Lack of α8-integrin aggravates podocyte injury in experimental diabetic nephropathy

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Submitted 1 February 2010; accepted in final form 31 August 2010

Hartner A, Cordasic N, Menendez-Castro C, Volkert G, Yabu JM, Kupraszewicz-Hutzler M, Rascher W, Hilgers KF. Lack of α8-integrin aggravates podocyte injury in experimental diabetic nephropathy. Am J Physiol Renal Physiol 299: F1151–F1157, 2010. First published September 8, 2010; doi:10.1152/ajprenal.00058.2010.—Development of diabetic nephropathy is accompanied by changes in integrin-mediated cell-matrix interactions. The α8-integrin chain is specifically expressed in mesangial cells of the glomerulus. During experimental hypertension, α8-integrin plays a protective role in the glomerulus. We hypothesized that α8-integrin is involved in maintaining the integrity of the glomerulus in diabetic nephropathy. Experimental streptozotocin (STZ) diabetes led to an increased expression and glomerular deposition of α8-integrin. To test the functional role of α8-integrin, STZ diabetes was induced in mice with a homozygous (α8/−/−) or heterozygous (α8+/−) deletion of the α8-integrin gene and in wild-type litters (α8+/+). Blood glucose and mean arterial blood pressure were not different in α8−/− and α8+/+ mice after 6 wk of diabetes. However, diabetic α8−/− mice developed significantly higher albuminuria and more glomerulosclerosis than diabetic α8+/+ mice. Moreover, in diabetic α8−/− mice, the number of glomerular cells staining positive for the podocyte markers WT-1 and vimentin were reduced more prominently than in diabetic α8+/+ mice. The filtration barrier protein nephrin was downregulated in diabetic glomeruli with the strongest reduction observed in α8−/− mice. Taken together, α8−/− mice developed more severe glomerular lesions and podocyte damage after onset of STZ diabetes than α8+/+ mice, indicating that α8-integrin is protective for the structure and function of the glomerulus and maintains podocyte integrity during the development of diabetic nephropathy.

streptozotocin diabetes; glomerular damage; podocyte injury

INTEGRINS ARE CELL MEMBRANE receptors for extracellular matrix components. These molecules mediate adhesion of cells to the adjacent matrix, migration, proliferation, and apoptosis (13). Integrins are heterodimers consisting of one α- and one β-chain. Most integrins responsible for cell-matrix interactions contain the β1-chain that can form heterodimers with several α-chains (4). The α8-integrin chain dimerizes solely with the β1-chain and serves as a receptor for fibronectin, vitronectin, tenascin C, osteopontin, and nephrin (6, 11, 35). The α8β1 is expressed in mesenchymal cells, including vascular smooth muscle cells, some fibroblasts, and mesangial cells of the glomerulus (34).

Diabetic nephropathy is the most common cause of end-stage kidney disease in developed countries, and its incidence continues to rise (33). Typical features of diabetic nephropathy are loss of functional podocytes and glomerulosclerosis. Thus, dysregulation of the expression of extracellular matrix components and their receptors is regularly observed during diabetic nephropathy (23, 29). Changes in glomerular integrin expression have been reported in human and animal models of diabetic nephropathy. In human diabetic kidneys, integrin chains α1, α2, α3, β1, and β3 are more abundant than in healthy controls (23). In glomerular tissue of diabetic rats, expression of the β1- and αv-integrin chains are induced (32, 40). In contrast, mesangial expression of αvβ3- and αvβ5-integrins is decreased in human diabetic nephropathy (16), and a reduction of α3β1-integrin expression is observed in the podocytes of rats with experimental diabetes (31).

