Development of Albuminuria and Glomerular Lesions in Normoglycemic B6 Recipients of *db/db* Mice Bone Marrow

The Role of Mesangial Cell Progenitors

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The pathologic hallmarks of diabetic nephropathy are excess mesangial extracellular matrix (ECM) and mesangial cell proliferation. We previously showed that mesangial cell phenotypic changes play an important role in the pathogenesis of diabetic nephropathy. We concluded that phenotypic changes were present in bone marrow (BM)-derived mesangial cell progenitors, as transplantation of BM from *db/db* mice, a model of type 2 diabetic nephropathy, transferred the db genotype and a nephropathy phenotype to naive B6 mice recipients. The recipients did not develop diabetes; however, they did develop albuminuria and glomerular lesions mirroring those in the donors (i.e., glomerular hypertrophy, increased ECM, and increased cell number with cell proliferation). We found that matrix metalloproteinase 2 (MMP-2) facilitated invasion of the mesangial cells into ECM and proliferation in vitro. Thus, increased MMP-2 activity in *db/db* mesangial cell progenitors may partially explain increased mesangial cell repopulation and proliferation in B6 recipients of *db/db* BM. In summary, BM-derived mesangial cell progenitors may play a crucial role in the development and progression of ECM accumulation and mesangial cell proliferation in this model of diabetic nephropathy in type 2 diabetes. *Diabetes* 53:2420–2427, 2004

I t is estimated that 90–95% of diabetic patients have type 2 diabetes, with diabetic nephropathy being one of the disease's major complications (1). Mesangial cell proliferation and mesangial sclerosis are pathologic hallmarks of diabetic nephropathy (2–4). The pathogenesis of glomerular lesions in diabetes includes changes in the phenotype of mesangial cells (5,6), such as increases in cell size and turnover and the deposition of excess extracellular matrix (ECM) (5–8). Hyperglycemia has been implicated as an important factor in the development of phenotypic changes, as many such changes have been induced in vitro by exposing mesangial cells to elevated glucose levels (8–12). We have found that mesangial cells isolated from nonobese diabetic mice, a model of type 1 diabetes, exhibit phenotypic changes, including increased type IV collagen synthesis and IGF-I secretion (5,6,13). Phenotypic changes have been reported in mesangial cells from other mouse models of type 2 diabetes with established nephropathy (7).

Phenotypic changes in mesangial cells may be a common feature of progressive glomerular diseases. We have found that mesangial cells isolated from three different strains of mice with glomerulosclerosis (ROP Os/+, mice transgenic for bovine growth hormone, and aging B6 mice) all exhibit an altered phenotype (14–16). Because the lesions in diabetic nephropathy are diffuse, bilateral, and prominent in the mesangial regions, we postulated that mesangial cells carrying the sclerosis phenotype could be derived from a common progenitor. Although the origin of mesangial cell progenitors in adults is not fully established, bone marrow (BM) has been shown to be one of the extraglomerular sites that contains mesangial cell progenitors (14,17–19). We postulated that phenotypic changes would be present in mesangial cell progenitors resident in the BM of mice with diabetic nephropathy. To test this hypothesis, BM from B6 *db/db* mice with established diabetic nephropathy was transplanted into naive syngeneic B6 mice. We found that the mesangial areas in recipient glomeruli were repopulated with mesangial cells containing the *db/db* genotype and phenotype and that the recipients developed albuminuria and severe glomerular lesions. These changes occurred in the absence of hyperglycemia in the recipients. Thus, BM mesangial cell progenitors may play an important role in the development and progression of diabetic nephropathy in *db/db* mice with type 2 diabetes.

RESEARCH DESIGN AND METHODS

Female B6 and B6 *db/db* mice were obtained from The Jackson Laboratories (cat. no. 000697; Bar Harbor, ME). We selected *db/db* mice with stable

hyperglycemia ($\geq$300 mg/dl), aged 8–9 weeks. Open renal biopsy was performed 6 weeks after the onset of hyperglycemia. The left kidney was exposed, and tissue was removed from the lower pole of the kidney, fixed in 4% paraformaldehyde, and processed for periodic acid Schiff (PAS) staining (16). There were $\geq$20 glomeruli in each section analyzed. Mice with established diabetic nephropathy, as evidenced by an increased urine albumin-to-creatinine ratio and the presence of glomerular lesions, were selected as BM donors. They were individually housed with free access to water and regular diet and followed for an additional 9 weeks to ensure the presence of stable diabetes with nephropathy. Total HbA$_{1c}$ was examined at the end of the study on whole blood by boronate affinity chromatography (Sigma, St. Louis, MO). Mice that reverted to normal glucose levels during the 9-week follow-up period (blood glucose levels stabilized at <200 mg/dl over a period of 3 consecutive weeks) were defined as regressors. They were not used as donors, but were included in the follow-up analyses. Urine samples were collected weekly and when mice were killed. Blood pressure was recorded 1 week before death.

Bone marrow transplantation. BM transplants were performed on three groups of mice: *1*) BM cells collected from two diabetic female B6 *db/db* mice, 9 weeks after diabetic nephropathy was established, were transplanted into nine female B6 mice (age 4 months), *2*) BM cells collected from two naive female B6 mice (age 6 months) were transplanted into five naive female B6 mice (age 4 months), and *3*) BM cells collected from two naive female B6 mice transgenic for green fluorescence protein (GFP) (age 4 months; The Jackson Laboratories) were transplanted into five naive male B6 mice (age 4 months). BM cells (1×10^7) from the femur and tibia were injected intravenously into lethally irradiated (950 cGy) mice (14). Recipients were followed for 4 months.

