The $\alpha 8$ Integrin Chain Affords Mechanical Stability to the Glomerular Capillary Tuft in Hypertensive Glomerular Disease

Andrea Hartner,* Nada Cordasic,* Bernd Klanke,*
Ulrich Müller,[†] R. Bernd Sterzel,* and
Karl F. Hilgers*

From the Medizinische Klinik IV,* Universität
Erlangen-Nürnberg, Erlangen, Germany; and the
Friedrich-Miescher-Institut,[†] Basel, Switzerland

In the kidney, the $\alpha 8$ integrin chain is expressed in glomerular mesangial cells. The $\alpha 8$ integrin plays a role in early nephrogenesis but its functional role in the adult kidney is unknown. We tested the hypothesis that $\alpha 8$ integrin-mediated cell-matrix interactions are important to maintain the integrity of the glomerulus in arterial hypertension. Desoxycorticosterone (DOCA)-salt hypertension was induced in mice homozygous for a deletion of the $\alpha 8$ integrin chain and wild-type mice. Blood pressure, albumin excretion, total renal mass, and glomerular filtration in DOCA-treated $\alpha 8$ -deficient mice were comparable to DOCA-treated wild types. DOCA-treated wild types showed increased glomerular immunostaining for $\alpha 8$ integrin compared to salt-loaded and untreated controls, whereas the glomeruli of $\alpha 8$ -deficient mice always stained negative. Morphometric studies revealed similar degrees of glomerulosclerosis in DOCA-treated $\alpha 8$ -deficient and DOCA-treated wild-type mice. However, DOCA-treated $\alpha 8$ -deficient mice had a higher score of capillary widening (mesangiolysis) than DOCA-treated wild-type mice, which was confirmed in two additional wild-type strains. Moreover, in DOCA-treated $\alpha 8$ -deficient mice, glomerular fibrin deposits were more frequent than in DOCA-treated wild types. The results show that lack of $\alpha 8$ is associated with increased susceptibility to glomerular capillary destruction in DOCA salt hypertension, whereas it does not seem to play a major role in the development of fibrosis or glomerulosclerosis. Our findings indicate that mesangial $\alpha 8$ integrin contributes to maintain the integrity of the glomerular capillary tuft during mechanical stress, eg, in hypertension. (*Am J Pathol* 2002, 160:861–867)

Alterations in matrix expression and composition as well as in abundance of matrix receptors are a hallmark of progressive glomerulosclerosis.^{1,2} Integrins are a large

family of cell surface matrix receptors. Induction of integrins was shown in various glomerulopathies suggesting a role for integrins during progression of disease.²

Integrins are heterodimers consisting of an α and a β subunit. The $\alpha 8$ integrin subunit was shown to be a partner for $\beta 1$.³ $\alpha 8\beta 1$ binds the RGD site of fibronectin, vitronectin, tenascin C,⁴ osteopontin,⁵ and the recently described nephronectin.⁶ In the kidney, we and others^{7,8} showed expression of $\alpha 8$ integrin in smooth muscle cells of renal arterioles and glomerular mesangial cells. Furthermore, glomerular $\alpha 8$ integrin was increased in a transient model of mesangioproliferative glomerulonephritis during matrix expansion.⁷ However, the function of $\alpha 8$ integrin in the mesangium of healthy and diseased glomeruli is still unclear.

During development, $\alpha 8$ integrin is induced in the mesenchyme surrounding the branching ureter tips.⁹ Mice deficient for the $\alpha 8$ integrin chain have severe defects in ureteral branching, frequently leading to agenesis or dysgenesis of the kidney. Many of these mice die shortly after birth. There are apparently no abnormalities in later stages of kidney development; however, surviving animals show unilateral kidneys or only small kidneys with normal appearing glomeruli.⁹ Thus, $\alpha 8$ does not seem to be essential for glomerulogenesis.

We analyzed the role of $\alpha 8$ in the maintenance of glomerular structure and function in the face of increased mechanical strain. Glomerular hypertension was induced in uninephrectomized wild-type and $\alpha 8$ integrin-deficient mice by administration of desoxycorticosterone-acetate (DOCA) and 1% NaCl as drinking water. This model was chosen because here the glomeruli of the kidney are uniquely exposed to high blood pressure.¹⁰ In view of the prominent glomerular abundance of $\alpha 8$ integrin, we hypothesized that a lack of $\alpha 8$ would aggravate glomerular injury, leading to more severe morphological alterations.

