Elevated glucose increases mesangial cell sensitivity to insulin-like growth factor I

Horney, Mark J., David W. Shirley, David T. Kurtz, and Steven A. Rosenzweig. Elevated glucose increases mesangial cell sensitivity to insulin-like growth factor I. Am. J. Physiol. 274 (Renal Physiol. 43): F1045–F1053, 1998.—To determine the effects of glucose on insulin-like growth factor I (IGF-I)-induced mesangial cell (MC) proliferation, we have examined the relationships between IGF binding protein 2 (IGFBP-2) secretion and proliferation in murine MCs (MMCs). MMCs incubated in high glucose (HG, 25 mM) exhibited a 25–30% reduction in IGFBP-2 secretion compared with cells in normal glucose (NG, 5.6 mM). This loss was not due to cell surface binding; it correlated with a 3.1-fold decrease in IGF-I receptor dependent. MMCs in HG displayed increased IGF-I-stimulated insulin receptor substrate-1/2 phosphorylation and activator protein-1 transcriptional activity compared with NG controls. Accordingly, although IGF-I was not proliferative in NG, it increased [3H]thymidine incorporation and cell number in HG to an extent proportional to the decrease in IGFBP-2. Thus hyperglycemia, as seen in diabetes, may increase MC IGF-I sensitivity by reducing IGFBP-2 expression, in turn increasing its proliferative and secretory responses and contributing to the development of diabetic glomerulosclerosis.

DIABETES IS THE MOST frequent cause of end-stage renal disease in the United States, currently accounting for more than 37% of all new cases annually (51). Diabetic glomerulosclerosis (DG) affects 30–40% of all diabetics (48) and is one of the leading causes of death among diabetic patients (39). The primary cause for the loss of kidney function is the development of sclerotic glomerular lesions, which result from expansion of the mesangium (48). Hallmarks of this expansion include increased cellularity, thickened basement membrane, and copious extracellular matrix (30, 48). The Diabetes Control and Complications Trial completed in 1993 (12), along with several animal studies (15, 29), demonstrated that improving glycemic control slows both the onset and progression of diabetic nephropathy. Hyperglycemia has been further implicated in the development of DG by the demonstration that it stimulates both mesangial cell (MC) proliferation (53) and the secretion of collagen, fibronectin, and laminin by MCs in vitro (1, 53).

The mechanisms whereby elevated glucose causes these responses are still not fully understood, but there is increasing evidence that several growth factors are involved (49). A number of studies have strongly implicated insulin-like growth factor I (IGF-I) as a key element in the development of DG; it is necessary for the early rapid growth phase (2, 20, 45) and can mimic elements of the protracted sclerosis phase, including decreased proteoglycan (32) and increased matrix component (17, 43) secretion. However, conflicting reports exist regarding changes in plasma and renal IGF-I levels (for review, see Ref. 3). In light of these findings, it seems prudent to investigate other events in IGF-I signaling that may be modified by glucose in diabetes, such as number and/or affinity of IGF-I receptors (IGF-IRs), secretion and/or cell surface association of IGF binding proteins (IGFBPs), availability of cytoplasmic signal transduction effectors, and/or expression of other growth factors that act in concert with IGF-I.

In the current study, we focused on defining the effects of elevated glucose on the IGF-BPs. There are at least seven IGFBPs that modulate the actions of IGF-I (for review, see Ref. 26). Recently, altered IGFBP regulation leading to abnormal cellular responses to IGF-I has been described in several diseases which involve accelerated proliferative rates (6, 8, 25). Thus dysregulation of IGFBPs may be a crucial component of the development of diseases involving the IGFs. In the present study, we demonstrate that murine MCs (MMCs) principally secrete IGFBP-2 and that increasing the glucose concentration decreases IGFBP-2 secretion and concomitantly increases MMC sensitivity to IGF-I, as demonstrated by increased signal transduction events and proliferation.

MATERIALS AND METHODS

Reagents. IGF-I was kindly supplied by Genentech (South San Francisco, CA). A cDNA specific for IGFBP-2 was a generous gift of Dr. Shunuchi Shimasaki (Scripps Research Institute, La Jolla, CA). All reagents were of reagent grade or higher.