Glycemic levels and glucose and insulin tolerance tests. Body weight, glycemia, and glycosuria were monitored biweekly. The glucose tolerance test (GTT) was administered as follows. Mice were fasted overnight (12 h), with free access to water, 1 week before being killed. After their baseline glycemic levels were measured, mice received 2 g/kg of glucose intraperitoneally. Blood glucose levels were monitored using a glucometer at 15, 30, 60, and 120 min. For the insulin tolerance test (ITT), mice were fasted for 3 h, with free access to water, and then injected with 0.5 unit/kg of regular insulin. Blood glucose levels were measured before, and 15, 30, and 60 min after insulin injection (16). The GTT and ITT were performed in all of the recipients. Total HbA$_{1c}$ levels were measured when mice were killed.

Serum insulin and leptin levels. Sera were obtained from recipients, age-matched naive female B6 mice, and diabetic *db/db* mice at the time of death. Serum insulin and leptin levels were measured by enzyme-linked immunosorbent assay based on the manufacturer's protocols (Crystal Chem, Downers Grove, IL).

Urine albumin. Freshly voided spot urine samples were collected weekly. Urine albumin was measured using a kit from Bethyl (Houston, TX) (16). Urine creatinine levels were measured in the same samples, and the urinary albumin excretion (UAE) rate was expressed as the ratio of albumin to creatinine.

Renal histology. Kidneys were flushed with saline via an aortic catheter and then perfused with 4% paraformaldehyde at mean arterial pressure levels to obtain in situ fixation (16). Tissues were embedded in glycol methacrylate. Sections (4-μm thick) were stained with PAS.

Morphometric analysis. Glomerular volume was assessed on methacrylate-embedded kidney tissue sections using a digitizing tablet and video camera (20). We recorded and assessed 50 glomeruli from each mouse, as previously described (16).

Glomerular labeling index. Mice received 0.25 mg/g bromodeoxyuridine (BrdU) intraperitoneally 1 h before being killed. Samples of kidney and small intestine (control tissue) were fixed with 4% paraformaldehyde. Paraffin sections were used for BrdU staining (16). The number of positively stained nuclei was counted in 40–50 successively encountered glomeruli in each section and the labeling index was expressed as the ratio of BrdU$^+$ cells to total glomerular cells multiplied by 100.

Glomerular mRNA levels. Glomeruli were microdissected from *db/db* mice, BM-engrafted B6 recipients, and age-matched naive female B6 mice. Glomerular α1 type IV collagen, laminin B1, and matrix metalloproteinase 2 (MMP-2) mRNA levels were determined by real-time PCR, as previously described (16,21). The levels of α1 type IV collagen, laminin B1, and MMP-2 mRNA expression were normalized to 18S mRNA levels.

Immunofluorescence microscopy. Frozen kidney sections embedded in Tissue-Tek were cut at 5-μm thickness and processed (16,22). Sections were coated with rabbit anti–type IV collagen or anti–laminin B (Biodesign, Kennebunkport, ME), then with biotin-conjugated goat anti-rabbit IgG (Sigma) and streptavidin-conjugated fluorescein isothiocyanate (Zymed, San Fran-

cisco, CA). Macrophages were identified with a rat anti-CD68 antibody (Serotec, Raleigh, NC). Spleen sections served as positive controls (14,16).

Mesangial cell culture. Mesangial cells isolated from recipients of BM from *db/db* or B6 mice were characterized as previously described (5,13,15). Mesangial cells were also isolated from age-matched diabetic *db/db* and naive B6 mice. Mesangial cells were grown in Dulbecco's modified Eagle's medium (DMEM)-Ham's F-12 mixture supplemented with 20% fetal bovine serum (FBS), 100 units/ml streptomycin, and 100 units/ml penicillin. Mesangial cell lines were obtained using cloning ring selection, followed by single-cell dilution and propagation. Mesangial cells were used between passages 5 and 11.

DNA analysis. DNA was isolated from microdissected glomeruli and cultured mesangial cells using Tri-reagent (Molecular Research Center, Cincinnati, OH) (23). To identify the cells with the leptin receptor mutation, primers (forward, 5′-CGGACACTCTTTGAAGTCTCTCA; reverse, 5′-CATTCAAACCATAGTTTA GGTTTGGTT) were used for PCR genotyping (24). Amplification was performed in a thermal cycler at 95°C for 3 min for denaturation, followed by 36 cycles of 30 s each at 94, 60, and 72°C. PCR products were cut with *Nia*IV or *Bst*EII to identify the db genotype (leptin receptor mutation). To quantitate the amount of mutant and/or wild-type DNA in samples, competitive PCR was performed by adding decreasing amounts of DNA (10–100 ng) from B6 *db/db* to a standard amount of B6 wild-type DNA. The ratio of *db* to wild-type genotype was calculated. The percentage of *db*$^+$ cells in glomeruli and cell outgrowths was derived using linear regression analysis from a standard curve (25).

MMP-2 activity and mesangial cell invasion. Mesangial cells isolated from BM engraftment and age-matched controls were seeded at the level of 45,000 cells/well in six-well plates. The medium was changed to DMEM/F12 with 0.1% FBS for 24 h. MMP-2 activity was assessed using 10% gelatin zymogram gels. Medium from samples, diluted to ensure that each represented an equal number of cells, was loaded onto gels and run under nonreducing conditions. Gels were washed for 1 h in 2.5% Triton X-100, incubated for 18 h in 50 mmol/l Tris buffer, and stained with Coomassie blue. The intensity of MMP-2 bands was quantitated using NIH Image 1.6 (14,15). Experiments were performed at least in duplicate.

