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

Andrea Hartner,1 Nada Cordasic,2 Carlos Menendez-Castro,1 Gudrun Volkert,2 Julie M. Yabu,1,3 Miroslava Kupraszewicz-Hutzler,2 Wolfgang Rascher,1 and Karl F. Hilgers2

1Departments of Pediatrics and Adolescent Medicine and 2Nephrology and Hypertension, University of Erlangen-Nürnberg, Erlangen, Germany; and 3Division of Nephrology, Stanford University School of Medicine, Palo Alto, California

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.

strenuous information processing; social cognition and decision; memory and learning; biology of aging; genetics of longevity; neurodegenerative disorders and aging; aging research.

strenuous information processing; social cognition and decision; memory and learning; biology of aging; genetics of longevity; neurodegenerative disorders and aging; aging research.

Address for reprint requests and other correspondence: A. Hartner, Dept. of Pediatrics, Loschengrassstrasse 15, D-91054 Erlangen, Germany. (e-mail: andrea.hartner@uk-erlangen.de).

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, tenasin 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 αβ1-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, AZ621-2531.31-1/01 and 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/6J/129Sv background. The α8-integ-
rin-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 con-
trols. 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 isoflurane 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/6J/129Sv background. Two days after STZ adminis-
tration, 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 pellet releasing 0.1 units insulin/day sc) which did not normalize blood glucose, but was
sufficient to avoid ketoacidosis. To normalize blood glucose levels, a
0.1 units insulin/day pellet was implanted subcutaneously (LinShin,
Toronto, Canada). Diabetic and control mice (solvent only) were
followed for 6 wk. At this time point, glomerular damage is readily
detectable in this animal model (21). Blood glucose was monitored
weekly (at 8 AM) using a reflectance meter (Glucometer Elite II).
After 4 wk, the mice were kept in metabolic cages for determination
of urinary albumin excretion (enzyme immunoassay kit; CellTrend,
Luckenwalde, Germany) for 24 h.

At the day of death, animals were equipped with a carotid artery
catheter under ketamine/xylazine anesthesia, and intra-arterial blood
pressure was measured in conscious mice 3 h after anesthesia. Ani-
mals were killed by dissecting the abdominal artery and bleeding in
deep ketamine/xylazine anesthesia. Tissue samples were prepared for
histochemistry and RNA extraction as described (19, 20).

Serum parameters. Creatinine, urea, and glucose were measured with
the automatic analyzer Integra 800 (Roche Diagnostics, Mann-
heim, Germany).

Real-time RT-PCR. Renal cortical tissue (10 mg) was homogenized in
500 μl of RNeasy lysis buffer reagent (Qiagen, Hilden, Germany)
with an ultraturrax for 30 s, and total RNA was extracted with RNeasy
Mini columns (Qiagen) according to the standard protocol. First-
strand cDNA was synthesized with TaqMan reverse transcription
reagents (Applied Biosystems, Darmstadt, Germany) using random
hexamers as primers. Final RNA concentration in the reaction mixture
was adjusted to 0.1 ng/μl. Reactions without Multiscribe reverse
transcriptase were used as negative controls for genomic DNA con-
tamination. RT-products were diluted 1:1 with dH2O before PCR
procedure. PCR was performed with an ABI PRISM 7000 Sequence
Detector System and SYBR Green reagents (Applied Biosystems)
according to the manufacturers instructions.

For amplification of the mouse α8-integrin cDNA, the forward
primer was 5′-AGA ATG ATT ACC CAG ATT TTC TGG T-3′, and
the reverse primer was 5′-GCT ACT TTC CTT CCA AAT GC-3′. The relative amount of the specific mRNA was normalized
with respect to 18S rRNA. Primers used for amplification of 18S
cDNA were: forward primer 5′-TG ATT AAG TCC CGT CTC TTC
GT-3′ and reverse primer 5′-CGA TCC GAG GGC CTC ACT A-3′.
All samples were run in triplicates.

Laser-assisted microdissection. Frozen renal tissue specimens were
cut in 10 μm-thick sections and mounted on membrane-coated slides.
To minimize degradation, slides were fixed with 70% ethanol for 1
min, then 100% ethanol for 1 min, and washed in diethylpyrocarbo-
mate-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) maintaining 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 instruc-
tions. Preparation of cDNA and detection of α8-integrin expression
was performed as described above.

Immunohistochemical analyses. Staining of cryostat and paraffin
sections was cloned 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 (Acris Antibodies,
Hiddenhauen, 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
examined using Metaview software (Visitron Systems, Puchheim, Ger-
many). 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
period 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 expan-
sion or sclerosis involving up the 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 parameter 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 com-
pared 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

F1152

α8-INTTEGRIN IN DIABETIC NEPHROPATHY

AJP-Renal Physiol • VOL 299 • NOVEMBER 2010 • www.ajprenal.org

Downloaded from http://ajprenal.physiology.org/ by 10.22033.6 on October 21, 2017
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 significant differences in 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 unspecified 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 mice developed tubulointerstitial damage (Table 1). Serum creatinine was significantly higher in diabetic wild-type mice compared with healthy controls. co, nondiabetic controls; α8+/−, wild-type mice; α8+/+, mice with a heterozygous deletion of the α8-integrin gene; α8−/−, mice with a homozygous deletion of the α8-integrin gene. Data are means ± SE. *P < 0.05 vs. respective controls.

