Reduction of Renal Mass Is Lethal in Mice Lacking Vimentin

Role of Endothelin–Nitric Oxide Imbalance

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Abstract

Modulation of vascular tone by chemical and mechanical stimuli is a crucial adaptive phenomenon which involves cytoskeleton elements. Disruption, by homologous recombination, of the gene encoding vimentin, a class III intermediate filament protein mainly expressed in vascular cells, was reported to result in apparently normal phenotype under physiological conditions. In this study, we evaluated whether the lack of vimentin affects vascular adaptation to pathological situations, such as reduction of renal mass, a pathological condition which usually results in immediate and sustained vasodilation of the renal vascular bed.

Ablation of 3/4 of renal mass was constantly lethal within 72 h in mice lacking vimentin ($Vim^{-/-}$), whereas no lethality was observed in wild-type littermates. Death in $Vim^{-/-}$ mice resulted from end-stage renal failure. Kidneys from $Vim^{-/-}$ mice synthesized more endothelin, but less nitric oxide (NO), than kidneys from normal animals. In vitro, renal resistance arteries from $Vim^{-/-}$ mice were selectively more sensitive to endothelin, less responsive to NO-dependent vasodilators, and exhibited an impaired flow (shear stress)-induced vasodilation, which is NO dependent, as compared with those from normal littermates. Finally, in vivo administration of bosentan, an endothelin receptor antagonist, totally prevented lethality in $Vim^{-/-}$ mice.

These results suggest that vimentin plays a key role in the modulation of vascular tone, possibly via the tuning of endothelin–nitric oxide balance. (*J. Clin. Invest.* 1997. 100: 1520–1528.) Key words: knockout mice • cytoskeleton • endothelin • nitric oxide • shear stress

Introduction

Adaptation of vascular tone to physiological and pathological situations is a key process in the maintenance of organ function. The vascular response to chemical or mechanical stimuli in terms of constriction or dilation is an integrative phenomenon that requires participation of endothelial and smooth muscle cells, and involves cytoskeleton elements among which intermediate filament proteins play a central role (1–4). The prominent reorganization of F-actin stress fibers, intermediate filaments, and microtubules to external forces (flow and/or pressure) implicates the cytoskeleton as a principal force transmission element in endothelial cells (5–7). Furthermore, the observation that filament disruption changes endothelial cell synthesis of bioactive molecules, such as endothelin-1 (ET-1),[1] nitric oxide (NO), or prostaglandins, argues in favor of a role of cytoskeleton in signal transduction (1). On the other hand, extracellular signals induce rearrangement of cytoskeleton through the activation of the small GTP-binding protein of the Rho family (8–10).

Vimentin is a class III intermediate filament protein mainly expressed in mesenchyme-derived cells including endothelial and vascular smooth muscle cells (11). This expression pattern suggests that vimentin should participate in the regulation of vascular tone. Indeed, it has been reported that mechanical stresses induce reorganization of vimentin filaments in endothelial cells (5, 12), whereas hormones, such as ET-1 and angiotensin II, induce vimentin protein phosphorylation in vascular smooth muscle cells (13). However, the role of vimentin in vascular responses remains to be elucidated. Recently, a strain of mice bearing a null mutation of vimentin gene has been established (14). Surprisingly, these animals develop and reproduce without an obvious phenotype.

Since vimentin is strongly expressed in normal kidneys, in both vessels and glomeruli, we evaluated, in a previous work (15), the function and structure of kidneys from mice lacking vimentin, and found that they did not differ from those of wild-type littermates. These findings prompted us to ask whether vimentin is involved in the vascular adaptation to pathological situations, such as nephron reduction. Indeed, reduction of renal mass is known to induce an immediate and sustained vasodilation which is paralleled by an increase of glomerular filtration in remaining glomeruli, in order to maintain renal function (16).

In this study, we used homozygous mice bearing a null mutation of vimentin gene to investigate: (*a*) in vivo, the consequences of a surgical reduction of renal mass; and (*b*) in vitro, the ability of renal resistance arteries to respond to chemical (contractile and relaxing agents) and mechanical (flow and pressure) stimuli.