Cell culture. MMCs were obtained from Dr. Fuad Ziyadeh (University of Pennsylvania School of Medicine) and grown in DMEM with 10% calf serum (GIBCO-BRL, Life Technologies, Grand Island, NY). This cell line was isolated from SLJ/J (H-2s) mice and simian virus SV40 transformed by Wolf et al. (53) and exhibits the characteristics of differentiated MCs.

Ligand blots. Subconfluent monolayers were washed twice with PBS, then incubated for 24 h in serum-free DMEM containing 1 g/l (5.6 mM) or 4.5 g/l (25 mM) glucose, with or without added IGF-I. At 24 h, the conditioned medium was removed, and, after addition of an equal volume of 20% trichloroacetic acid (TCA), the samples were rocked overnight at 4°C. The samples were centrifuged, and the pellets were washed with ice-cold acetone. The precipitates were solubilized in SDS sample buffer without dithiothreitol, run on a 12.5% nonreducing polyacrylamide gel, and transferred to nitrocellulose, using a Semi-Phor semi-dry transfer unit (Hoefer Scientific Instruments, San Francisco, CA). Ligand
blots were performed according to Hossenlopp et al. (24). Briefly, membranes were washed in Tris-buffered saline (TBS; 10 mM Tris-HCl, pH 7.4, 150 mM NaCl, 0.5 g/l Na₂SO₄) containing 3% Nonidet P-40 at 4°C for 30 min, then in TBS containing 1% BSA at 4°C for 2 h, and finally in TBS containing 0.1% Tween 20 at 4°C for 10 min. The nitrocellulose was then incubated overnight at 4°C in TBS/0.1% Tween 20 with 100,000 cpm/ml ¹²⁵I-labeled IGF-I (2,000 Ci/mmol; Amersham Life Science, Arlington Heights, IL). The nitrocellulose was then washed twice with TBS/0.1% Tween 20 at 4°C for 30 min and three times with TBS at 4°C for 30 min. The membrane was then placed on Kodak XAR film for 1 wk at -80°C and developed using a Kodak X-OMAT film developer. To confirm the identity of IGFBP-2, membranes were subjected to the immunoblot procedure described below, using anti-IGF-BP-2 (Upstate Biotechnology, Lake Placid, NY).

Immunoblots. Subconfluent monolayers were serum starved for 24 h in DMEM containing 5.6 mM or 25 mM glucose, followed by addition of 100 nM IGF-I for 1–15 min. The stimulated monolayers were washed with ice-cold PBS containing 2 mM sodium orthovanadate and then collected, pelleted, resuspended in lysis buffer (50 mM HEPES, pH 8.0, containing 10 mM sodium pyrophosphate, 100 mM sodium fluoride, 4 mM EDTA, 1% Triton X-100, 1 mM phenylmethylsulfonyl fluoride, 2 mM sodium orthovanadate, 100 µM benzamidine, 1 µg/ml aprotinin, and 25 µM leupeptin), and incubated at 4°C for 15 min. Insoluble material was removed by microcentrifugation at 13,000 rpm for 30 min at 4°C. Fifty micrograms of each lysate were dissolved in SDS sample buffer and resolved on 10% SDS-polyacrylamide gels according to the procedure of Laemmli (27), using a Hoefer apparatus. Immunoblot analysis was carried out as described by Oenar et al. (34). Tyrosine-phosphorylated proteins were detected with affinity-purified anti-phosphotyrosine antibodies (34) at 5 µg/ml. Blots were developed with peroxidase-conjugated goat anti-rabbit immunoglobulin (Chemicon International, Temecula, CA) and the enhanced chemiluminescence (ECL) system (Amersham) and exposed to Kodak XRP X-ray film for 1–10 min. The autoradiographs were scanned by transillumination using an XRS OmniMedia 3cx scanner, followed by densitometric analysis with NIH Image software version 1.61.