As the upregulation of certain integrins parallels matrix expansion, integrins could play a profibrotic role in chronic kidney disease (1). On the other hand, integrins have an important function in maintaining tissue integrity, which is a prerequisite to normal organ structure and function. Studies in integrin-deficient mice revealed that the lack of integrins is associated with a higher incidence of organ damage during experimental disease. For example, induction of glomerular injury in α1-integrin-deficient mice led to more severe glomerulosclerosis than in wild-type mice (9). Similarly, we have previously shown that induction of DOCA-salt hypertensive nephropathy in α8-integrin-deficient mice resulted in more pronounced glomerular damage compared with wild-type mice (17). Thus, in the present study, we hypothesized that an underexpression of the α8-integrin chain aggravates the progression of diabetic nephropathy. We compared the effects of diabetes on renal outcome in homozygous α8-integrin-deficient mice to heterozygous α8-integrin-deficient and wild-type littermates, which were uninephrectomized to adjust for the reduced renal mass in homozygous α8-integrin-deficient mice. Heterozygous α8-integrin-deficient and wild-type littermates that were uninephrectomized and a normoglycemic diabetic wild-type littermate group served as additional control groups for the possible effects of uninephrectomy and of streptozotocin (STZ) treatment (7), respectively.

MATERIALS AND METHODS

Animal procedures. Animals were housed in a room maintained at 22 ± 2°C, exposed to a 12:12-h light-dark cycle with free access to standard chow (cat. no. 1320, Altromin, Lage, Germany) and tap water. All procedures performed on animals were done in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the local government authorities (Regierung von Mittelfranken, AZ621-2531.3-18/94). The α8-integrin-deficient mice were a generous gift of U. Muller (Scripps Institute, LaJolla, CA) (30) and were bred from a heterozy-
gous colony on a mixed C57BL/6J295Sv background. The α8-integ

-GENIC DEFICIENT MICE (α8-/−) DEVELOP ONLY ONE KIDNEY OR TWO SMALLER KIDNEYS, LEADING TO A 50% REDUCED TOTAL RENAL MASS. STRUCTURE AND FUNCTION OF THE GLomerulus, HOWEVER, IS NOT IMPAIRED. Heterozygous littermates (α8+/−) do not display any reductions in renal mass. Heterozygous and wild-type littermates (α8+/+) were used as controls. The offspring from the heterozygous colony was genotyped and assigned to the experimental groups. At an average weight of 16–18 g, a part of α8+/− and α8+/+ mice underwent right unilateral nephrectomy in ketamine/xylazine anesthesia to adjust for the reduced kidney mass in α8−/− mice. The α8−/− mice were not nephrecto-

mized. After 2 wk of recovery, diabetes was induced by a single intraperitoneal injection of 200 mg/kg STZ (Sigma, Deisenhofen, Germany) dissolved in 0.1 M sodium citrate buffer (pH 4.5) under light iso- fluorane anesthesia as described before (10). This diabetes model of single-shot high-dose STZ was chosen because in pilot experiments, the 5 × 50 mg/kg multiple-shot STZ model (7) did not lead to consistent development of diabetes in the α8−/− mice bred on a mixed C57BL/6J295Sv background. Two days after STZ administration, blood was obtained from the tail vein in isoflurane anesthesia, and diabetes was confirmed by measurement of blood glucose using a reflectance meter (Glucometer Elite II; Bayer, Leverkusen, Germany). Only mice with consistent blood glucose > 250 mg/dl were included. Mice were then divided into the following groups: 1) control, α8−/− mice (n = 7); 2) uninephrectomized control, α8+/− mice (n = 6); 3) uninephrectomized control, α8+/+ mice (n = 8); 4) diabetic, α8−/− mice (n = 8); 5) uninephrectomized diabetic, α8+/− mice (n = 6); 6) uninephrectomized diabetic, α8+/+ mice (n = 8); 7) diabetic, α8+/− mice (n = 7); 8) diabetic, α8+/+ mice (n = 8); 9) normoglycemic diabetic, α8+/+ mice (n = 6). Diabetic mice received a small amount of insulin (1/2 ml) injection. The slides were kept in 100% ethanol for 1 min, then 100% ethanol for 1 min, and washed in diethylpyrocarbonate-treated deionized water. The sections were air-dried for 10 min at room temperature and used immediately. Glomerular structures were identified and selected. Laser-assisted microdissection (LCM; Palm Systems, Germany) was performed by transferring the samples into adhesive caps and into tubes containing 40 μl of RLT lysis buffer (Qiagen) containing RNase-free conditions. Then 100 glomeruli per kidney were microdissected. Total RNA was isolated using the RNeasy Micro Kit (Qiagen) according to the manufacturer’s instructions. Preparation of cDNA and detection of α8-integrin expression was performed as described above.