We show that (*a*) reduction of renal mass is lethal in 100%

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1. *Abbreviations used in this paper:* ET, endothelin; MAP, mean arterial blood pressure; NADPH-d, NADPH diaphorase; NO, nitric oxide; L-NOARG, N^{ω}-nitro-L-arginine.

of mice lacking vimentin, but not in wild-type littermates, a phenotype which is accounted for by ET-1–NO imbalance and by impairment of flow-induced vasodilation; and (*b*) lethality in mutant mice subjected to reduction of renal mass is totally prevented by infusion of an ET receptor antagonist.

Methods

Mice lacking vimentin. The details of targeted inactivation of vimentin gene in mice have been reported previously (14). All experiments were performed on Vim^{l} knockout mice, in which the endogenous vimentin gene has been disrupted by an in-frame insertion of *Escherichia coli* β-galactosidase coding sequences into exon 1 of vimentin gene. Therefore, X-gal staining, which reveals β-galactosidase activity (see below), could permit analysis of the pattern of renal vimentin expression after reduction of renal mass.

To identify wild-type ($Vim^{+/+}$) and homozygous ($Vim^{-/-}$) mice of different crosses, DNA from mouse tail was extracted, and the presence of targeted vimentin alleles was detected using PCR, as previously described (15).

Experimental protocol. Adult (3–4 mo) $Vim^{+/+}$ and $Vim^{-/-}$ mice, originating from the same litter, were studied. Subtotal nephrectomy was performed as previously described (17). Briefly, in 2,2,2 tribromoethanol (Janssen, Beerse, Belgium; 37 μg/g of body weight) anesthetized mice, 75% of total renal mass was removed by excision of the right kidney and the two poles of the left kidney.

In a first set of experiments, we evaluated the survival rate in 15 $Vim^{-/-}$ mice and 10 $Vim^{+/+}$ littermates subjected to subtotal nephrectomy. The surviving animals were killed after 21 d after surgery. In a second step, because of the early and massive lethality observed in $Vim^{-/-}$ mice, time controls were performed in $Vim^{+/+}$ mice which were killed between 24 and 72 h after surgery for morphological analysis and renal vimentin expression ($n = 6$ for each group). For determination of ET-1 mRNA and protein content and of NADPH diaphorase (NADPH-d), a marker of NO synthesis (18, 19), in kidney from $Vim^{-/-}$ and $Vim^{+/+}$ mice, 16 animals of each group were killed 24 h after surgery. Nonoperated kidneys, removed at time of subtotal nephrectomy, were compared with remnant kidneys removed at time of killing.

In eight $Vim^{-/-}$ mice subjected to subtotal nephrectomy, bosentan (F. Hoffmann-La Roche Laboratories, Basel, Switzerland) (10 mg/kg/d) was infused continually via osmotic minipumps (Alzet; Alza Corp., Palo Alto, CA) (0.5 μl/h) from the day of surgery to day 14. Surviving animals were killed 21 d after surgery. Three $Vim^{-/-}$ mice were treated with placebo in the same conditions, and died within 48 h after surgery.

Mean arterial blood pressure (MAP) was measured 24 h before and after surgery in conscious $Vim^{+/+}$ mice and $Vim^{-/-}$ mice treated with or without bosentan ($n = 5$ in each group), by the tail-cuff method using an electrosphygmomanometer (Phymep, Paris, France). Plasma creatinine and electrolyte concentrations were determined on blood samples obtained by intracardiac puncture 24 h after surgery in each of these three groups of animals. Values were compared with those obtained from five normal, nonoperated $Vim^{+/+}$ and $Vim^{-/-}$ mice and from five $Vim^{+/+}$ binephrectomized mice. At time of killing, heart, lung, and kidney weights were measured in each animal. Lung dry weight was measured after overnight dehydration of lungs in acetone/dyethylether (1:1, vol/vol) solution. Lung water content was calculated as the difference between wet and dry weights.

All animal procedures were conducted in accordance with French government regulations (Services Vétérinaires de la Santé et de la Production Animale, Ministère de l'Agriculture).

Renal function. Plasma creatinine and electrolyte concentrations were determined with a multiparametric autoanalyzer (Hitachi 717; Boehringer-Mannheim, Melun, France).

