composition. The effects of glucose on fibronectin and laminin content in labeled mesangial cells and matrix were determined according to the method of Ayo et al. (4). Briefly, the medium was removed after labeling the cells for 24 h with [^{35}S]methionine. The labeled mesangial cells and matrix were washed three times with cold PBS and extracted with PBS containing 2% NP-40, 1 mM EDTA, and 2 mM PMSF at pH 7.2. [^{35}S]fibronectin and [^{35}S]laminin were determined by immunoprecipitation using anti-human fibronectin (Calbiochem-Behring, La Jolla, CA) and anti-human laminin (Collaborative Research, Bedford, MA) antisera. The reacted fibronectin and laminin were precipitated with protein-A agarose beads (Pansorbin, Boehringer Mannheim, Indianapolis, IN), and the pellets were washed two times with PBS (as above) and four times with washing buffer (50 mM Tris-HCL, pH 8.3, containing 0.45 M NaCl, 0.5% NP-40). Pellets were then solubilized in 5% sodium dodecyl sulfate (SDS) buffer by heating at 100°C for 5 min. An aliquot was counted, and the results were expressed as dpm/well. The identity of the immunoprecipitated material was confirmed by SDS polyacrylamide gel electrophoresis using fibronectin or laminin as standards.

TABLE 1
The effect of glucose on the production of extracellular matrix proteins

	Laminin (dpm/well)	Fibronectin (dpm/well)	Collagen (hydroxyproline μM/μg amino acid)
Glucose (9 mM)	52,670 ± 500	83,640 ± 2,145	0.14 ± 0.02
Glucose (30 mM)	64,070 ± 680*	111,080 ± 3,425*	0.25 ± 0.08*

Data are means ± SD. * P <0.05, significantly different from 9 mM glucose.

For the measurement of collagen, the labeled mesangial cells and matrix were scraped from the plate, washed in PBS, and hydrolyzed in 6 N HCl at 110°C for 18 h. An aliquot of the hydrolysate was then derivatized with phenyl isothiocyanate, and the hydroxyproline content was measured by high-performance liquid chromatography as described previously (5). Data are expressed as micromoles hydroxyproline per microgram amino acid.

Statistical analysis. Results were expressed as means ± SD and compared by analysis of variance with post-hoc comparison using Duncan's multiple range test. Significance was accepted at the $P < 0.05$ level.

RESULTS

Mesangium matrix composition. Similar in behavior to their adult counterparts, fetal mesangial cells and their matrix contain more collagen, laminin, and fibronectin when exposed to a high glucose concentration (4,7,9). Results are shown in Table 1.

Effects of a high glucose concentration on mesangium degradation. The degradation of mesangium matrix made in a high-glucose environment is slower and is not normalized by the presence of aminoguanidine during synthesis. Results are shown in Fig. 1*A*. The rate of mesangium degradation is not affected by mannitol, sorbitol, or ribose (Fig. 1*B*). Degradation of mesangium matrix is also slower when a high glucose concentration is only present during the degradation process. However, in this situation, the effect of 30 mM glucose is abolished by aminoguanidine and can be mimicked by mannitol, ribose, and partially by sorbitol. Results are shown in Fig. 2*A* and *B*.

Effects of modulating protein kinase C activity on mesangium degradation. PMA, added in a manner to antagonize protein kinase C activity, retarded the rate of mesangium degradation in a normal glucose concentration. PDBU, an agonist of protein kinase C activity, increased the rate of mesangium degradation. Results are shown in Fig. 3*A*. In the presence of a high glucose concentration, PMA further reduced the rate of mesangium degradation, although this decrease was not statistically significant (Fig. 3*B*).

Effects of a high glucose concentration on mesangial cell growth. Mesangium matrix synthesized in the presence of a normal or high glucose concentration supported the growth of mesangial cells to the same extent (Fig. 4*A*). High glucose concentration in the culture medium also had no effect on the growth rate of mesangial cells plated on matrix made in a normal glucose concentration (Fig. 4*B*).