**Immunohistochemical analysis.** Staining of cryostat and paraffin sections was performed as previously described (18). The primary rabbit antibody to α8-integrin (gift from U. Muller, Scripps Institute, LaJolla) was used on cryopreserved tissue at a dilution 1:500. CY3-

labeled anti-rabbit IgG (DAKO Diagnostica, Hamburg, Germany) was used as secondary antibody. Detection of podocyte markers WT-1 (NeoMarkers, Fremont, CA) and vimentin (Progen, Heidelberg, Germany), and the filtration barrier protein nephrin (Arcis Antibodies, Hiddendenhausen, Germany) was carried out in paraffin-embedded renal tissue after antigen retrieval (TRS; DAKO Diagnostica) as described (19).

Counting of WT-1 and vimentin-positive glomerular cells and evaluation of the expansion of glomerular α8-integrin and vimentin was done in a Leitz Aristoplan microscope (Leica Instruments) in every third glomerulus. Staining for α8-integrin and nephrin was evaluated by Metaview software (Visi
tron Systems, Puchheim, Germany). The stained area was expressed as percentage of the total area of the glomerular tuft.

**Renal histology.** Glomerulosclerosis, tubulointerstitial injury, and glomerular perimeter was evaluated in kidney sections stained with periodic acid-Schiff’s reagent. For glomerulosclerosis, a score of 0 to 4 based on the sclerotic area of the glomerulus was used. A score of 0 indicated normal glomerulus, a score of 1 indicated mesangial expansion or sclerosis involving up to 25% of the glomerular tuft, a score of 2 indicated sclerosis 25 to 50%, a score of 3 indicated sclerosis of 50 to 75%, and a score of 4 indicated global sclerosis (> 75%). At least 50 consecutive glomeruli per section were evaluated, and the glomerulosclerosis index is given as the mean score per animal. Tubulointerstitial injury was assessed in 10 randomly sampled kidney fields using a tubulointerstitial damage score as described (2). Glomerular perimeter was traced and evaluated by Metaview software.

**Analysis of data.** ANOVA, followed by a post hoc Bonferroni test, was used to test significance of differences between groups. A P value of < 0.05 was considered significant. The procedures were carried out using the SPSS software (release 15.0; SPSS, Chicago). Values are displayed as means ± SE.

**RESULTS**

In diabetic nephropathy, glomerular α8-integrin expression is increased. Six weeks after induction of STZ diabetes, mRNA expression of the α8-integrin chain was increased in cortical tissue homogenates of wild-type mice (Fig. 1A). In mice heterozygous for the α8-integrin deficiency, the total amount of cortical α8-integrin mRNA was somewhat reduced compared with wild-type mice (Fig. 1A), but an induction of mRNA expression was still observed (Fig. 1A). To confirm the glomerular localization of this increase, α8-integrin expression
in microdissected glomeruli was determined (Fig. 1B). In glomerular microdissections of renal tissue from α8-integrin-deficient mice, expression of α8-integrin mRNA was not detected (Fig. 1B). Moreover, the glomeruli of diabetic wild-type mice revealed expanded and more intense mesangial staining for α8-integrin compared with the glomeruli of control mice (Fig. 1C). Densitometric evaluation revealed a significantly higher percentage of glomerular area stained positive for α8-integrin in STZ diabetic mice than in control animals.