Supported by grant SFB 423/A2 from the Deutsche Forschungsgemeinschaft.

Prof. R. Bernd Sterzel died August 6, 2001.

Accepted for publication November 14, 2001.

Address reprint requests to Karl F. Hilgers, M.D., Nephrology Research Laboratory, Loschgestrasse 8, 91054 Erlangen, Germany. E-mail: karl.hilgers@rzmail.uni-erlangen.de.

Materials and Methods

DOCA Salt Hypertension

Mice were housed in a room maintained at $22 \pm 2^\circ\text{C}$, exposed to a 12-hour dark/light cycle. The animals were allowed unlimited access to chow (no.1320; Altromin, Lage, Germany) and tap water (or 1% saline, see below). All procedures performed on animals were done in accordance with guidelines of the American Physiological Society and were approved by the local government authorities (Regierung von Mittelfranken, AZ no. 621-2531.31-1/01).

Male homozygous $\alpha 8$ integrin-deficient mice generally develop only one kidney or two smaller kidneys, leading to a reduced total renal mass. In this study, only $\alpha 8$ -deficient mice with two smaller kidneys were used. $\alpha 8$ -deficient mice were not backcrossed into C57/BL6 but maintained as a homozygous line derived from the first generation of homozygotes.⁹ Age- and weight-matched male 129 mice (Charles River, Sulzfeld, Germany) were used as the primary wild-type controls. Being aware that 129 mice are not the ideal wild-type control for $\alpha 8$ -deficient mice, we additionally investigated two more wild-type strains: C57/BL6 mice and 129xC57/BL6 intercrosses.

At an average weight of 16 g to 18 g, wild-type mice underwent right unilateral nephrectomy to adjust for the reduced kidney mass in $\alpha 8$ -deficient mice. $\alpha 8$ -deficient mice were not nephrectomized. After 2 weeks of recovery, 21-day-release DOCA pellets containing 50 mg of DOCA (Innovative Research of America, Sarasota, FL) were implanted subcutaneously by incision of the right flank under light ether anesthesia. Control animals were sham operated. After 21 days they received a replacement pellet. All animals (DOCA and control groups) received isotonic saline (10 g NaCl/L) for drinking for 6 weeks, starting with the first day of DOCA treatment. The animals were then followed by weekly measurements of weight and systolic blood pressure (Visitech Systems, Apex, NC).

After 6 weeks of treatment, the mice were put into metabolic cages, and urine was collected for 24 hours for measurement of albuminuria. The urinary albumin content was measured by an enzyme immunoassay kit (EIA) (CellTrend, Luckenwalde, Germany). Finally, animals were sacrificed (6 DOCA-treated 129 mice, 10 DOCA-treated $\alpha 8$ -deficient mice, 6 129 control mice, and 10 $\alpha 8$ -deficient controls). At the day of sacrifice, animals were equipped with a carotid artery catheter under ketamine/xylazine anesthesia and intra-arterial blood pressure was measured in conscious mice 4 hours after anesthesia. Animals were sacrificed by dissecting the abdominal artery and bleeding in deep ketamine/xylazine anesthesia.

After measuring kidney weight, the organs were decapsulated. Kidneys were put in methyl-Carnoy solution (60% methanol, 30% chloroform, and 10% glacial acetic acid) for fixation.

Renal Histology

After overnight fixation in methyl-Carnoy solution, tissues were dehydrated by bathing in increasing concentrations of methanol, followed by 100% isopropanol. After embedding in paraffin, 2- μm sections were cut with a Leitz SM 2000 R microtome (Leica Instruments, Nussloch, Germany). Before any staining procedure, sections were deparaffinized by bathing in xylol and rehydrated in decreasing concentrations of alcohol. For evaluation of general renal histology, kidney sections were periodic acid-Schiff (PAS) stained.