DISCUSSION

Enlargement of the mesangium is the most consistent morphological finding in diabetic nephropathy and correlates well with the severity of the clinical disease (1,2,10). It is

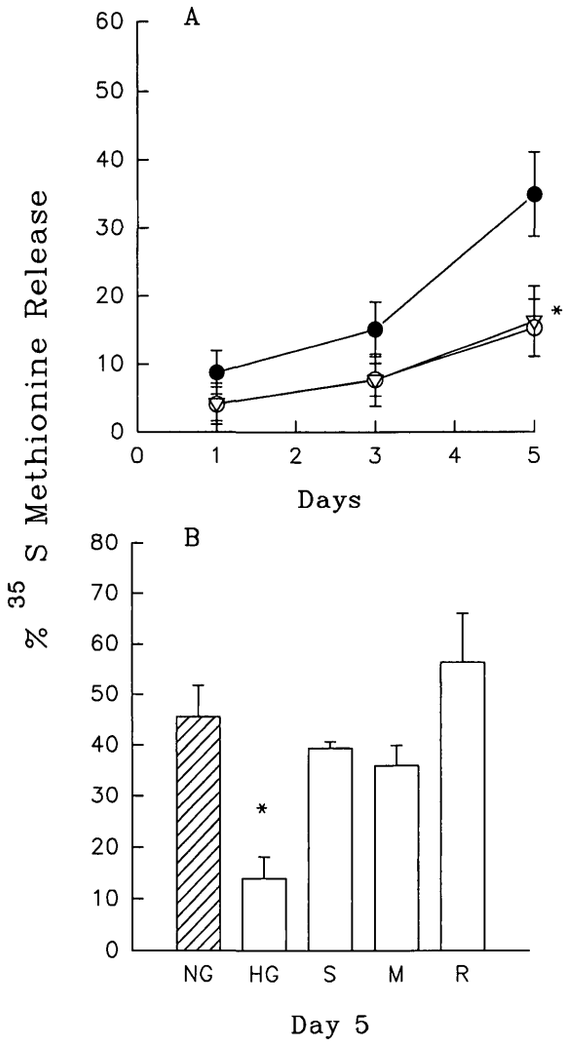

FIG. 1. *A*: degradation of mesangial matrix by mesangial cells grown in 9 mM glucose. Matrix was made by cells grown in 9 mM glucose (●), 30 mM glucose (○), or 30 mM glucose plus 12.5 mM aminoguanidine (▽). Each point represents the mean ± SD of 6–8 wells. *B*: degradation of mesangial matrix by mesangial cells growing in 9 mM glucose for 5 days. Matrix was made by cells grown in 9 mM glucose (NG), 30 mM glucose (HG), 21 mM sorbitol (S), 21 mM mannitol (M), and 21 mM ribose (R). Each point represents the mean ± SD of 6–8 wells. *$P < 0.05$, significantly different from 9 mM glucose.