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>
</tr>
<tr>
<td>STZ</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 stain, 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.
mice were affected similarly to wild-type mice (Table 1). Tubulointerstitial injury was not reverted by insulin treatment (Table 2).

Mice deficient for α8-integrin develop more severe glomerular injury during STZ-induced diabetes compared with wild types. The relative kidney weights of nondiabetic homozygous α8-integrin-deficient mice as well as nondiabetic uninephrectomized heterozygous α8-integrin-deficient and wild-type mice were similar (Table 1). Induction of diabetes led to an increase in relative kidney weight, especially in homozygous α8-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 α8-integrin-deficient, heterozygous α8-integrin-deficient, and wild-type mice with significantly higher levels in homozygous α8-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 α8-integrin-deficient mice (Fig. 2B). Insulin treatment normalized glomerulosclerosis scores (Table 2). Albuminuria and glomerulosclerosis of heterozygous α8-integrin-deficient and wild-type mice were not different (Fig. 2), despite the reduced expression of α8-integrin in heterozygous α8-integrin-deficient mice compared with wild-type mice (Fig. 1). The differences in albuminuria and glomerulosclerosis of homozygous α8-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 α8-integrin-deficient mice (Fig. 2) or whether they were not subjected to operation procedures (Table 2).

Mice deficient for α8-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 α8-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 α8-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 α8-integrin-deficient mice, the reduc-

Table 2. Comparison of physiological and histological parameters in the additional nonuninephrectomized control groups

<table>
<thead>
<tr>
<th>Group</th>
<th>α8−/− STZ</th>
<th>α8−/+ STZ</th>
<th>α8+/+ STZ</th>
<th>α8+/+ STZ Normoglyc</th>
<th>α8−/− STZ vs. α8+/+ STZ</th>
<th>α8−/− STZ vs. α8+/+ STZ Normoglyc</th>
</tr>
</thead>
<tbody>
<tr>
<td>No./group</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</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>
<td>7.2 ± 13.5†</td>
<td>127.3 ± 13.7†</td>
</tr>
<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>
<td>3.72 ± 1.35†</td>
<td>3.72 ± 1.35†</td>
</tr>
<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>
<td>0.26 ± 0.13†</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>237.8 ± 3.3</td>
<td>195.0 ± 3.2</td>
<td>195.0 ± 3.2</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>
<td>0.39 ± 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>7.04 ± 0.08†</td>
<td>7.04 ± 0.08†</td>
<td>7.04 ± 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>
<td>6.9 ± 0.3</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>
<td>30.5 ± 1.0†</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>
<td>1.08 ± 0.37</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.
Fibrotic alterations (1) and might thus be detrimental for normal
reported. Upregulation of many integrins is associated with
mental rat diabetic nephropathy (8, 16, 23, 32, 40). However,
lation of integrins is a common feature of human and experi-
diabetic glomeruli of mice after treatment with STZ. Dysregu-
results suggest that the upregulation of integrins in glomeruli undergoing mechanical or metabolic stress. These
leads to more severe glomerular lesions and aggravated podo-
cyte injury during the development of diabetic nephropathy.
Thus, increased amounts of TGF-
amounts in response to mesangial cell stimulation with TGF-
previous studies de-
that the induction of integrin in diabetic nephropathy is due
to the direct effects of high glucose concentrations (data not
showed). 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 α- 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).

Discussion
Our data show that 1) the α8-integrin chain is more abundant in glomeruli of diabetic kidneys, and 2) the lack of α8-integrin leads to more severe glomerular lesions and aggravated podocyte injury during the development of diabetic nephropathy. Thus, α8-integrin seems to have an important function in the maintenance of glomerular structure and function, especially in glomeruli undergoing mechanical or metabolic stress. These results suggest that the upregulation of α8-integrin in diabetic nephropathy may be protective rather than contributing to fibrotic alterations.
Increased α8-integrin immunoreactivity was observed in diabetic glomeruli of mice after treatment with STZ. Dysregulation of integrins is a common feature of human and experimental rat diabetic nephropathy (8, 16, 23, 32, 40). However, while αv- and α3-integrins have been investigated in detail in this context, the involvement of α8-integrin has not been reported. Upregulation of many integrins is associated with fibrotic alterations (1) and might thus be detrimental for normal
tissue homeostasis. For example, inhibition of α1β1-integrin by blocking antibodies in a rat model of glomerulonephritis prevents hypercellularity and matrix expansion in the glomerulus (25). On the other hand, integrins may be protective for tissue homeostasis in the kidney. Lack of α3β1 results in disorganization of the glomerular filtration barrier (27).
Several factors could account for the induction of α8-integrin expression in mesangial cells during diabetic nephropathy. High glucose concentrations are known to be able to regulate expression of integrins in glomerular cells. In mesangial cells, high glucose concentrations led to an increase of α2-integrin expression whereas α1-integrin was downregulated (36). Upregulation of αv- 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).

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