To quantify glomerulosclerosis a score of 0 to 4 was used as described before.¹¹ 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 50 to 75% and/or segmental extracapillary fibrosis or proliferation, and a score of 4 indicated global sclerosis (>75%) or global extracapillary fibrosis or proliferation, or complete collapse of the glomerular tuft. To assess the extent of the formation of microaneurysms, capillary widening was scored. Similar to the score for sclerosis, a score 0 to 4 was applied. Score 0 indicated no capillary widening in the glomerular tuft, score 1 indicated a capillary widening involving up to 25% of the glomerular tuft, score 2 indicated a capillary widening 25 to 50%, score 3 indicated a capillary widening 50 to 75%, and a score of 4 indicated capillary widening involving >75% of the glomerular tuft.

Antibodies

The rabbit polyclonal antiserum to $\alpha 8$ integrin was used at a dilution of 1:200 as described before.⁷ A monoclonal antibody to smooth muscle actin (DAKO Diagnostika, Hamburg, Germany) was used at a dilution of 1:50 to stain activated myofibroblasts. A mouse monoclonal antibody to fibrin (American Diagnostica, Pfungstadt, Germany) was used at a dilution of 1:1000 to stain fibrin exudates after blocking of endogenous mouse IgG by a mouse-on-mouse kit (MOM) (Linaris, Wertheim, Germany). Rabbit polyclonal antibodies to fibronectin (Life Technologies, Eggenstein, Germany), collagen I (Biogenesis, Poole, England), and collagen IV (Southern Biotechnology Associates, Birmingham, AL) were used at a dilution 1:1000.

Immunohistochemistry

In deparaffinized kidney sections, endogenous peroxidase activity was blocked with 3% H_2O_2 in methanol for 20 minutes at room temperature.

Sections were then layered with the primary antibody and incubated at 4°C overnight. After addition of the secondary antibody (dilution, 1:500; biotin-conjugated goat anti-rabbit IgG or rabbit anti-mouse IgG, all Dianova, Hamburg, Germany), the sections were incubated with avidin horseradish peroxidase complex and exposed to 0.1% diaminobenzidine tetrahydrochloride and 0.02%

Figure 1. Immunohistochemical detection of $\alpha 8$ in glomeruli of control wild-type mice (A) and in DOCA-treated wild-type mice (B and C). Original magnifications, $\times 600$.

H_2O_2 as a source of peroxidase substrate. The Vectastain DAB kit (Vector Laboratories, Burlingame, CA) was used as a chromogen. Each slide was counterstained with hematoxylin. As a negative control, we used equimolar concentrations of preimmune rabbit or mouse IgG.

Expansion of interstitial collagen I was measured in a Leitz Aristoplan microscope (Leica Instruments) by Meta-view software (Visitron Systems, Puchheim, Germany) in 10 nonoverlapping medium-power cortical views per section excluding glomeruli and was expressed as percentage of stained area per cross-section. Glomerular $\alpha 8$, fibronectin, and collagen IV staining was measured by Metaview in every third glomerulus per cross-section and was expressed as percentage of stained area per glomerular tuft.

Analysis of Data

Two-way analysis of variance, followed by post hoc Newman-Keuls test with adjustment for multiple comparisons, was used to test significance of differences between groups. A P value < 0.05 was considered significant. The procedures were performed using the Statistica software (StatSoft, Tulsa, OK). Values are displayed as means $\pm$ SEM.

Results

DOCA-Salt Hypertensive Animals Display Increased Glomerular $\alpha 8$ Immunoreactivity

Wild-type mice had expanded and more intense mesangial staining for $\alpha 8$ integrin when treated with DOCA as compared to salt-treated controls (Figure 1). In wild-type controls, $\alpha 8$ staining covered $5.0 \pm 0.8\%$ of glomerular area, whereas in DOCA salt-treated wild types $17.7 \pm 1.7\%$ of the glomerular tuft stained positive for $\alpha 8$. Another cell-type staining with the $\alpha 8$ integrin antibody in the mouse kidney was the smooth muscle cell of the renal vasculature. Treatment with DOCA salt increased vascular smooth muscle staining for $\alpha 8$ (not shown). No staining for $\alpha 8$ in renal interstitial fibroblasts was detected in control animals. However, in some DOCA-treated animals $\alpha 8$ immunoreactivity was detected in clusters of renal interstitial fibroblasts (not shown). All $\alpha 8$ -deficient mice were negative for $\alpha 8$ immunoreactivity in all renal

cells, confirming the lack of $\alpha 8$ expression in this mouse strain.