For invasion assays, mesangial cells isolated from BM-engrafted and age-matched controls were suspended in medium containing 0.5% FBS and plated onto a matrigel-coated invasion chamber (BD Biosciences, Bedford, MA) at the level of 2×10^5 cells/chamber. There were no differences in the efficiency of attachment to matrigel among the different mesangial cell lines. A chamber was placed in each well of a 24-well plate, which was then filled with 0.8 ml of medium containing 2% FBS. The plate was then incubated at 37°C, 5% CO$_2$, for 22 h. Noninvading cells were removed from the upper surface of the membrane. Invading cells, which appeared on the lower surface of the membrane, were stained with 1% toluidine and counted using a 100× ocular. To determine whether MMP-2 affected mesangial cell invasion, a specific inhibitor of MMP-2, *cis*-octadecenoyl-N-hydroxylamide oleoyl-N-hydroxylamide (OA-Hy, 0.4 μg/ml; Calbiochem, San Diego, CA), was added to one group of wells. Experiments were performed in triplicate.

MMP-2 and mesangial cell proliferation. Mesangial cells isolated from 5-month-old wild-type female B6 mice were seeded at a density of 30,000 cells/well in 24-well plates. Cells were made quiescent by incubating them with DMEM/F12 with 0.1% FCS for 48 h. After the cell layers were washed with PBS, new medium with 0.1% FBS containing pro- or active MMP-2 (Calbiochem) was added. Active MMP-2 was obtained by incubating pro-MMP-2 enzyme with 1 mmol/l *p*-aminophenylmercuric acetate (APMA) in 150 mmol/l NaCl, 10 mmol/l CaCl$_2$, 50 mmol/l Tris-HCl (pH 7.4) at 37°C for 2 h (26). Activation of MMP-2 was confirmed by gelatin zymography (data not shown). APMA and buffer salts were removed by passing the mixture through a 30-kDa cut filter spin column (Microcon; Millipore, Bedford, MA). Proliferation was assessed 24 h after the addition of MMP-2 by ^{3}H-thymidine uptake (27).

Statistical analysis. Data are expressed as means $\pm$ SE. One-way ANOVA or unpaired *t* tests were used to evaluate the differences among experimental groups.

RESULTS

***db/db* mice with stable type 2 diabetes and established diabetic nephropathy as BM donors.** The female *db/db* donors were obese and developed diabetes by age 8–9 weeks. In addition, ~75% of B6 *db/db* mice obtained from The Jackson Laboratories developed stable hyperglycemia. Renal biopsy was performed 6 weeks after diabetes onset. Diabetic nephropathy was defined as increased UAE (urinary albumin/creatinine: 0.27 ± 0.1 [*db/db*; $n = 7$] vs. 0.03 ± 0.01 [B6; $n = 6$]); $P < 0.01$) and biopsy-proven glomerular lesions (Fig. 1). The lesions progressed in BM
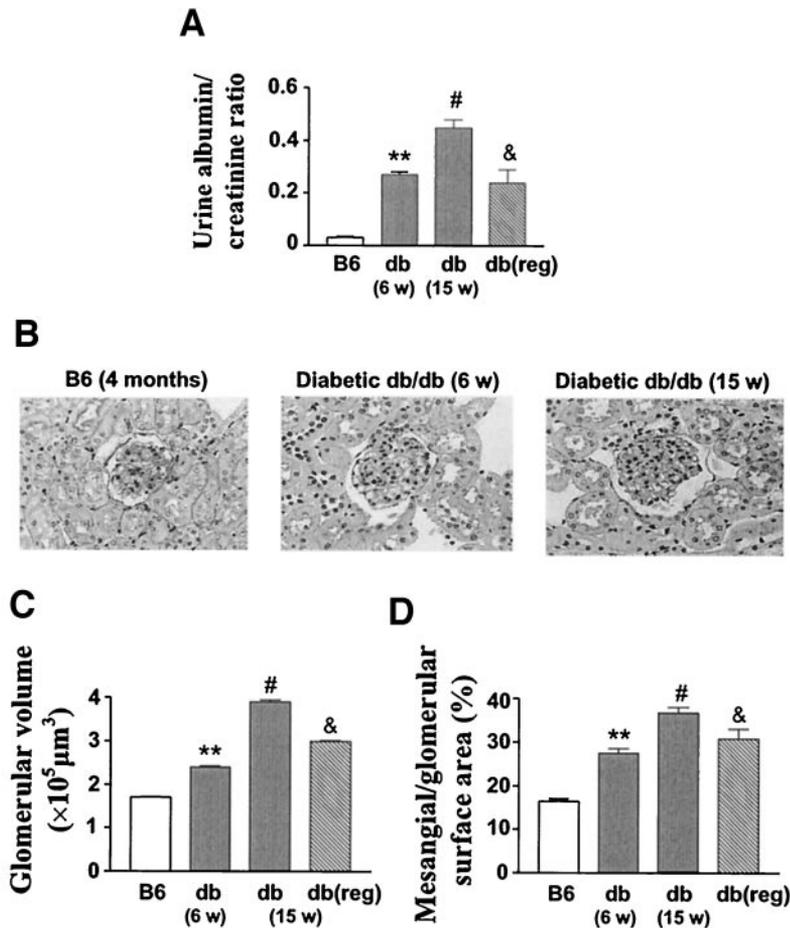
FIG. 1. Nephropathy in donor B6 *db/db* mice. *A*: *db/db* mice with sustained hyperglycemia had increasing UAE, but UAE was stable in regressors. **$P < 0.01$ vs. B6 mice; #$P < 0.05$ vs. db (6 w); &$P < 0.05$ vs. db (15 w). *B*: The mesangial areas were expanded in *db/db* mice 6 weeks after diabetes onset and were further increased at 15 weeks (PAS; magnification ×400). *C*: Increased glomerular volume in diabetic *db/db* mice from 6 to 15 weeks, but not in db (reg). **$P < 0.01$ vs. B6 mice; #$P < 0.01$ vs. db (6 w); &$P < 0.05$ vs. db (15 w). *D*: Mesangial fractional area was increased in *db/db* mice with sustained hyperglycemia, but not in db (reg). **$P < 0.01$ vs. B6 mice; #$P < 0.05$ vs. db (6 w); &$P < 0.05$ vs. db (15 w). B6, B6 mice; db (6 w), *db/db* mice 6 weeks after diabetes onset; db (15 w), *db/db* mice 15 weeks after diabetes onset; db (reg), *db/db* mice in which diabetes regressed.