Northern blots. Cells stimulated and collected as described above were resuspended in 500 µl of Ultraspec reagent (Biotec Laboratories, Houston, TX) and vortexed for 1 min at high speed. Chloroform (100 µl) was added, and the samples were vortexed for 15 s at high speed, incubated on ice for 5 min, and then microcentrifuged for 15 min at 13,000 rpm at 4°C. The upper aqueous phase was removed, an equal volume was added, and the RNA was allowed to precipitate for 10 min at 4°C. After centrifugation, the pellet was washed with 70% ethanol, dissolved in 10 µl of diethyl pyrocarbonate-treated H₂O containing 1 mM EDTA and vortexed for 5 s at low speed. RNA content was measured by absorbance at 260 nm. Five micrograms of total RNA from each sample were resolved by electrophoresis on a 1% formaldehyde-agarose gel and transferred to charged nylon membranes by overnight capillary transfer. The membranes were baked for 2 h at 80°C. After overnight prehybridization, the membranes were hybridized overnight in 50% formamide at 42°C with a 32P-labeled probe for IGF-BP-2, prepared from the cDNA obtained from Dr. Shunichi Shimakasi using a Prime-A-Probe DNA labeling kit (Ambion, Austin, TX), according to the manufacturer’s instructions. The membranes were washed, sealed in a bag, and exposed to Kodak XAR film.

Competition binding assays. Binding assays were performed essentially as previously described (33). Briefly, cells were grown to confluence in 12-well plates, washed with HEPES-magnesium saline (HMS) buffer (50 mM HEPES, pH 7.4, containing 0.15 M NaCl, 5 mM MgCl₂, 0.2% BSA, and 0.01% soybean trypsin inhibitor), and incubated at room temperature for 30 min with 20,000 cpm of ¹²⁵I-IGF-I (2,000 Ci/mmol; Amersham) and various concentrations of cold IGF-I (10⁻¹⁰–10⁻⁵ M). The monolayers were then washed with HMS and solubilized in 2 N NaOH, and bound radioactivity was quantified in a gamma spectrometer (Compugamma; Pharmacia Biotech, Piscataway, NJ). Data were subjected to Scatchard analysis using Radlig software (Biosoft, Ferguson, MO).

Transient transfections. With the use of the calcium phosphate method (42), subconfluent MMCs in 60-mm dishes were transfected with 1–2 µg 3x-tumor-promoting agent response element-chloramphenicol acetyltransferase (3xTRE-CAT) reporter construct (31). After 5 h, the medium was aspirated, and the monolayers were shocked for 1 min with medium containing 10% fetal bovine serum and 25% glycerol. The monolayers were rinsed three times with Puck's and fed with DMEM/10% calf serum. Eighteen hours after refeeding, cells were rinsed and infected with DHEM containing experimental additions was added. Twenty-four hours later, the cells were rinsed with PBS, harvested, and centrifuged. The pellet was resuspended in 60 µl of reporter lysis buffer (Promega, Madison, WI) and incubated at 23°C for 15 min. After centrifugation, 8.75 µl of the supernatant were removed and heat inactivated at 70°C for 15 min, followed by addition of 2.5 µl of n-butylryl-CoA (Sigma Chemical, St. Louis, MO) and 1.25 µl of [³²P]chloramphenicol (25 µCi/ml, Amersham). This mixture was incubated for 18 h at 37°C, followed by extraction of n-butylryl-[³²P]chloramphenicol with tetramethylpentadecane/mixed xylenes (2:1) and quantification by liquid scintillation counting (44). Variation in transfection efficiency was normalized by protein assay of the lyses.

[³H]thymidine incorporation. Monolayers grown in 24-well plates were starved of serum for 24 h, then incubated in serum-free DMEM containing 0.1% BSA, with or without IGF-I in 1 g/l or 4.5 g/l glucose. After 1–15 min, the monolayers were washed with ice-cold PBS and lysed in 1 ml of 0.1% SDS for 1 h at 37°C. The lysates were precipitated by addition of 1 ml of 20% TCA, and the precipitates were collected on glass filters. The precipitate was dissolved using 500 µl 0.1 N KOH, followed by the addition of 5 ml scintillation cocktail (Ecoscint A; National Diagnostics, Atlanta, GA), and counted using a Beckman LS-6000SC scintillation counter.

Cell counting. Cell monolayers were washed with PBS, lifted using 1 x trypsin/EDTA (Life Technologies), and diluted with DMEM containing 10% calf serum. Cells were counted using a hemocytometer.