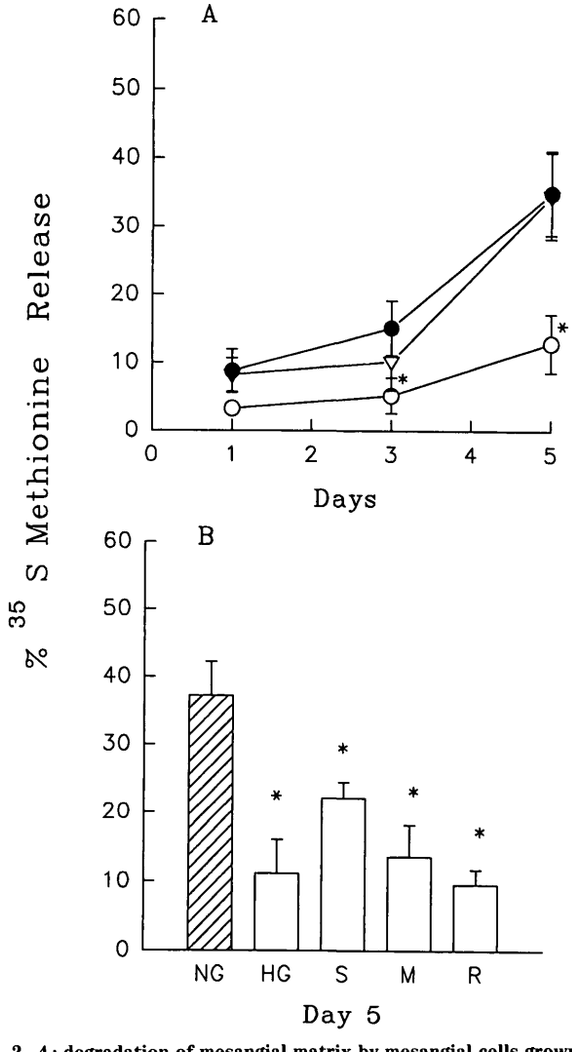

FIG. 2. *A*: degradation of mesangial matrix by mesangial cells grown in 9 mM glucose (●), 30 mM glucose (○), or 30 mM glucose plus 12.5 mM aminoguanidine (▽). Matrix was made by cells grown in 9 mM glucose. Each point represents the mean ± SD of 6–8 wells. *B*: degradation of mesangial matrix by mesangial cells grown for 5 days in 9 mM glucose (NG), 30 mM glucose (HG), 21 mM sorbitol (S), 21 mM mannitol (M), or 21 mM ribose (R). Matrix was made by cells grown in 9 mM glucose. Each point represents the mean ± SD of 6–8 wells. *$P < 0.05$, significantly different from 9 mM glucose.

likely to play a central pathogenetic role in this diabetic complication. The enlarged mesangium can compress the glomerular capillaries and thereby alter intraglomerular hemodynamics. Alteration in the quantity and composition of the mesangium in diabetes can also change cell-matrix interaction, permeability, and many other aspects of glomerular function. These considerations make it vitally important to understand more fully the phenomenon of mesangium enlargement in diabetes.

Many in vitro studies have shown an increase in the synthesis of several matrix components by mesangial cells cultured in high-glucose environments, including that of collagen, fibronectin, and laminin (4,7,10,11). Although from a fetal source, the mesangial cells used for experimental studies in this study showed the same behavior. There is much less information on whether the degradation of mesangium matrix is abnormal in diabetes, partly because of the lack of a suitable model in which the process of degradation can be assessed separately from that of synthesis. In this study, we have overcome this problem by using pre-synthesized radioactively labeled matrix as substrate for measuring the rate of mesangium degradation. This is a different experimental design from the one described by Ayo et al. (4), who found no effect of glucose on the intracellular degradation of matrix proteins. There are two important methodological differences. First, Ayo et al. (4) used a pulse-chase system combined with immunoprecipitation to follow the intracellular synthesis and turnover of fibronectin and laminin, whereas we have studied the degradation of preformed matrix components. We have not identified which matrix components are degraded but have studied the overall degradation process. The relative rates of degradation of the various matrix components are currently being investigated. Second, Ayo et al. (4) used cell/matrix plus media to assess degradation, whereas in our present study, the release of radioactivity into the media was chosen as the endpoint. These methodological differences could contribute to the varying effects of a high glucose concentration on degrada-