donors in which hyperglycemia persisted until the mice were killed (15 weeks after diabetes onset); that is, the UAE increased (0.45 ± 0.03 vs. 0.27 ± 0.1 at 6 weeks; $P < 0.05$) (Fig. 1*A*) as did glomerular lesions (Fig. 1*B*−*D*). Mice in which glycemic levels reverted to normal levels did not develop progressive nephropathy (~25% of the total) and were labeled as regressors. UAE glomerular volume, and fractional mesangial area remained at 6-week levels in regressors.

Repopulation of B6 recipient glomerular resident cells with *db/db* BM cells. Because cells derived from BM contained the db genotype, a leptin receptor mutation, we identified donor cells in recipient glomeruli by PCR genotyping. We found the db genotype in all nine B6 recipients of *db/db* BM (Fig. 2*A*); 9–21% of glomerular cells had the db genotype as determined by quantitative PCR using DNA isolated from B6 *db/db* as the standard. Immunofluorescence microscopy revealed that there was less than one macrophage per glomerulus, a number not different from that seen in wild-type B6 controls. Thus, the cells derived from donor *db/db* BM were predominantly indigenous to the glomerulus. The origin of cells repopulating the glomeruli was established by isolating mesangial cells from microdissected recipient glomeruli. The initial cellular outgrowth from these glomeruli contained mainly mesangial cells, with a few endothelial cells, but no identifiable epithelial cells. We found the db genotype in seven of nine in the initial glomerular outgrowths; ~20–30% of the cells in the seven db+ outgrowths had the db genotype. Lines of either the B6 or the db genotype were

obtained from the outgrowths (Fig. 2*B*). A small amount of the wild-type genotype was also present in the line that was selected to be predominantly of the db genotype. Gene fusion of nonparenchymal cells with mesangial cells was unlikely to be the cause of these findings because both mesangial and endothelial cells remained diploid after multiple passages, as determined by flow cytometry analysis. BM from female B6 GFP mice was transplanted into male B6 mice to further explore the question of gene fusion. We found GFP+ cells in recipient B6 glomeruli and in mesangial cells isolated from recipients. We identified cells that contained either the GFP or male genotype, but did not find cells that contained both the GFP and male genotype. These observations support the conclusion that donor BM-derived mesangial cell progenitors repopulated the glomeruli and that gene fusion was not identifiable under these conditions (17). The number of donor cells repopulating the glomeruli of B6 recipients of *db/db* BM (9–21%) was increased compared with B6 recipients of GFP BM (0.2–5%).