Statistical analysis. Data are presented as means ± SE and were evaluated using the Student's t-test. Results were considered significant when P < 0.05. The correlation between the effect of high glucose (HG) on IGF-BP-2 secretion and on cell proliferation was established using standard linear regression.

RESULTS

Identification of mesangial cell IGFBPs. To determine which IGFBPs are expressed by MMCs, MMC conditioned medium was subjected to ligand blot analysis. As shown in Fig. 1A, MMCs secrete two IGFBPs with masses of 32 and 24 kDa (Fig. 1A). The 32-kDa IGFBP is the predominant species, whereas the 24-kDa...
IGFBP was undetectable in some ligand blot analyses. Immunoblot analysis of the 32-kDa species confirmed its identity as IGFBP-2 (Fig. 1B). Based on its molecular weight, the 24 kDa was presumed to be IGFBP-4. This composition and ratio of IGFBPs is consistent with what has been described for other smooth muscle cells (10). A small amount of both binding proteins was also detected in PBS-washed, solubilized monolayers (data not shown), indicating their presence on the cell surface and/or within the cell.

Kinetics of IGFBP-2 secretion. Figure 2 shows the pattern of accumulation of IGFBP-2 in the medium over a 48-h period, indicating that IGFBP-2 is constitutively secreted. Increasing the ambient glucose concentration from 5.6 mM [1 g/l; normal glucose (NG)] to 25 mM (4.5 g/l; HG) resulted in a 35% reduction in total IGFBP-2 secretion. To rule out the possibility that the reduction in soluble IGFBP-2 was the result of increased adherence to the cell surface, cell-associated IGFBP-2 was measured at 24 h in NG and HG. As shown in Fig. 3, cell-associated IGFBP-2 accounts for <15% of the total IGFBP-2. In elevated glucose, the level of cell-associated IGFBP-2 decreased to the same extent (29%) as soluble IGFBP-2 (25%), demonstrating that the decrease in soluble IGFBP-2 is not due to its increased cell association. Addition of 100 µM cycloheximide abolished the production of cell-associated and soluble IGFBP-2 (data not shown), indicating that both are synthesized and secreted in a constitutive manner during the course of the experiment.

Northern blot analysis was carried out to determine whether HG affected the steady-state levels of IGFBP-2 mRNA. As shown in Fig. 4, the steady-state level of IGFBP-2 mRNA was decreased 3.1-fold after 24 h in HG. This indicates that elevated glucose alters the transcription of the IGFBP-2 gene and/or destabilizes the mRNA, reducing its half-life.

IGF-I regulation of IGFBP-2 secretion. It has been reported that IGFBP-2 secretion is increased in response to IGF-I stimulation in a number of different cell types (5, 16, 47). MMCs cultured in NG exhibited a similar response (Fig. 5). Stimulation with 1, 10, or 100 nM IGF-I caused a significant increase in IGFBP-2 secretion per cell over 24 h, with a maximal increase of 1.8-fold over basal secretion in response to 100 nM IGF-I. In the presence of HG, MMCs exhibited a 25%
reduction in IGFBP-2 secretion per cell over 24 h. When further challenged with IGF-I, they did not exhibit increased IGFBP-2 secretion.

To determine whether IGF-I binding to IGFBP-2 is a necessary requirement or whether IGF-IR activation alone is sufficient for stimulation of IGFBP-2 secretion, MMCs were stimulated with 10 and 100 nM insulin. In the absence of insulin receptors [as has been demonstrated for murine MCs by Oemar et al. (33)], insulin binds to and activates the IGF-IR with ~100-fold lower affinity than IGF-I (25) but does not interact with the IGFBPs (3, 26). One hundred nanomolar insulin stimulated IGFBP-2 approximately as well as 1 nM IGF-I (data not shown), indicating that IGF-IR activation but not IGF-I/IGFBP-2 interaction is required for stimulation of IGFBP-2 secretion.