$\alpha 8$ -Deficient and Wild-Type Mice Develop a Comparable Degree of Hypertension and Hypertensive Nephrosclerosis

$\alpha 8$ -Deficient mice of the control group had a slightly higher blood pressure than 129 wild-type controls. However, DOCA treatment led to a significant and comparable increase in mean arterial blood pressure both in $\alpha 8$ -deficient and wild-type mice (Figure 2). DOCA-treated wild-type and $\alpha 8$ -deficient mice developed hypertension-dependent alterations to a similar degree, comprising a marked rise in relative weights of kidney and left ventricle, a decrease of creatinine clearance, and augmented albuminuria (Table 1). Dramatic histological changes were detected in all DOCA-treated animals that reached comparable values in wild-type and $\alpha 8$ -deficient mice. Interstitial fibrosis reflected as interstitial collagen I expansion was prominent in both DOCA-treated groups (Figure 3). Glomerulosclerosis was marked after DOCA treatment as assessed by a semiquantitative score of PAS-stained glomeruli and computer-based analysis of staining for fibronectin and glomerular collagen IV (Figure 4; A, B, and C).

Figure 2. Arterial blood pressure measurements in wild-type and $\alpha 8$ -deficient mice with and without DOCA treatment. Data are means $\pm$ SEM. *, $P < 0.05$, DOCA-treated mice *versus* respective controls. #, $P < 0.05$, $\alpha 8$ -deficient controls *versus* wild-type controls.

Table 1. Body Weights, Relative Weights of Kidneys and Left Ventricles, Creatinine Clearance, and Albumin Excretion of All Experimental Groups

	Body weight, g	Relative kidney weight, mg/g	Relative weight of left ventricle, mg/g	Creatinine clearance, ml/min	Albumin excretion, mg/day
Wild type	25.5 ± 1.6	10.9 ± 1.2	4.4 ± 0.3	0.43 ± 0.1	0.4 ± 0.2
Wild type/DOCA	23.8 ± 1.3	14.2 ± 1.3*	5.5 ± 0.2*	0.19 ± 0.02*	3.3 ± 1.4*
$\alpha 8^{-/-}$	24.4 ± 0.4	9.9 ± 0.3	4.2 ± 0.2	0.30 ± 0.03	0.2 ± 0.1
$\alpha 8^{-/-}$ /DOCA	20.9 ± 0.5*	14.4 ± 0.6*	5.8 ± 0.2*	0.17 ± 0.03*	3.1 ± 0.9*

Data are means ± SEM.

* $P < 0.05$ DOCA-treated mice versus controls.

The two additional wild-type strains (C57/BL6 and 129xC57/BL6 intercrosses) studied revealed a smaller increase in blood pressure and weaker fibrosis and glomerulosclerosis than $\alpha 8$ -deficient and 129 wild-type mice (not shown). For that reason C57/BL6 and 129xC57/BL6 intercrosses were not chosen for further investigations.

DOCA-Treated $\alpha 8$ Integrin-Deficient Mice Suffer from Extensive Glomerular Disruption

In the $\alpha 8$ -deficient mice, glomerular cells were in a more activated state compared to wild types, as shown by immunoreactivity for smooth muscle actin. This was particularly prominent after treatment with DOCA (Figure 5A). There was a tendency toward formation of microaneurysms (as assessed by a score for capillary widening) in the salt-loaded $\alpha 8$ -deficient mice and most prominently in DOCA-treated $\alpha 8$ -deficient mice, compared to wild types (Figure 5B). The glomerular tuft appeared much more disrupted in DOCA-treated $\alpha 8$ -deficient mice than in wild types where DOCA treatment did not lead to mesangiolytic lesions (Figure 6, B and E). The DOCA salt-treated $\alpha 8$ integrin-deficient group had the highest degree of glomerular fibrin deposition ($7.2 \pm 1.1\%$ of glomeruli), indicating widespread exudation of serum from the glomerular capillaries (Figure 6F). Much less fibrin-positive glomeruli were detected in DOCA salt-treated wild types ($2.4 \pm 0.8\%$) as depicted on Figure 6E.