B6 recipients of *db/db* BM developed albuminuria and glomerular lesions, but no changes in glucose levels or insulin responses. The body weight of B6 recipients of *db/db* BM was unchanged, blood glucose levels remained normal, and the response to glucose and insulin infusion was normal (Fig. 2*C* and *D*). B6 recipients of B6 BM also had normal GTT and ITT results. Although diabetic B6 *db/db* mice had increased serum insulin (153 ± 69 ng/ml) and leptin (108 ± 29 ng/ml) levels, the levels were normal in B6 recipients of *db/db* BM (insulin: $0.6 \pm$
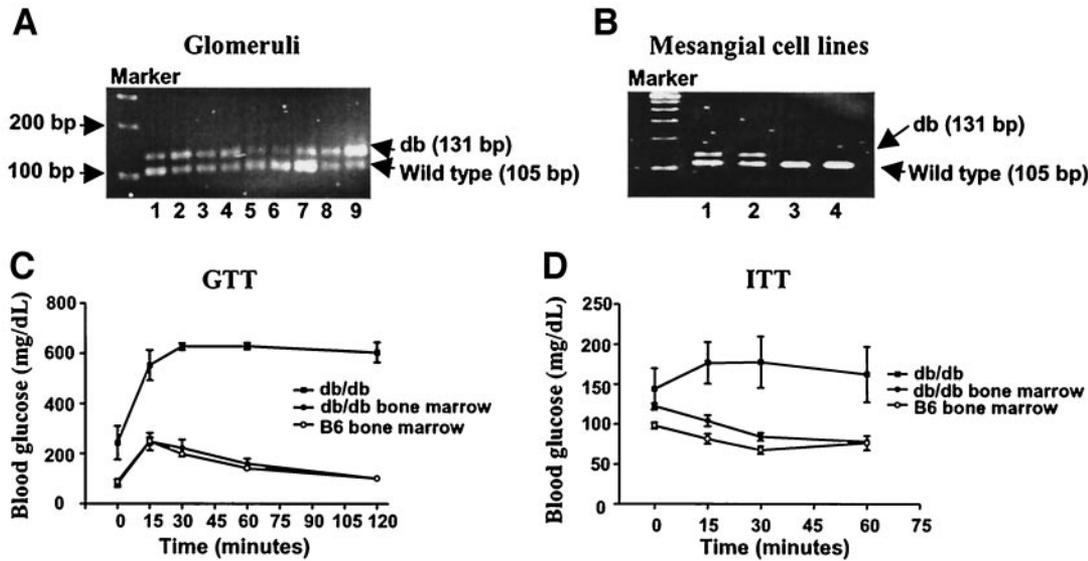
FIG. 2. Transfer of the db genotype but not diabetes. *A*: db (131 bp) and wild-type (105 bp) genotypes were both present in glomeruli of B6 recipients of *db/db* BM. Shown are samples of glomeruli of *db/db* BM recipients (*lanes 1–8*) and a B6 *db/db* mouse (*lane 9*). A band representing the wild-type genotype was consistently present in B6 *db/db* mice. *B*: The two mesangial cell lines from the same B6 recipient of *db/db* BM had the db genotype (*lanes 1* and *2*), whereas the two other cell lines from the same recipients were wild type (*lanes 3* and *4*). *C*: B6 recipients of *db/db* BM had a normal glucose response. *D*: B6 recipients of *db/db* BM, but not *db/db* donors, had a normal insulin response.
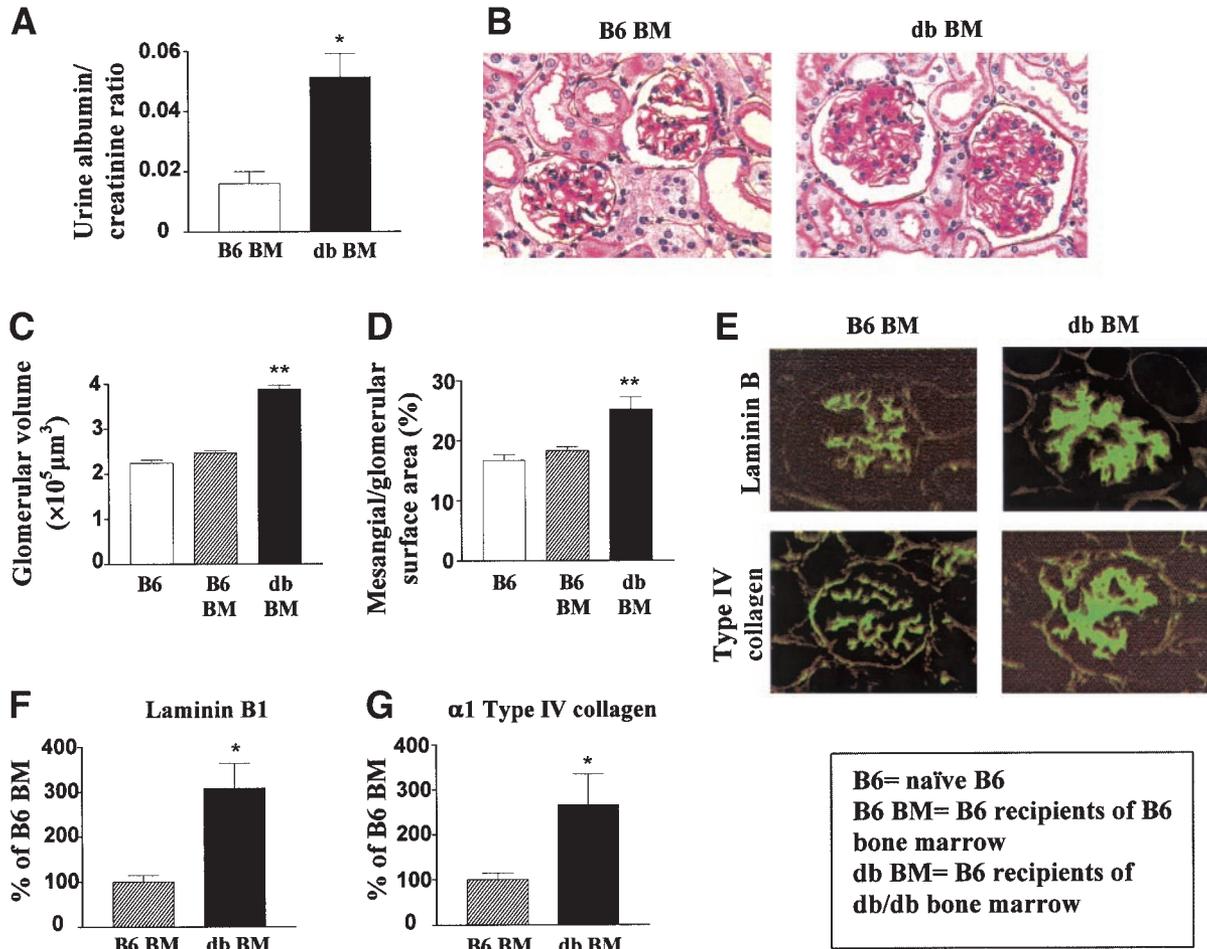