STZ-induced diabetes is comparable in mice deficient for α8-integrin and wild-type mice. Blood glucose levels after onset of STZ diabetes were increased in all genotypes, with no significant differences in α8-integrin-deficient and wild-type litters (Table 1) by glucometer analysis. To confirm these measurements, serum analyses using an automatic analyzer were performed in diabetic animals. No differences in serum glucose levels were observed in α8-integrin-deficient and wild-type litters (415.3 ± 76.9 mg/dl in α8−/− and 474.0 ± 93.5 mg/dl in α8+/− vs. 420.3 ± 111.2 mg/dl in α8+/+ STZ-treated mice, not significant). Mean arterial blood pressure and relative left ventricular weight was similar in all groups, regardless of whether diabetic or not (Table 1). To control for the effects of uninephrectomy on the development of diabetes and renal outcome, untreated (uninephrectomized) heterozygous and wild-type mice also received STZ to induce diabetes. Similar to the results from the uninephrectomized groups, blood glucose levels of these mice were not significantly different from blood glucose levels in homozygous α8-integrin-deficient mice (Table 2). Another group of diabetic wild-type mice received a high dose of insulin to normalize blood glucose levels (Table 2) to control for unspecific effects of STZ administration.

STZ induced tubulointerstitial injury to a comparable degree in mice deficient for α8-integrin and wild-type mice. Tubulointerstitial changes were observed after administration of STZ in all genotypes (Table 1). Homozygous α8-integrin-deficient

Table 1. Comparison of physiological and histological parameters in mice with or without a deficiency for α8-integrin

<table>
<thead>
<tr>
<th>Treatment</th>
<th>α8−/−</th>
<th>α8+/−</th>
<th>α8+/+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>STZ</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>No/group</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood glucose, mg/dl</td>
<td>114.5 ± 2.1</td>
<td>504.2 ± 43.6*</td>
<td>115.9 ± 3.7</td>
</tr>
<tr>
<td>Glucometer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean arterial blood pressure, mmHg</td>
<td>119.3 ± 2.7</td>
<td>116.1 ± 0.7</td>
<td>119.0 ± 5.5</td>
</tr>
<tr>
<td>Body weights, g</td>
<td>25.7 ± 0.6</td>
<td>23.7 ± 0.9</td>
<td>26.2 ± 0.5</td>
</tr>
<tr>
<td>Relative left ventricular weight, % (g/g)</td>
<td>0.323 ± 0.012</td>
<td>0.309 ± 0.005</td>
<td>0.310 ± 0.012</td>
</tr>
<tr>
<td>Relative kidney weight, % (g/g)</td>
<td>0.746 ± 0.030</td>
<td>1.162 ± 0.080*</td>
<td>0.748 ± 0.020</td>
</tr>
<tr>
<td>Serum creatinine, mg/dl</td>
<td>0.14 ± 0.02</td>
<td>0.18 ± 0.02</td>
<td>0.12 ± 0.01</td>
</tr>
<tr>
<td>Serum urea, mg/dl</td>
<td>54.7 ± 5.3</td>
<td>93.6 ± 9.1*</td>
<td>52.2 ± 4.5</td>
</tr>
<tr>
<td>Urine volume, mg/dl</td>
<td>2.20 ± 0.46</td>
<td>10.68 ± 1.52*</td>
<td>1.63 ± 0.28</td>
</tr>
<tr>
<td>Glomerular perimeter, μm</td>
<td>206.6 ± 3.2</td>
<td>251.9 ± 3.3*</td>
<td>203.5 ± 2.1</td>
</tr>
<tr>
<td>Vimentin staining, vimentin-positive podocytes/glomerulus</td>
<td>5.92 ± 0.21</td>
<td>3.39 ± 0.48*</td>
<td>7.04 ± 0.26</td>
</tr>
<tr>
<td>Tubulointerstitial damage index, score</td>
<td>0.16 ± 0.04</td>
<td>1.59 ± 0.17*</td>
<td>0.23 ± 0.19</td>
</tr>
</tbody>
</table>