Effect of reduced IGFBP-2 secretion on IGF-I signaling and proliferation

The observation that HG impairs IGFBP-2 secretion in response to IGF-I implies that, in HG, IGF-I will have greater access to the IGF-IR. In addition to this increased availability of IGF-I and in good agreement with previously published studies (33, 37, 52), Scatchard analysis indicated a 20% upregulation in IGF-IR number per cell in HG (data not shown). The combined effects of increased IGF-1Rs and decreased IGFBP-2 secretion should result in a state of “hypersensitivity” to IGF-I. This was confirmed by examining IGF-I-stimulated insulin receptor substrate (IRS)-1/2 phosphorylation by anti-phosphotyrosine (anti-pY) immunoblot analysis. Acute tyrosyl phosphorylation of IRS-1/2 (1–5 min post-IGF-I stimulation) was elevated as much as 2.5-fold. In addition, return to the baseline level of phosphorylation was prolonged in HG, compared with NG (Fig. 6). It is unlikely that the 20% increase in IGF-IR number alone accounts for such a dramatic increase in IRS-1/2 phosphorylation, suggesting that the reduction in IGFBP-2 contributes to this increase in IGF-I sensitivity. Alternatively, this may reflect changes in other cell signaling effectors in HG. Due to the relatively low levels of IGF-1Rs on MMCs, tyrosyl phosphorylation of the IGF-IR β-subunit was not detectable on anti-pY immunoblots of MMC whole cell lysates; it could only be detected after immunoprecipitation with anti-IGF-IR antibodies and subsequent anti-pY immunoblot. The presence of IRS-1 in the 185-kDa tyrosine phosphorylated band has been confirmed by immunoprecipitation with anti-IRS-1 with subsequent anti-pY immunoblot. However, the relative contributions of IRS-1 and IRS-2 to this band have not yet been determined.

To obtain additional functional information concerning the increase in IGF-I sensitivity, we examined a downstream target of IGF-I signaling involved in the proliferative response. The activator protein-1 (AP-1) complex is an IGF-I-responsive transcription factor associated with cell proliferation (31, 40). To assess the effects of IGF-I and HG on AP-1 activity in MMCs, cells were transiently transfected with 3×TRE-CAT, a reporter construct consisting of 3 tandem repeats of the tumor-promoting agent response element (TRE; AP-1 binding site), followed by the chloramphenicol acetyltransferase (CAT) gene. Changes in CAT activity were
quantified after 24 h of incubation in NG or HG with or without 100 nM IGF-I. As shown in Fig. 7, neither IGF-I in NG nor HG alone stimulated AP-1 transcriptional activation. This is consistent with the weak mitogenic activity of IGF-I in NG (see below). However, when cells were treated with a combination of IGF-I and HG, a 2.5-fold increase in AP-1 activation was observed. These findings are in agreement with our hypothesis that elevated glucose, by reducing the secretion of IGFBP-2, increases MC sensitivity to IGF-I action.

We next directly assessed the effect of IGF-I in the context of HG on MMC proliferation by examining changes in \[^{3}H\]thymidine incorporation and by measuring increases in cell number. As shown in Fig. 8, the increase in IRS-1/2 tyrosine phosphorylation and AP-1 activity we observed correlated with an increased proliferative response to IGF-I in HG. At concentrations of 1, 10, and 100 nM, IGF-I had no significant impact on MMC \[^{3}H\]thymidine incorporation or cell number in NG. Similarly, HG alone had no effect on proliferation. However, stimulation with 1, 10, and 100 nM IGF-I in HG resulted in significant increases in \[^{3}H\]thymidine incorporation and cell number. The magnitude of this proliferative response directly correlated with the relative diminution in IGFBP-2 secretion at each dose of IGF-I (Fig. 9).

**DISCUSSION**

In a fashion comparable to other cell types (5, 16, 47), MMCs secrete IGFBP-2 in response to IGF-I stimulation. Because IGFBP-2 has, with few exceptions, been shown to be inhibitory to IGF-I action (26), the secretion of IGFBP-2 is presumed to represent a mechanism for limiting the cellular response to IGF-I (19, 35, 41). In MMCs cultured in NG, IGFBP-2 secretion appears to negate the proliferative influence of exogenous IGF-I. However, in elevated glucose, IGFBP-2 secretion is impaired. The resulting decrease in its inhibitory influence correlates with increased intracellular signaling events leading up to and including an increased proliferative response to IGF-I. In addition, although we have observed increases in IGF-I-induced collagen secretion similar to those reported by others in NG (1.5- to 2.5-fold; Refs. 17, 36), we have measured three- to fourfold increases in HG (unpublished observations). These findings indicate that IGF-I can cause the hallmarks of glomerulosclerosis and that these cellular responses are exaggerated in HG in a coordinate fashion with decreased IGFBP-2 secretion. Thus glucose regulation of IGFBP-2 secretion by MCs plays a potential role in both the initial IGF-I-dependent renal hypertrophy and the protracted glomerular sclerosis observed in diabetes.