Figure 3. Evaluation of interstitial fibrosis by measurement of interstitial collagen I staining in wild-type and $\alpha 8$ -deficient mice with and without DOCA treatment. Data are means ± SEM. *, $P < 0.05$, DOCA-treated mice versus respective controls. #, $P < 0.05$, $\alpha 8$ -deficient controls versus wild-type controls.

Salt-loaded control groups were always negative for fibrin (not shown).

Discussion

Our data provide evidence that $\alpha 8$ integrin is important for the maintenance of structural integrity of the glomerulus after mechanical injury: 1) $\alpha 8$ was more abundant in glomeruli of DOCA salt-treated mice than in control mice; and 2) $\alpha 8$ -deficient mice displayed a degree of glomerular disruption in this model of glomerular hypertension that was not found in any other mouse strain investigated.

Cell-matrix interactions are conceivably important in the adaptation of the vascular wall to high blood pressure. However, surprisingly little information has been reported on integrins in hypertension. Most of these studies focused on leukocyte adhesion,¹² platelet glycoproteins,¹³ and the cellular transduction of mechanical signals.¹⁴ Intengan and co-workers¹⁵ reported that $\alpha v\beta 3$ and $\alpha 5\beta 1$ integrins are up-regulated in the vascular wall of a genetic model of hypertension. Together with our observation that $\alpha 8\beta 1$ is induced by DOCA salt hypertension, these data suggest that the induction of integrin receptors that mediate contact to extracellular matrix proteins such as fibronectin or vitronectin is part of the adaptive response of the blood vessel wall to high blood pressure.

Our data suggest that lack of $\alpha 8\beta 1$ leads to a destruction of capillary tufts in the presence of high blood pressure. The absence of $\alpha 8\beta 1$ can apparently not entirely be compensated for by other integrins. We are not aware of functional studies on the role of other integrins in hypertension. One recent study reported that endothelin-1 decreased αv integrin expression.¹⁶ In view of our results, it is intriguing to speculate that the down-regulation of integrin expression may contribute to the vasculotoxic effects of endothelin-1.¹⁷

Altered glomerular expression patterns of integrins have been more extensively described in various forms of glomerulopathies. In IgA nephropathy, the vitronectin receptor $\alpha v\beta 3$ ¹⁸ and the fibronectin receptor $\alpha 5\beta 1$ ¹⁹ were strongly enhanced in the mesangium. Similar changes were observed in lupus nephritis.^{18,19} During development of diabetic nephropathy, the glomerular expression of integrins such as $\alpha 3$, $\alpha 5$, or αv is profoundly changed.²⁰ These findings suggest an important role for integrins in pathological rearrangements of the matrix and alterations in cell behavior during glomerular dis-

Figure 4. Matrix expansion in glomeruli of wild-type and $\alpha 8$ -deficient mice after DOCA treatment. **A:** Total glomerulosclerosis assessed by a semiquantitative score. **B:** Measurement of glomerular fibronectin staining. **C:** Measurement of glomerular collagen IV staining. Data are means $\pm$ SEM. *, $P < 0.05$, DOCA-treated mice *versus* respective controls. #, $P < 0.05$, $\alpha 8$ -deficient controls *versus* wild-type controls.

Figure 5. Glomerular cell activation and microaneurysm formation in wild-type and $\alpha 8$ -deficient mice after DOCA treatment. **A:** Glomerular cell activation evaluated after staining for α -smooth muscle actin. **B:** Mesangiolytic score assessed by scoring capillary widening in glomeruli. Data are means $\pm$ SEM. *, $P < 0.05$, DOCA-treated mice *versus* respective controls. #, $P < 0.05$, $\alpha 8$ -deficient controls *versus* wild-type controls. §, $P < 0.05$, DOCA treated $\alpha 8$ -deficient mice *versus* DOCA-treated wild-type mice.

eases. Whether or not $\alpha 8$ integrin expression also undergoes pathological changes in human glomerulopathies has not yet been studied. In a transient rat model of glomerulonephritis, an induction of mesangial $\alpha 8$ integrin after the onset of glomerular injury was found that was reduced to control levels after complete healing of the glomerulus.⁷