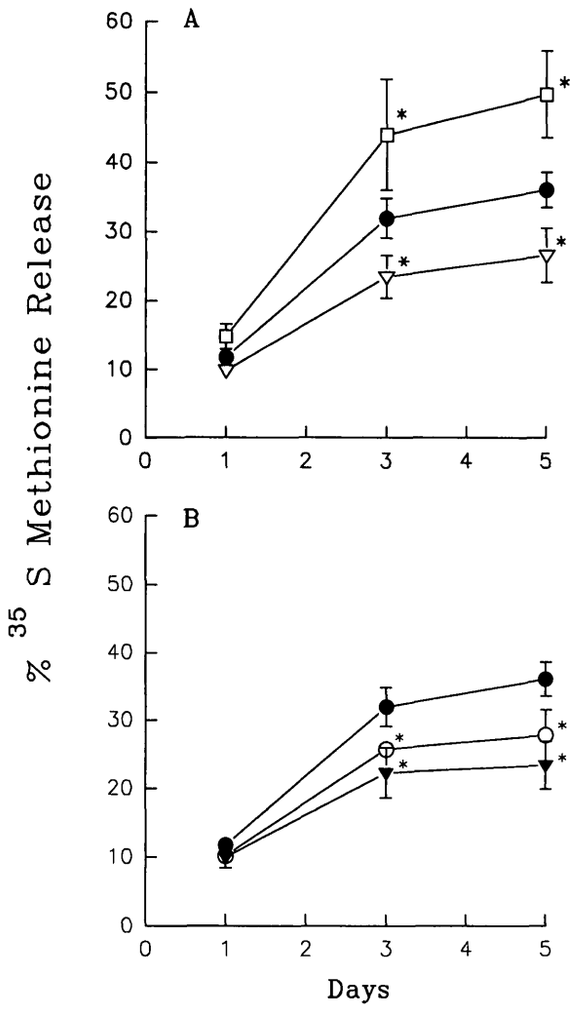

FIG. 3. *A*: degradation of mesangium matrix by mesangial cells grown in 9 mM glucose (●) with the addition of 0.1 μM PDBU (□) or 1.0 μM PMA (▽). Matrix was made by cells grown in 9 mM glucose. Each point represents the mean ± SD of 6–8 wells. *B*: degradation of mesangium matrix by mesangial cells grown in 9 mM glucose (●), 30 mM glucose (○), or 30 mM glucose with the addition of 1.0 μM PMA (▼). Matrix was made by cells grown in 9 mM glucose. Each point represents the mean ± SD of 6–8 wells. *$P < 0.05$, significantly different from 9 mM glucose.

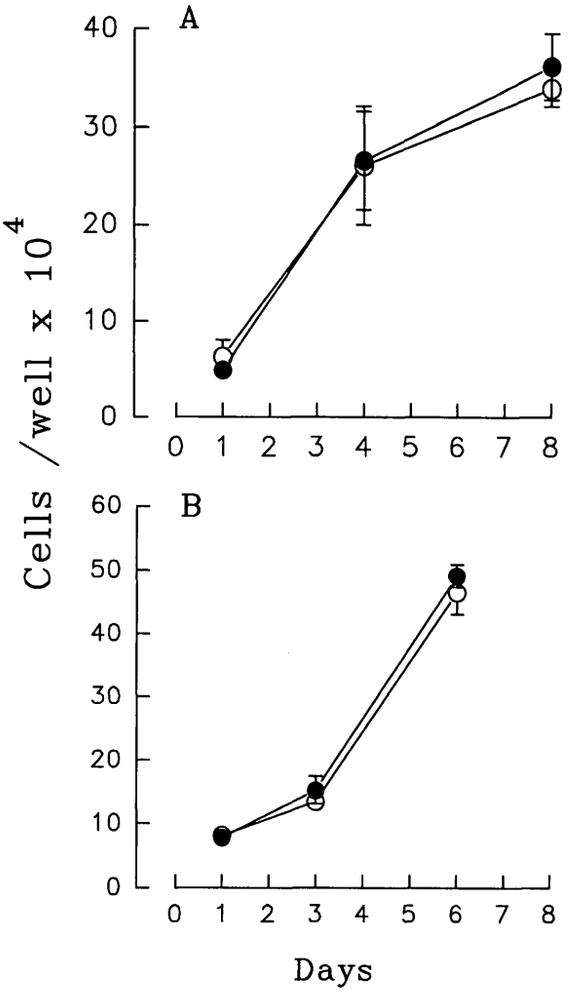

FIG. 4. *A*: growth rate of mesangial cells on matrix made in 9 mM glucose (●) or 30 mM glucose (○). Each point represents the mean ± SD of 6–8 wells. *B*: effect of 9 mM glucose (●) or 30 mM glucose (○) on the growth rate of cells on matrix made in 9 mM glucose. Each point represents the mean ± SD of 6–8 wells.

tion observed in these two studies. For example, Ayo's system would measure the net effect of changes due to synthesis and degradation, whereas in our system, degradation is studied in isolation. Moreover, lysosomal enzymes play a major role in intracellular degradation, but metalloproteinases are likely to be the important enzymes extracellularly.

Our method has proven to be a versatile system that can be used in studying mesangium degradation by live cells or cell extracts. Although only results obtained from [^{35}S]methionine labeling have been shown, we have carried out experiments using [^{3}H]proline- and [^{35}S]sulphuric acid–labeled matrix for specific monitoring of collagen and proteoglycan degradation, respectively. In addition, it is possible to manipulate the composition of the matrix and investigate the effects of these changes on mesangium degradation. Thus far, we have only used TCA precipitation to show that the radioactive materials released into the media are of low molecular weight. We have yet to identify if any component of the matrix is preferentially degraded. Previous studies investigating degradation of the extracellular matrix have used radioactively labeled Matrigel as a substrate (12). In that system, degradation of the matrix occurred only when plasminogen was added, which suggests that a matrix factor may be removed in the preparation of Matrigel.