Data are means ± SE. α8−/−, mice with a homozygous deficiency for α8-integrin; α8+/−, mice with a heterozygous deficiency for α8-integrin; α8+/+, wild-type mice; STZ, treatment with streptozotocin. *P < 0.05 vs. respective control; †P < 0.05 vs. wild-type mice.
Table 2. Comparison of physiological and histological parameters in the additional nonuninephrectomized control groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>a8+/− STZ</th>
<th>a8/+ STZ</th>
<th>a8+/+ STZ</th>
<th>a8+/+ STZ Normoglyc</th>
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</thead>
<tbody>
<tr>
<td>No./group</td>
<td>8</td>
<td>7</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>Blood glucose, mg/dl</td>
<td>504.2 ± 43.6</td>
<td>394.2 ± 28.6</td>
<td>481.5 ± 27.4</td>
<td>127.3 ± 13.7†</td>
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<tr>
<td>Urine volume, mg/dl</td>
<td>10.68 ± 1.52</td>
<td>8.01 ± 1.44</td>
<td>12.90 ± 1.39</td>
<td>3.72 ± 1.35†</td>
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<tr>
<td>Albuminuria, mg/day</td>
<td>3.96 ± 1.31*</td>
<td>0.76 ± 0.36</td>
<td>1.45 ± 0.22</td>
<td>0.26 ± 0.13†</td>
</tr>
<tr>
<td>Glomerular perimeter, µm</td>
<td>251.9 ± 3.3</td>
<td>232.8 ± 5.0</td>
<td>237.3 ± 3.3</td>
<td>195.0 ± 3.2†</td>
</tr>
<tr>
<td>Glomerulosclerosis index, score</td>
<td>1.03 ± 0.03*</td>
<td>0.65 ± 0.03</td>
<td>0.63 ± 0.04</td>
<td>0.39 ± 0.04†</td>
</tr>
<tr>
<td>WT-1 stain, WT-1-positive cells/glomerulus</td>
<td>3.03 ± 0.19*</td>
<td>5.4 ± 0.19</td>
<td>5.29 ± 0.15</td>
<td>0.74 ± 0.08†</td>
</tr>
<tr>
<td>Vimentin stain, vimentin-positive podocytes/glomerulus</td>
<td>3.39 ± 0.48*</td>
<td>5.24 ± 0.29</td>
<td>5.3 ± 0.29</td>
<td>6.9 ± 0.3</td>
</tr>
<tr>
<td>Nephrin stain, % positive glomerular area</td>
<td>13.7 ± 1.1*</td>
<td>20.3 ± 0.9</td>
<td>20.9 ± 1.2</td>
<td>30.5 ± 1.0†</td>
</tr>
<tr>
<td>Tubulointerstitial damage index, score</td>
<td>1.59 ± 0.17</td>
<td>1.06 ± 0.25</td>
<td>1.40 ± 0.27</td>
<td>1.08 ± 0.37</td>
</tr>
</tbody>
</table>

Data are means ± SE. *P < 0.05 vs. wild-type mice; †P < 0.05 vs. wild-type mice exposed to high levels of glucose.

mice were affected similarly to wild-type mice (Table 1). Tubulointerstitial injury was not reverted by insulin treatment (Table 2).

Mice deficient for a8-integrin develop more severe glomerular injury during STZ-induced diabetes compared with wild types. The relative kidney weights of nondiabetic homozygous a8-integrin-deficient mice as well as nondiabetic uninephrectomized heterozygous a8-integrin-deficient and wild-type mice were similar (Table 1). Induction of diabetes led to an increase in relative kidney weight, especially in homozygous a8-integrin-deficient mice, while in the other groups only a tendency toward increased relative kidney weights was observed (Table 1). Urine volume was comparably increased in all diabetic mice regardless of the genetic background (Table 1) and was nearly normalized by insulin treatment (Table 2). Serum urea was increased in all diabetic groups to a similar extent (Table 1), while serum creatinine only tended to be higher 6 wk after induction of diabetes, without reaching statistical significance (Table 1). Glomerular size, as determined by measuring the glomerular perimeter, was expanded in all diabetic groups and normalized in insulin-treated mice (Tables 1 and 2). After 6 wk of diabetes, albumin excretion was increased in homozygous a8-integrin-deficient, heterozygous a8-integrin-deficient, and wild-type mice with significantly higher levels in homozygous a8-integrin-deficient mice compared with the other genotypes (Fig. 2A). Insulin treatment significantly reduced albuminuria in diabetic mice (Table 2). Glomerulosclerosis indices were significantly increased in all diabetic mice (Fig. 2B and Table 2). The highest glomerulosclerosis scores were obtained in homozygous a8-integrin-deficient mice (Fig. 2B). Insulin treatment normalized glomerulosclerosis scores (Table 2). Albuminuria and glomerulosclerosis of heterozygous a8-integrin-deficient and wild-type mice were not different (Fig. 2), despite the reduced expression of a8-integrin in heterozygous a8-integrin-deficient mice compared with wild-type mice (Fig. 1). The differences in albuminuria and glomerulosclerosis of homozygous a8-integrin-deficient mice and both other genotypes were detected regardless of whether the other genotypes were uninephrectomized to adjust for the reduced renal mass in homozygous a8-integrin-deficient mice (Fig. 2) or whether they were not subjected to operation procedures (Table 2).