Several mechanisms were considered whereby elevated glucose could result in decreased levels of soluble IGFBP-2. These include redistribution to the cell surface, accelerated degradation, or decreased synthesis. Elevated glucose did not cause redistribution of IGFBP-2 from the extracellular environment to the cell surface (cell-associated IGFBP-2 was commensurately...
decreased). Although a protease specific for IGFBP-2 has been described for vascular smooth muscle cells (22), we have been unable to detect changes in proteolytic activity toward IGFBP-2 in conditioned medium taken from cells incubated in HG. On the other hand, MMCs cultured in HG did exhibit a decrease in steady-state IGFBP-2 mRNA, indicating decreased transcription of the IGFBP-2 gene and/or decreased stability of its mRNA. This may explain how HG reduces IGFBP-2 production in MMCs. The molecular details of this process remain to be determined. This represents a rare example of HG reducing a cellular function and occurs concomitantly with increased release of other constitutively secreted proteins (e.g., collagen).

Although the finding of increased MC expression of IGF-IRs has been documented in model systems of diabetes, including db/db mice (33), streptozotocin (STZ)-treated rats (52), and cultured human and rat mesangial cells (37), there are few in vitro studies detailing the secretion and regulation of IGFBPs by individual renal cell types in diabetes. Landau et al. (28) recently reported the presence of IGFBP-2 mRNA in glomeruli based on in situ hybridization of renal sections from STZ-treated rats. In their study, IGFBP-2 mRNA decreased 25% within the 1st wk of diabetes and remained reduced for at least 90 days. A more specific in vitro study by Pugliese et al. (37) was performed on human and rat mesangial cells in culture using extended incubations in 30 mM glucose. In that study, HG increased IGF-I secretion, increased IGF-IR expression decreased.)

Fig. 8. Effect of elevated glucose on proliferative response to IGF-I. After 24 h of serum starvation, MMCs were treated with the indicated concentrations of IGF-I in NG or HG for 24 h. A: cells were then incubated for 2 h with 1 mCi/ml [3H]thymidine, and thymidine incorporation was measured. Data shown were obtained from 6 replicate experiments. B: cells were trypsinized and counted using a hemocytometer. Data shown represent results of 20 experiments. Data are expressed as average fold change compared with unstimulated cells in NG ± SE. *P < 0.05 and **P < 0.01, statistically significant. IGF-I stimulated increases in NG were not statistically significant.

Fig. 9. Correlation between decreased IGFBP-2 and increased proliferation. After 24 h of serum starvation, MMCs were treated for 24 h with 0, 1, 10, or 100 nM IGF-I in NG or HG. Ligand blot analysis of conditioned medium and cell counting were used to assess differences in IGFBP-2 secretion and cell number in HG vs. NG for each dose of IGF-I. A: data points from 6 experiments were plotted, and linear regression was performed (r = 0.89). B: results for each dose of IGF-I were averaged and plotted ± SE.
(16%) and decreased IGFBP levels with no change in response to isosmolar mannitol. The decrease in IGFBPs was assessed by ligand blot but was not quantified, nor were the IGFBPs secreted by rat MCs identified. A more recent study of the rat renal cortex 7 days after induction of diabetes with STZ revealed a 35% decrease in IGFBP-2 mRNA (18). Our studies corroborate these findings and extend them by analyzing the impact of these changes on IGF-I signaling and MC responses.