The structure of the glomerulus is established and maintained by interactions of glomerular cells with the adjacent extracellular matrix. In the mesangial matrix surrounding mesangial cells, several possible ligands for $\alpha 8$ are present, including fibronectin as the most abundant and vitronectin as well as tenascin C.²¹ During DOCA-induced glomerulosclerosis, many matrix constituents are up-regulated and the $\alpha 8$ ligand osteopontin is expressed *de novo* by glomerular cells.¹¹ $\alpha 8$ can promote cell attachment to matrix,²² and ligands for $\alpha 8$ are up-regulated in glomeruli of hypertensive animals. Presumably, lack of $\alpha 8$ in our model weakens the attachment of mesangial cells to the mesangial matrix, leading to a loss

Figure 6. Glomeruli of kidney sections of wild-type (A, B, and C) and $\alpha 8$ -deficient (D, E, and F) mice. PAS staining of salt-loaded controls (A and D) and DOCA-treated mice (B and E). C and F: Immunohistochemical detection of fibrin in DOCA-treated mice. Original magnifications, $\times 600$.

of tissue integrity in the presence of mechanical stress. Further, signaling events induced by matrix-integrin interaction contribute to cell differentiation in many instances.^{23,24} Therefore, lack of $\alpha 8$ on mesangial cells could contribute to the activation of these cells to a fibroblast-like phenotype (induction of smooth muscle actin) as described by Johnson and colleagues.²⁵

Interestingly, the increase in fibronectin is comparable in DOCA-treated wild-type and $\alpha 8$ -deficient mice and very similar to the increase of collagen IV, which is not a ligand for $\alpha 8$. Thus, the most abundant ligand for $\alpha 8$ in the mesangial matrix is regulated independently of the presence of its integrin receptor $\alpha 8$.

Transforming growth factor (TGF)- β is thought to play a central role in renal fibrotic events.²⁶ This seems also to be true for the DOCA salt model of hypertension in which expression of TGF- β is up-regulated after 6 weeks of disease.²⁷ TGF- β can induce expression not only of matrix constituents but also of matrix receptors in glomerular cells.^{21,28} Several studies have shown that expression of $\alpha 8$ is enhanced by TGF- β in cultured mesenchymal cells,^{7,29} including renal mesangial cells.⁷ Whether the increase in glomerular immunoreactivity for $\alpha 8$ in DOCA salt hypertension is because of the up-regulated TGF- β expression in this disease model remains to be shown. Other factors, such as mechanical stress, could possibly also contribute to the increase in glomerular $\alpha 8$.

Similar to $\alpha 3$ -deficient mice, in which capillary loop widening was described,³⁰ $\alpha 8$ -deficient mice showed a tendency toward microaneurysm formation that was clearly enhanced by superimposing mechanical stress on glomerular capillaries. However, in $\alpha 3$ -deficient mice, this defect seems to be produced by podocyte failure,³⁰

whereas in $\alpha 8$ -deficient mice probably the integrity of the mesangium is weakened.

To compensate for the lower renal mass, we uninephrectomized wild-type but not $\alpha 8$ -deficient mice. The measurements of relative kidney weight and creatinine clearance indicate that uninephrectomy in wild types led to very similar renal mass and function as in $\alpha 8$ -deficient mice. We cannot exclude the possibility that the subtle glomerular alterations induced by the lack of $\alpha 8$ influence the degree of hypertensive glomerular damage. However, we consider it unlikely that these alterations alone can account for the extensive destruction of the glomerular capillary architecture in $\alpha 8$ -deficient DOCA-treated mice.

A recent publication by Levine and colleagues³¹ investigated $\alpha 8$ integrin expression in the context of fibrotic mechanisms in fibroblast cells of lung and liver. $\alpha 8$ expression by lung alveolar interstitial cells was greatly enhanced in fibrosis. Hepatic stellate cells displayed a *de novo* expression of $\alpha 8$ when fibrosis was induced by bile duct ligation or carbon tetrachloride injury. Many of the $\alpha 8$ -positive myofibroblast cells co-expressed smooth muscle actin, which has been implicated in the development of fibrosis in several organs.^{32,33} Integrins could play an important role in the activation of fibroblasts by signals from the extracellular matrix. In the normal kidney of mouse, rat, and man $\alpha 8$ was not detected in the tubulointerstitium. However, in the renal interstitium of some DOCA-treated animals clusters of fibroblasts became reactive for $\alpha 8$, similar to the reported findings in liver,³¹ suggesting a role of $\alpha 8$ also in renal interstitial fibrosis.