Mice deficient for a8-integrin display an aggravated podocyte damage during STZ-induced diabetes compared with wild types. Two markers of normal podocytes, WT-1 and vimentin, were investigated: 1) the number of WT-1 positive cells per glomerulus was reduced in diabetic mice (Fig. 3) and 2) the reduction of WT-1 positive cells was most prominent in homozygous a8-integrin-deficient mice (Fig. 3). Likewise, the number of vimentin-positive glomerular cells was reduced in diabetic mice, with the lowest number of vimentin-positive cells counted in homozygous a8-integrin-deficient mice (Table 2). Downregulation of nephrin, a protein of the podocyte filtration barrier, was considered as a marker of podocyte injury. The immunoreactivity of nephrin was reduced in all diabetic mice; however, in homozygous a8-integrin-deficient mice, the reduc-
fibrotic alterations (1) and might thus be detrimental for normal results suggest that the upregulation of α2-integrin expression whereas α1-integrin was downregulated (36). Upregulation of αν- and α5-integrins in response to hyperglycemia was also detected in glomerular epithelial cells, while at the same time, expression of α2- and α3-integrins was reduced (26). Our studies in mesangial cells exposed to different glucose concentrations, however, do not support the notion that the induction of α8-integrin in diabetic nephropathy is due to the direct effects of high glucose concentrations (data not shown). During diabetic nephropathy, TGF-β is upregulated in kidney tissue and mediates pathologic alterations in the kidney (37). Moreover, TGF-β stimulates the expression of α1- and α5-integrins in rat mesangial cells (24). Previous studies detected increased expression of α8-integrin mRNA and protein amounts in response to mesangial cell stimulation with TGF-β (22). Therefore, increased amounts of TGF-β in the diabetic kidney of STZ-treated animals (14) could account for the mesangial induction of α8-integrin in this model.

Mice with a homozygous deletion of α8-integrin are born with a 50% reduced renal mass due to impaired epithelial-mesenchymal interactions during kidney development (30).
For this reason, wild-type mice and mice with a heterozygous deletion of the α8-integrin gene were uninephrectomized to adjust for the reduced renal mass of homozygous α8-integrin-deficient mice in this study. To control for effects caused by uninephrectomy itself, we also induced diabetes in wild-type and heterozygous α8-integrin-deficient mice that did not undergo uninephrectomy. In the adult kidney, glomerular expression of α8-integrin is confined to mesangial cells (22). Despite its prominent expression in the glomerulus, lack of α8-integrin per se does not lead to major alterations in glomerular structure or function (15). However, if mechanical stress is induced in glomeruli of α8-integrin-deficient mice by exposing them to high blood pressure, disruption of the glomerular capillary tuft is observed (17). On the other hand, glomerular hypertension resulted in an increase in albuminuria and glomerular matrix expansion with no differences observed between wild-type and α8-integrin-deficient mice (17). Thus, these findings are in contrast with the present study in diabetic nephropathy, where a significantly higher amount of albuminuria and increased glomerulosclerosis were detected. Enhanced diabetic glomerulosclerosis in homozygous α8-integrin-deficient mice could be a direct consequence of the lack of α8-integrin, although it is unknown whether or not signaling via α8-integrin can lead to reduced deposition of matrix proteins or increased activity of matrix proteases. Several studies reported an association of fibrotic alterations and α8-integrin expression in different tissues, but no causal relationship has been determined (5, 17, 28).