We found that mesangium synthesized in a high-glucose environment is degraded at a slower rate when reexposed to growing mesangial cells. This supports the notion that decreased degradation plays a role in causing the mesangium enlargement that occurs in diabetes. There may indeed be a reciprocal relationship between extracellular matrix production and the degradation process. Although the precise mechanism has not been defined, from the experimental design it can be concluded that a high glucose concentration can lead to abnormalities in the synthesis or posttranslational modifications of mesangium matrix, which becomes less susceptible to degradation. These effects of glucose were specific because they were not reproduced by adding other sugars. Because the aminoguanidine present during synthesis of the mesangium did not abolish the effects of high glucose, it is unlikely that increased glycation of matrix components plays a significant role.

Moreover, a high-glucose milieu present after the matrix has already been synthesized but at a time when mesangial cells are degrading the matrix also inhibits mesangium degradation. In this system, the high glucose concentration

must be exerting its effects directly on the degradative capacity of the mesangial cells or on the preformed matrix, independent of the other actions it has during synthesis of the mesangium. These actions of glucose appeared less specific, because mannitol and ribose were equally effective. This does not necessarily render our observation physiologically irrelevant, because hyperglycemia in vivo may indeed have some of its action mediated by an osmotic mechanism. The effects of a high glucose concentration during degradation were abolished by aminoguanidine. In addition to blocking formation of advanced glycation end products, this compound is known to inhibit the synthesis of nitric oxide and also to bind free glucose (13,14). Which of its actions plays a role in the observed changes of mesangium degradation remains to be evaluated.

Collectively, these results indicate that decreased degradation can indeed be playing a causative role in the mesangium enlargement of diabetes. This is consistent with the findings of Reckelhoff et al. (15), who showed a decrease in the activities of two classes of proteolytic enzymes in the glomeruli of diabetic rats. The changes in mesangium degradation that we have observed in a high glucose concentration cannot be due to alterations in cell numbers. Although Crowley et al. (16) showed that proliferation is reduced when cells are grown on matrix glycated in vitro, in our system (where matrix is made by cells grown in a high glucose concentration), high glucose concentration has no effect on the growth rate of mesangial cells.

Mesangium matrix degradation is a complex process involving interaction of proteolytic enzymes, their inhibitors, and the mesangium. Mesangial cells have been shown to produce several components of this cascade, including metalloproteinases, tissue inhibitors of metalloproteinases, plasminogen activators, and plasminogen activator inhibitors (17–19). In addition, the serine proteinases, such as plasmin derived from plasma and leukocytes, are also likely to be important, both by acting directly on the mesangium and also synergistically with metalloproteinases (20). Which of these enzymes and their inhibitors are affected by the high-glucose milieu is not clear at this stage, and more specific examination of each will be required. How high glucose concentration and differing duration of exposure affect the mesangium matrix degradation also remain to be determined.

High glucose concentration is known to increase protein kinase C activity in mesangial cells, and many studies have demonstrated the importance of protein kinase C activity in regulating the function of mesangial cells, including the synthesis of various matrix components (8,21–23). Protein kinase C agonists have also been shown to alter the turnover of matrix proteins and to promote metalloproteinase activities (24,25). These considerations led us to investigate whether the effects of high glucose on mesangium degradation were mediated by increased protein kinase C activities. Our results showed that when protein kinase C activity is stimulated or inhibited, mesangium degradation rate changed in the same direction. Moreover, when PMA was added to antagonize protein kinase C activity, it did not nullify the effects of high glucose on mesangium degradation. These observations make it unlikely that the effect of a high glucose concentration on reducing mesangium degradation is mediated by an increase in protein kinase C activity.

Diabetic nephropathy is a major cause of morbidity and mortality in diabetes. Its treatment remains unsatisfactory despite the availability of modern antihypertensive agents, intensive insulin treatment regimen, dialysis, and transplantation. Improvement in the understanding of its pathogenesis will lead to more rational strategies in treatment and prevention.