Together, these findings indicate a role for $\alpha 8$ integrin in fibrotic and/or sclerotic processes in many organs, including liver, lung, and kidney. Moreover, our data support the notion that $\alpha 8$ may also contribute to adaptive changes that are necessary in the presence of mechanical stress.

Acknowledgments

We thank Elisabeth Buder and Rainer Wachtveitl for their expert technical assistance.

References

- Fogo AB: Mesangial matrix modulation and glomerulosclerosis. *Exp Nephrol* 1999, 7:147–159
- Adler S, Brady HR: Cell adhesion molecules and the glomerulopathies. *Am J Med* 1999, 107:371–386
- Bossy B, Bossy-Wetzel E, Reichardt LF: Characterization of the integrin alpha 8 subunit: a new integrin beta 1-associated subunit, which is prominently expressed on axons and on cells in contact with basal laminae in chick embryos. *EMBO J* 1991, 10:2375–2385
- Schnapp LM, Hatch N, Ramos DM, Klimanskaya IV, Sheppard D, Pytela R: The human integrin alpha 8 beta 1 functions as a receptor for tenascin, fibronectin, and vitronectin. *J Biol Chem* 1995, 270:23196–23202
- Denda S, Reichardt LF, Muller U: Identification of osteopontin as a novel ligand for the integrin alpha8 beta1 and potential roles for this integrin-ligand interaction in kidney morphogenesis. *Mol Biol Cell* 1998, 9:1425–1435
- Brandenberger R, Schmidt A, Linton J, Wang D, Backus C, Denda S, Muller U, Reichardt LF: Identification and characterization of a novel extracellular matrix protein nephronectin that is associated with integrin alpha8beta1 in the embryonic kidney. *J Cell Biol* 2001, 154:447–458
- Hartner A, Schocklmann H, Prols F, Muller U, Sterzel RB: Alpha8 integrin in glomerular mesangial cells and in experimental glomerulonephritis. *Kidney Int* 1999, 56:1468–1480
- Schnapp LM, Breuss JM, Ramos DM, Sheppard D, Pytela R: Sequence and tissue distribution of the human integrin alpha 8 subunit: a beta 1-associated alpha subunit expressed in smooth muscle cells. *J Cell Sci* 1995, 108:537–544
- Muller U, Wang D, Denda S, Meneses JJ, Pedersen RA, Reichardt LF: Integrin alpha8beta1 is critically important for epithelial-mesenchymal interactions during kidney morphogenesis. *Cell* 1997, 88:603–613
- Dworkin LD, Hostetter TH, Rennke HG, Brenner BM: Hemodynamic basis for glomerular injury in rats with desoxycorticosterone-salt hypertension. *J Clin Invest* 1984, 73:1448–1461
- Hartner A, Porst M, Gauer S, Prols F, Veelken R, Hilgers KF: Glomerular osteopontin expression and macrophage infiltration in glomerulosclerosis of DOCA-salt rats. *Am J Kidney Dis* 2001, 38:153–164
- Mai M, Hilgers KF, Geiger H: Experimental studies on the role of intercellular adhesion molecule-1 and lymphocyte function-associated antigen-1 in hypertensive nephrosclerosis. *Hypertension* 1996, 28:973–979
- Frank MB, Reiner AP, Schwartz SM, Kumar PN, Pearce RM, Arbogast PG, Longstreth Jr WT, Rosendaal FR, Psaty BM, Siscovick DS: The Kozak sequence polymorphism of platelet glycoprotein Ibalpha and risk of nonfatal myocardial infarction and nonfatal stroke in young women. *Blood* 2001, 97:875–879
- Li C, Xu Q: Mechanical stress-initiated signal transductions in vascular smooth muscle cells. *Cell Signal* 2000, 12:435–445