A number of studies have shown IGF-I to be a MC mitogen in vitro (20, 36, 43). In these studies, MCs were grown in RPMI 1640, which contains 2 g/l (11.1 mM) glucose, twice the concentration we use as NG. This level of glucose could be impacting IGF-1R and IGFBP-2 levels to increase IGF-I sensitivity and push the cells into a proliferative phase. Indeed, the American Diabetes Association has recently recommended a diagnosis of diabetes mellitus for patients having a fasting plasma glucose of 126 mg/dl (1.26 g/l or 7 mM) or greater based on the increased rate of development of microvascular complications, such as nephropathy, observed at this glucose concentration (21).

There is considerable data implicating transforming growth factor-β (TGF-β) as a key mediator of the HG-induced increase in MC extracellular matrix component secretion (for review, see Ref. 46). However, it is clear that TGF-β is not solely responsible for all of the observed MC responses, as it cannot account for the decreased proteoglycan content nor the increased cellularity of the diabetic glomerulus. Although TGF-β stimulates proteoglycan release from MCs (4), IGF-I-induced proteoglycan synthesis is completely eliminated in HG (32). Furthermore, TGF-β inhibits MC proliferation (36, 53) but has been reported to enhance the proliferative potential of IGF-I in myoblasts overexpressing the IGF-1R (38). IGF-I alone stimulates differentiation but in conjunction with TGF-β causes proliferation. In the present study, IGF-I was shown to be proliferative in HG in direct proportion to the degree of reduction of IGFBP-2 secretion. It is possible that TGF-β is responsible for the reduced IGFBP-2 secretion and that, as a result, small changes in glomerular or plasma IGF-I levels may have a more dramatic impact on MC growth, particularly during the IGF-I spike that has been demonstrated to occur during the first few days post-STZ treatment of rats (2). Similarly, TGF-β has been shown to act synergistically with IGF-I in articular chondrocytes (50) and to decrease IGFBP-4 mRNA in human osteoblast-like cells (14). Our preliminary data indicate that TGF-β (1 ng/ml) causes a decrease in IGFBP-2 secretion similar to that observed with HG. Clearly, a more detailed investigation of the interactions between TGF-β and IGF-I signaling components is warranted.

Although considerable evidence exists supporting a role for IGF-I in the development of diabetic glomerulosclerosis, its involvement has been questioned by studies showing that growth hormone (GH)-transgenic mice develop full-blown glomerulosclerosis, while IGF-I-transgenic mice only develop glomerular hypertrophy (13). In that study, serum IGF-I was elevated in both sets of transgensics but to a greater extent in the IGF-I mice. Unfortunately, serum IGF-I was measured after separation from IGFBPs, and no measurements of serum IGFBPs nor free IGF-I were made. The discrepancy in the observed sclerotic outcome may be explainable by considering the differential effects of GH and IGF-I on IGFBP-2 secretion. The results of the present study suggest that, in response to IGF-I under normoglycemic conditions, the glomerulus (MCs) increases production of IGFBP-2 to limit the response to IGF-I. In contrast, a number of studies have shown that GH inhibits IGFBP-2 production and impairs the normal IGFBP-2 secretory response to IGF-I in a fashion similar to HG (7, 9, 11, 23). We suggest that MCs in IGF-I-transgenic mice secrete normal levels of IGFBP-2 in response to elevated levels of IGF-I and thus limit IGF-I-induced proliferation and extracellular matrix secretion, whereas MCs in GH-transgenic mice have a much higher sensitivity to IGF-I as a result of decreased levels of IGFBP-2. Thus the present findings provide a potential mechanism for testing in future analyses.

In summary, elevated glucose was found to decrease basal and block IGF-I-induced IGFBP-2 secretion by mesangial cells. The decrease in IGFBP-2 production correlated with increased IRS-1/2 phosphorylation, AP-1 transcriptional activity, and MMC proliferation in response to IGF-I. Because IGF-I plays a role in MC proliferation and extracellular matrix production, glucose inhibition of IGFBP-2 secretion and the consequent increase in MC sensitivity to IGF-I may contribute to the hypercellular and sclerotic lesions that develop in the diabetic glomerulus leading to end-stage renal disease.

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Address for reprint requests: S. A. Rosenzweig, Dept. of Pharmacology, Medical Univ. of South Carolina, 171 Ashley Ave. Charleston, SC 29425.

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REFERENCES