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REFERENCES

1. Steffes MW, Osterby R, Chavers B, Mauer SM: Mesangial expansion as a central mechanism for loss of kidney function in diabetic patients. *Diabetes* 38:1077–1081, 1989
2. Mauer SM, Steffes MW, Ellis EN, Sutherland DER, Brown DM, Goetz FC: Structural functional relationships in diabetic nephropathy. *J Clin Invest* 97:1143–1155, 1984
3. The Diabetes Control and Complications Trial Research Group: The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *N Engl J Med* 329:977–986, 1993
4. Ayo SH, Radnik RA, Glass WF II, Garoni JA, Rampt ER, Appling DR, Kreisberg JI: Increased extracellular matrix synthesis and mRNA in mesangial cells grown in high glucose medium. *Am J Physiol* 260:F181–F191, 1991
5. Fisher E, McLennan SV, Tada H, Heffernan S, Yue DK, Turtle JR: Interaction of ascorbic acid and glucose on the production of collagen and proteoglycan by fibroblasts. *Diabetes* 40:371–376, 1991
6. Williams B, Schrier RW: Characterization of glucose induced in situ protein kinase C activity in cultured vascular smooth muscle cells. *Diabetes* 41:1464–1472, 1992
7. Danne T, Spiro MJ, Spiro RJ: Effect of high glucose on type IV collagen production by cultured epithelial, endothelial, and mesangial cells. *Diabetes* 42:170–177, 1993
8. Studer RK, Craven PA, DeRubertis FR: Role for protein kinase C in the mediation of increased fibronectin accumulation by mesangial cells grown in high glucose medium. *Diabetes* 42:118–126, 1993
9. Ishimura E, Sterzel RB, Budde K: Formation of extracellular matrix by cultured rat mesangial cells. *Am J Path* 134:843–855, 1989
10. Williamson JR, Tilton RG, Chang K, Kilo C: Basement membrane abnormalities in diabetes: relationship to clinical microangiopathy. *Diabetes Metab Rev* 4:339–370, 1988
11. Kreisberg JI, Ayo SH: The glomerular mesangium in diabetes mellitus. *Kidney Int* 43:109–113, 1993
12. Wong AP, Cortez SL, Baricos WH: Role for plasmin and gelatinase in extracellular matrix degradation by cultured rat mesangial cells. *Am J Physiol* 32:F1112–F1118, 1992
13. Bucala R, Tracy KJ, Cerami A: Advanced glycosylation end products quench nitric oxide and mediate defective endothelium-dependent vasodilation in experimental diabetes. *J Clin Invest* 87:432–438, 1991
14. Requena JR, Vidal P, Cabezas-Cerrato J: Aminoguanidine inhibits protein browning without extensive Amadori carbonyl blocking. *Diabetes Res Clin Pract* 19:23–30, 1993
15. Reckelhoff JF, Tygart VL, Mitias MM, Walcott JL: STZ-induced diabetes results in decreased activity of glomerular cathepsin and metalloprotease in rats. *Diabetes* 42:1425–1432, 1993
16. Crowley ST, Brownlee M, Edelstein D, Satriano JA, Mori T, Singhal PC, Schlondorff D: Effects of nonenzymatic glycosylation of mesangial matrix on proliferation of mesangial cells. *Diabetes* 40:540–547, 1991
17. Alexander CM, Werb Z: Proteinases and extracellular matrix remodelling. *Curr Opin Cell Biol* 1:974–982, 1989
18. Baricos WH, Shah SV: Proteolytic enzymes as mediators of glomerular injury. *Kidney Int* 40:161–173, 1991
19. Davies M, Coles GA, Thomas GJ, Martin J, Lovett DH: Proteinases and the glomerulus: their role in glomerular diseases. *Klin Wochenschr* 68:1145–1149, 1990
20. Thomas GJ, Davies M: The potential role of human kidney cortex cysteine proteinases in glomerular basement membrane degradation. *Biochim Biophys Acta* 990:246–253, 1988
21. DeRubertis FR, Craven PA: Protein kinase C is activated in glomeruli from streptozocin diabetic rats: possible mediation by glucose. *J Clin Invest* 83:1667–1675, 1989
22. Ayo SH, Radnik R, Garoni JA, Troyer DA, Kreisberg JI: High glucose increases diacylglycerol mass and activates protein kinase C in mesangial cell cultures. *Am J Physiol* 261:F571–F577, 1991
23. Dunlop M, Keogh R, Larkins RG: Fibronectin induced increase in mesangial cell prostaglandin release: effect of hyperglycemia and PKC inhibition. *Diabetes* 42:183–190, 1993
24. Burrous BA, Wolf G: The kinetics of fibronectin synthesis and release in normal and tumor promoter-treated human lung fibroblasts. *Mol Cell Biochem* 96:57–67, 1990
25. Herron GS, Werb Z, Dwyer K, Banda MJ: Secretion of metalloproteinases by stimulated capillary endothelial cells. *J Biol Chem* 261:2810–2813, 1986