- Intengan HD, Thibault G, Li JS, Schiffrin EL: Resistance artery mechanics, structure, and extracellular components in spontaneously hypertensive rats: effects of angiotensin receptor antagonism and converting enzyme inhibition. *Circulation* 1999, 100:2267–2275
- Doi M, Shichiri M, Yoshida M, Marumo F, Hirata Y: Suppression of integrin alpha(v) expression by endothelin-1 in vascular smooth muscle cells. *Hypertens Res* 2000, 23:643–649
- Schiffrin EL: Endothelin and endothelin antagonists in hypertension. *J Hypertens* 1998, 16:1891–1895
- Shikata K, Makino H, Morioka S, Kashitani T, Hirata K, Ota Z, Wada J, Kanwar YS: Distribution of extracellular matrix receptors in various forms of glomerulonephritis. *Am J Kidney Dis* 1995, 25:680–688
- Kuhara T, Kagami S, Kuroda Y: Expression of beta 1-integrins on activated mesangial cells in human glomerulonephritis. *J Am Soc Nephrol* 1997, 8:1679–1687
- Jin DK, Fish AJ, Wayner EA, Mauer M, Setty S, Tsilibary E, Kim Y: Distribution of integrin subunits in human diabetic kidneys. *J Am Soc Nephrol* 1996, 7:2636–2645
- Rupprecht HD, Schocklmann HO, Sterzel RB: Cell-matrix interactions in the glomerular mesangium. *Kidney Int* 1996, 49:1575–1582
- Muller U, Bossy B, Venstrom K, Reichardt LF: Integrin alpha 8 beta 1 promotes attachment, cell spreading, and neurite outgrowth on fibronectin. *Mol Biol Cell* 1995, 6:433–448
- Giancotti FG, Ruoslahti E: Integrin signaling. *Science* 1999, 285:1028–1032
- Kreidberg JA, Symons JM: Integrins in kidney development, function, and disease. *Am J Physiol* 2000, 279:F233–F242
- Johnson RJ, Floege J, Yoshimura A, Iida H, Couser WG, Alpers CE: The activated mesangial cell: a glomerular “myofibroblast”? *J Am Soc Nephrol* 1992, 2:S190–S197
- Border WA, Noble NA: Interactions of transforming growth factor-beta and angiotensin II in renal fibrosis. *Hypertension* 1998, 31:181–188
- Kim S, Ohta K, Hamaguchi A, Omura T, Yukimura T, Miura K, Inada Y, Wada T, Ishimura Y, Chatani F: Role of angiotensin II in renal injury of deoxycorticosterone acetate-salt hypertensive rats. *Hypertension* 1994, 24:195–204
- Kagami S, Kondo S, Urushihara M, Loster K, Reutter W, Saijo T, Kitamura A, Kobayashi S, Kuroda Y: Overexpression of alpha1beta1 integrin directly affects rat mesangial cell behavior. *Kidney Int* 2000, 58:1088–1097
- Thibault G, Lacombe MJ, Schnapp LM, Lacasse A, Bouzeghrane F, Lapalme G: Upregulation of alpha(8)beta(1)-integrin in cardiac fibroblast by angiotensin II and transforming growth factor-beta1. *Am J Physiol* 2001, 281:C1457–C1467
- Kreidberg JA, Donovan MJ, Goldstein SL, Rennke H, Shepherd K, Jones RC, Jaenisch R: Alpha 3 beta 1 integrin has a crucial role in kidney and lung organogenesis. *Development* 1996, 122:3537–3547
- Levine D, Rockey DC, Milner TA, Breuss JM, Fallon JT, Schnapp LM: Expression of the integrin alpha8beta1 during pulmonary and hepatic fibrosis. *Am J Pathol* 2000, 156:1927–1935
- Knittel T, Kobold D, Piscaglia F, Saile B, Neubauer K, Mehde M, Timpl R, Ramadori G: Localization of liver myofibroblasts and hepatic stellate cells in normal and diseased rat livers: distinct roles of (myo-) fibroblast subpopulations in hepatic tissue repair. *Histochem Cell Biol* 1999, 112:387–401
- Zeisberg M, Strutz F, Muller GA: Role of fibroblast activation in inducing interstitial fibrosis. *J Nephrol* 2000, 13(Suppl 3):S111–S120