FIG. 3. Glomerular lesions in B6 recipients of *db/db* BM. *A*: Increased UAE in B6 recipients of *db/db* BM. *$P < 0.05$ vs. B6 BM. *B*: Glomeruli were normal in B6 recipients of B6 BM. Increased glomerular size and marked expansion of mesangial areas in B6 recipients of *db/db* BM (PAS; magnification ×400). *C*: Glomerular volume was increased in B6 recipients of *db/db* BM. **$P < 0.01$ vs. B6 BM. Naive B6 mice and B6 recipients of B6 BM were similar. *D*: Fractional mesangial area was increased in B6 recipients of *db/db* BM. **$P < 0.01$ vs. B6 BM. *E*: Glomerular laminin B1 and type IV collagen were increased in B6 recipients of *db/db* BM compared with B6 recipients of B6 BM (magnification ×400). *F*: Increased laminin B1 mRNA levels in the glomeruli of B6 recipients of *db/db* BM compared with B6 recipients of B6 BM. *$P < 0.05$ vs. B6 BM. *G*: Increased α1 type IV collagen mRNA levels in glomeruli of B6 recipients of *db/db* BM compared with B6 recipients of B6 BM. *$P < 0.05$ vs. B6 BM. *F* and *G*: There were no differences in glomerular 18S mRNA levels between B6 recipients of *db/db* or B6 BM. Laminin B1 and α1 type IV collagen mRNA levels in B6 recipients of B6 BM were arbitrary defined as 100%.
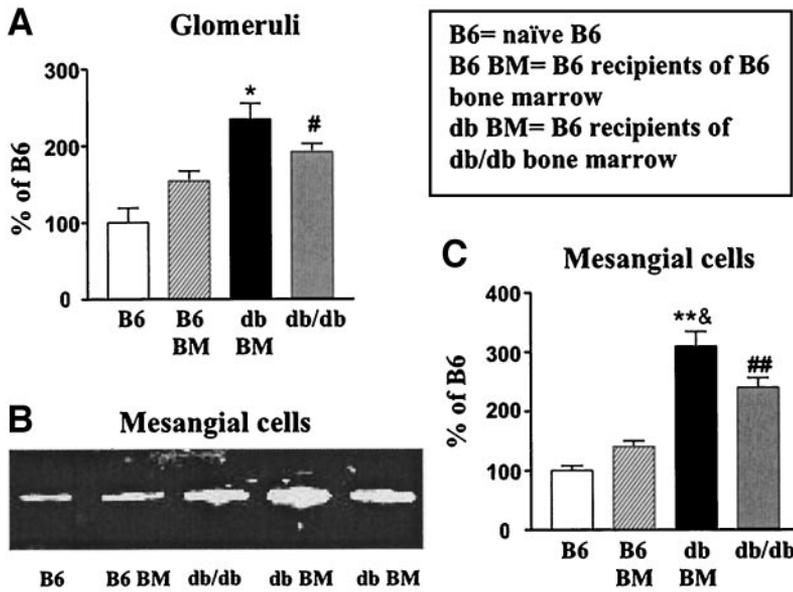
FIG. 4. Increased MMP-2 mRNA expression and activity in glomeruli and mesangial cells of recipients of *db/db* BM. *A*: Increased MMP-2 mRNA levels in glomeruli of B6 recipients of *db/db* BM. Data are percentage of MMP mRNA in naive B6 mice. *$P < 0.05$ vs. B6 BM; $P < 0.05$ vs. B6. *B*: MMP-2 zymography. Increased MMP-2 activity in two single mesangial cell lines from B6 recipients of *db/db* BM and in mesangial cells isolated from naive *db/db*. *C*: Scans of gelatin gels. Increased MMP-2 activity in mesangial cells from naive *db/db* and in lines from recipients of *db* BM. Levels in recipients were higher than in naive mice. Data are percentages of MMP activity in mesangial cell lines from B6 mice. **$P < 0.01$ vs. B6 BM; ##$P < 0.01$ vs. B6; &$P < 0.05$ vs. *db/db*.

0.2 ng/ml; leptin: 7.5 ± 2.9 ng/ml). UAE was increased in B6 recipients of *db/db* BM compared with age-matched B6 recipients of naive B6 BM ($n = 6$) (Fig. 3*A*). The glomeruli of B6 recipients of *db/db* BM were large, and the mesangial areas were expanded (Fig. 3*B*). The glomerular volume and mesangial areas were increased in B6 recipients of *db/db* BM, but not in B6 recipients of B6 BM (Fig. 3*C* and *D*). The enlarged mesangial areas in B6 recipients of *db/db* BM contained type IV collagen and laminin, as determined by immunofluorescence microscopy (Fig. 3*E*). The levels of α1 type IV and laminin B1 mRNA in glomeruli were increased in B6 recipients of *db/db* BM, compared with recipients of B6 BM (Fig. 3*F* and *G*). No differences were found in glomerular α1 type IV and laminin B1 mRNA levels between normal B6 mice and B6 recipients of B6 BM.

Increased MMP-2 expression in B6 recipients of *db/db* BM. MMP-2 mRNA expression was upregulated in the glomeruli of donor diabetic B6 *db/db* mice (Fig. 4*A*). Furthermore, mesangial cells isolated from glomeruli of B6 *db/db* donors had increased MMP-2 levels compared with mesangial cells isolated from B6 donors. We exam-

ined glomerular MMP-2 mRNA expression in B6 recipients of BM from B6 *db/db* donors to determine if the transfer of diabetic nephropathy also transferred the MMP-2 phenotype. Glomerular MMP-2 mRNA levels were significantly increased in B6 recipients of BM from *db/db* donors (Fig. 4*A*). We isolated mesangial cell lines in the cellular outgrowths of B6 recipients of BM from B6 *db/db* donors that had the db genotype and of B6 recipients of BM from B6 donors (Fig. 2*B*). We evaluated the MMP-2 levels in both types of lines and found that it was increased only in lines with the db genotype (Fig. 4*B* and *C*).