Furthermore, it is unclear, how a lack of α8-integrin in mesangial cells can lead to podocyte injury. We used WT-1 and vimentin as markers of normal podocytes and observed a reduction of WT-1 and vimentin-positive glomerular cells in diabetic kidneys with the most prominent reduction in mice with a homozygous deletion of α8-integrin. Several factors are discussed to contribute to diabetic podocyte failure, including hyperglycemia, mechanical stress, or TGF-β (39). Lack of α8-integrin did not lead to significant increases in blood glucose levels in our study. Moreover, TGF-β expression was increased in diabetic nephropathy but was not different in wild-type and α8-integrin-deficient mesangial cells (14; A. Hartner, unpublished observations). We have previously shown that α8-integrin is important in maintaining adhesion of mesangial cells to mesangial matrix components, as mesangial cells lacking α8-integrin adhere much more weakly to ligands of α8-integrin compared with wild-type cells (3). Increased deposition of the α8-integrin ligands fibronectin and osteopontin has been described in diabetic nephropathy (12, 29). Lack of α8-integrin might reduce the firm adhesion of mesangial cells to their surrounding matrix, leading to loss of structural integrity especially in diseased glomeruli, which are exposed to increased mechanical stress. This might in turn lead to podocyte failure (39) and downregulation of nephrin, leading to a disruption of the filtration barrier. Interestingly, all podocyte markers studied were somewhat decreased in non diabetic glomeruli of mice with a homozygous deletion of α8-integrin. This could be due to a lower number of podocytes or to dedifferentiation of podocytes. Previous studies revealed equal amounts of podocytes in wild-type and α8-integrin-deficient mice evaluated by morphometric analyses (15). Thus, it seems that a lack of α8-integrin per se leads to a certain degree of dedifferentiation of podocytes, possibly rendering them more vulnerable to injury caused by diabetes. On the other hand, in mice with a heterozygous deletion for the α8-integrin gene, the expression of α8-integrin is somewhat attenuated, but this does not lead to an increased vulnerability to diabetic nephropathy, as diabetic mice of this genotype develop albuminuria and glomerular damage to a similar degree to diabetic wild-type mice. Thus, α8-integrin expression in mice with a heterozygous deletion of the α8-integrin gene seems to be sufficient to prevent more severe glomerular injury.

A limitation of the present study is the previously described toxic effect of the single-shot high-dose STZ administration (7). Tubulointerstitial damage was not reverted by insulin treatment. This type of injury is therefore probably not mediated by high glucose, but rather is due to a direct toxic effect of STZ. In contrast, the glomerular changes observed were all ameliorated by insulin treatment. Thus it seems unlikely that these glomerular changes are a consequence of a direct toxic effect in the present study. However, one has to keep in mind that there is some evidence for a feedback on glomerular function by tubulointerstitial damage in diabetes (38).

Taken together, our data point to a protective role for α8-integrin in diabetic nephropathy. Upregulation of α8-integrin in glomeruli of diabetic kidneys might serve to ameliorate glomerular damage.

ACKNOWLEDGMENTS
Part of this work was presented in abstract form at the 37th Annual Meeting of the American Society of Nephrology, St. Louis, MO. Present address of J. M. Yabu: Division of Nephrology, Department of Medicine, Stanford University School of Medicine, Palo Alto, California.

GRANTS
This study was supported by a grant from the Deutsche Forschungsgemeinschaft, Bonn, Germany and Sonderforschungsbereich 423, TP A2.

DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

REFERENCES
8-INTEGRIN IN DIABETIC NEPHROPATHY