MMP-2 facilitates BM-derived mesangial cell invasion through ECM. MMP-2 has been shown to play an important role in the invasion of cells into the ECM, especially tumor cells (28). Therefore, we examined whether MMP-2 might facilitate the entry of BM-derived mesangial cell progenitors into the mesangium using matrigel-coated membranes as a model. Matrigel contains several components found in the mesangial matrix, including type IV collagen, laminin, and proteoglycan. Two mesangial cell lines with the db genotype (Fig. 2*B*) and
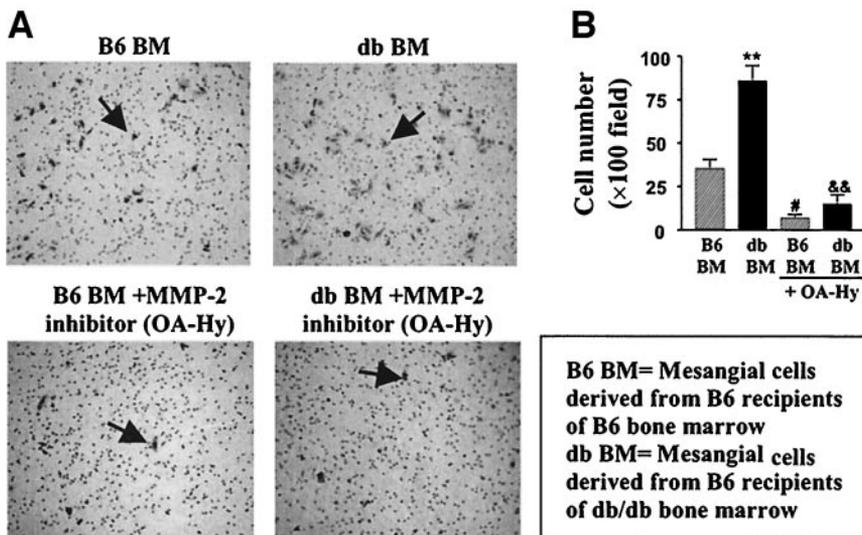

FIG. 5. MMP-2 and mesangial cell invasion of membranes coated with matrigel. Invading cells appear as stained objects on the undersurface of the membranes (magnification ×100). *A*: There was more invasion by mesangial cells (denoted by arrow) from B6 recipients of *db/db* BM than from B6 recipients of B6 BM. Invasion of both was largely blocked by the MMP-2 inhibitor, OA-Hy. *B*: There was increased invasion by mesangial cells from B6 recipients of *db/db* BM compared with B6 recipients of B6 BM. Invasion of both was decreased by OA-Hy. **$P < 0.01$ vs. B6 BM; #$P < 0.05$ vs. B6 BM not treated with OA-Hy; &&$P < 0.01$ vs. db BM not treated with OA-Hy.
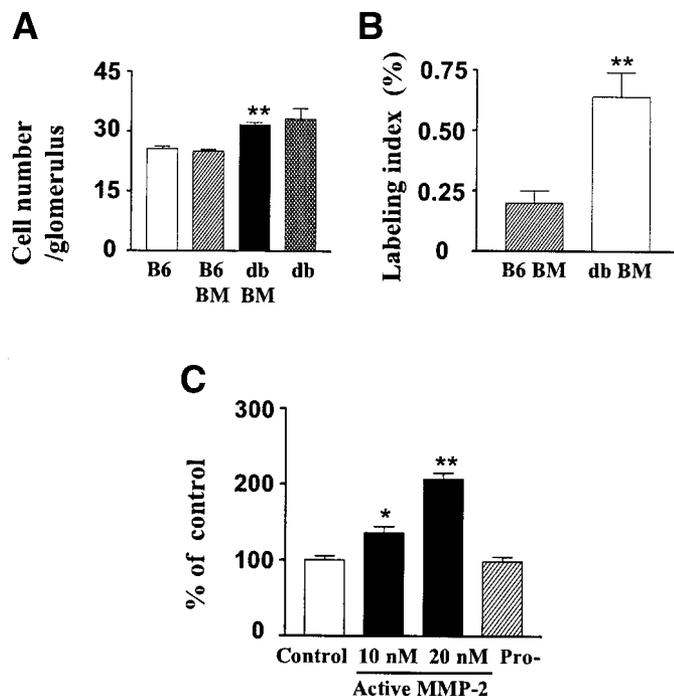
FIG. 6. MMP-2 induces proliferation. *A*: Glomerular cell number was increased in B6 recipients of *db/db* BM and in naive *db/db*. **$P < 0.01$ vs. B6 BM. *B*: The number of BrdU-labeled cells was increased in the glomeruli of B6 recipients of *db/db* BM. **$P < 0.01$ vs. B6 BM. *C*: Although pro-MMP-2 (Pro-, ▨) did not alter ^{3}H-thymidine uptake, active MMP-2 (■) induced a dosage-dependent increase in mesangial cell proliferation in vitro. *$P < 0.05$, **$P < 0.01$ vs. untreated cells.

mesangial cells isolated from wild-type or B6 recipients of B6 BM were used. More mesangial cells derived from the glomeruli of B6 recipients of *db/db* BM invaded matrigel than did those derived from recipients of B6 BM (Fig. 5*A* and *B*). There were no differences in the ability to invade matrigel among any of the cell lines, except those with the db/db genotype. The degree of invasiveness was directly correlated with the levels of MMP-2 in the mesangial cell lines (Fig. 4*B*). This result may partially explain the observation that a greater number of cells were found to repopulate the glomeruli in recipients of *db/db* BM compared with recipients of B6 GFP BM. The invasion of BM-derived mesangial cells through matrigel was decreased ($\geq$80%) by OA-Hy, an MMP-2 inhibitor (Fig. 5*A* and *B*). Thus, MMP-2 may play a critical role in the repopulation of recipient glomeruli by BM-derived mesangial cell progenitors.

MMP-2 induces mesangial cell proliferation. There was an increase in the number of glomerular cells and in cell proliferation in the glomeruli of B6 recipients of BM from diabetic B6 *db/db* donors (Fig. 6*A* and *B*). Because MMP-2 directly affects the phenotype of mesangial cells (26), we examined the effect of MMP-2 on mesangial cell proliferation. Active MMP-2, but not the proenzyme, caused a dosage-dependent increase in mesangial cell proliferation, as assessed by ^{3}H-thymidine incorporation (Fig. 6*C*).

DISCUSSION

The current study demonstrated that phenotypic changes in BM-derived mesangial cell progenitors transmit diabetic nephropathy from donors with type 2 diabetes to naive,

normoglycemic recipients. We selected B6 *db/db* mice as a model of type 2 diabetic nephropathy because they are obese and ~75% have sustained hyperglycemia, hyperinsulinemia, and hyperleptinemia. We have found that those with sustained hyperglycemia develop increasingly severe albuminuria and progressive glomerulosclerosis. We selected only those *db/db* mice that had sustained diabetes, albuminuria, and biopsy-proven diabetic nephropathy as BM donors. BM from this group of donors was transplanted into naive, syngeneic B6 recipients. The db genotype was found in the glomeruli of all nine recipients and was identified in the initial cellular outgrowths of seven recipients. Mesangial cell lines obtained from these outgrowths contained either the B6 or the db genotype and the corresponding phenotype. Gene fusion was unlikely to be a major cause of our finding the db genotype in mesangial cells for three reasons: *1*) lines with or without the db genotype were isolated from the glomerular outgrowths; *2*) cells with or without the db genotype remained diploid after several passages; and *3*) among the mesangial cell lines isolated from the glomeruli of male B6 recipients that had received BM from female GFP donors, we found cells containing either the GFP or y genotype, but not cells with the combined genotypes. This result is consistent with those in previous reports (17).

The reconstitution of normoglycemic B6 recipient glomeruli with *db/db* BM-derived mesangial cells was associated with the development of albuminuria and severe glomerular lesions in recipients. The glomerular lesions included hypertrophy, mesangial cell proliferation, and ECM expansion containing laminin and type IV collagen. The lesions in recipient glomeruli were similar to those in the diabetic *db/db* donors. Because the glomeruli of B6 recipients of BM from B6 mice or B6 GFP mice were initially normal, mesangial cell progenitors in *db/db* mice BM appear to have phenotypic changes that serve to both initiate and perpetuate diabetic glomerular lesions. However, we cannot rule out a role for glomerular endothelial cell progenitors in transmitting the lesions. It has been reported that increased macrophage infiltration may contribute to glomerular lesions in type 2 diabetic patients (29). However, we did not find an increase in macrophages in recipient glomeruli. Therefore, it appears unlikely that glomerulosclerosis in the recipients of *db/db* mice BM was transmitted by BM-derived macrophages.

The transfer of diabetic glomerulopathy to B6 recipients by *db/db* BM occurred in the absence of hyperglycemia and hyperleptinemia, suggesting that the phenotypic changes were present in BM-derived mesangial cell progenitors. Hyperglycemia may play a central role in the phenotypic change of BM mesangial cell progenitors because *1*) glomerular lesions in *db/db* regressors, in which glycemic levels returned to nondiabetic levels during the follow-up period, did not progress; and *2*) glycemic control with peroxisome proliferator−activated receptor-α or -γ antagonists decreased the progression of diabetic nephropathy in *db/db* mice (30,31). Other factors such as high leptin levels and the leptin receptor mutation may also contribute to phenotypic changes (32,33). The comparison of mesangial cells from the regressors and controls, as well as the nature and extent of the phenotypic

changes transferred by BM of the regressors to controls, is the subject of future studies.

There was increased repopulation of glomerular cells in recipients of *db/db* BM compared with recipients of B6 GFP BM. We found that *db/db* glomeruli and mesangial cells isolated from these glomeruli had increased levels of MMP-2. MMP-2 expression was also increased in glomeruli of B6 recipients of *db/db* BM and in mesangial cells isolated from recipients that had the db genotype, an indication that they were derived from BM. Because MMP-2 correlates with tumor invasion (28,34), we examined the role of MMP-2 on the entry of BM-derived mesangial cells into the mesangium. We found that the ability of BM-derived mesangial cells to invade through ECM directly correlated with MMP-2 activity. Blockade of MMP-2 activity sharply inhibited cell invasion. These data suggest that increased MMP-2 may play an important role in the entry of BM-derived mesangial cell progenitors into glomeruli. The high MMP-2 activity in mesangial cells derived from *db/db* mice BM may partially explain the enhanced reconstitution of glomeruli found in recipients of *db/db* mice BM compared with recipients of BM from naive B6 mice. There may also be interactions between resident glomerular cells and the newly arriving progenitors from the BM. Although of interest, we did not address this question in the current study.

Glomerular cell proliferation was increased in B6 recipients of *db/db* mice BM. This may have accounted for the increased glomerular cell number in these recipients. Because inhibition of MMP-2 in rat mesangial cells alters cell proliferation and type I and IV collagen expression, we examined the effect of MMP-2 on mesangial cell proliferation (26). Active MMP-2, but not the proenzyme, increased mesangial cell proliferation in a dosage-dependent manner. Because the mesangial cells were isolated from the glomeruli of naive B6 mice in these experiments, we hypothesized that increased MMP-2 expression by *db/db* BM-derived mesangial cells could serve as a mitogenic stimulus to recipient mesangial cell progenitors, resulting in cell proliferation and increased cell number in recipient glomeruli.

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