Localization of β-galactosidase activity. X-gal staining was performed as previously described (15). Briefly, renal tissue was frozen in 2-methylbutane, kept at −80°C, and cut on cryostat (Shandon, Dublin, Ireland) to obtain 7-μm-thick sections. Sections were fixed in fresh 2% formaldehyde/0.2% glutaraldehyde, then incubated overnight at 35°C in a staining solution containing 2 mM $MgCl_2$, 4 mM $K_3Fe(CN)_6$, 4 mM $K_4Fe(CN)_6$, and 0.4 mg/ml 4-chloro-5-bromo-3-indolyl-β-D-galactopyranoside (X-gal; GIBCO BRL, Cergy Pontoise, France) buffered with PBS. The slides were counterstained with eosin and mounted in Eukitt (Labonord, Villeneuve d'Ascq, France).

Immunohistochemistry. Immunostaining was performed using the ABC procedure (20) as modified by Maunoury et al. (21). Briefly, kidneys were fixed in Kryofix (Merck, Nogent-sur-Marne, France), ethanol dehydrated, and paraffin embedded. 5-μm-thick sections were incubated first with 10% donkey nonimmune serum for 30 min, then with a rabbit polyclonal anti–rat vimentin antiserum (kindly provided by Dr. A.M. Hill, Orsay, France), diluted 1:2,000 overnight at room temperature, and finally with a biotinylated donkey anti–rabbit antibody (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA) diluted 1:200 for 30 min. This was followed by incubation with the avidin–biotin–peroxidase complex (Vector Laboratories, Inc., Burlingame, CA) and then with 0.05% 3.3′-diamino-benzidine-tetrahydrochloride (DAB; Fluka, Mulhouse, France) and 0.01% freshly prepared hydrogen peroxide (Perydrol; Merck). The sections were counterstained with alcian blue and mounted in Eukitt (Labonord).

Negative controls were obtained by replacing specific antisera with normal nonimmune sera; no labeling was observed, indicating that all the procedures and reagents used resulted in specific labeling.

Renal morphology. Serial sections, processed as for immunohistochemistry, were stained with hematoxylin and eosin and periodic acid Schiff (PAS).

Northern blot analysis. Total RNA was extracted from kidneys using the RNAzol kit (Bioprobe, Montreuil-sous-Bois, France). RNA (20 μg/lane) was fractionated on a 1.2% agarose-formaldehyde gel and transferred onto nylon membrane (Zetabind; CUNO, Inc., Meriden, CT). Prehybridization, hybridization, and washing were carried out according to the manufacturer's recommendations. RNA was quantified by densitometric computer analysis in a series 400 PhosphorImager (Molecular Dynamics, Inc., Sunnyvale, CA). cDNA probes were labeled by a random priming method (Boehringer-Mannheim) using [α-^{32}P]dCTP. The following probes were used: the human pre-pro-ET-1, kindly provided by Dr. F. Pinet (Paris, France) and the murine GAPDH. The GAPDH probe was used in order to quantify the RNA loading on the gel and to compare the intensity of hybridization obtained in the different lanes.

ET-1 enzyme immunoassay. Renal ET-1 was extracted according to the method of Matsumoto et al. (22) with minor modifications. Briefly, kidneys were homogenized with a polytron homogenizer in 10 vol of 1 M acetic acid containing 10 μg/ml pepstatin for 60 s and immediately boiled for 10 min. The homogenates were centrifuged at 14,000 rpm for 35 min at 4°C and the supernatants were applied to Sep-Pak plus C18 cartridges (Waters, Milford, MA), and the absorbed peptides were eluted with methanol/water/trifluoroacetic acid (90:10:0.1) and then lyophilized. ET-1 concentration was assessed by ELISA, using the ET-1 (human) enzyme immunoassay kit (Cayman Chemical, Ann Arbor, MI).

NADPH-d biochemical assay. To test NADPH-d activity, kidneys were homogenized in 10 vol of 50 mM Tris chloride, pH 7.4, 1 mM EDTA, 1 mM PMSF, 1 μM leupeptin, 1 μM pepstatin, and 0.2% Triton X-100. The homogenates were centrifuged at 14,000 rpm for 15 min at 4°C and the supernatants were used for analysis. An equal amount of proteins was used for each determination. NADPH-d activity was determined according to the method of Hope et al. (18) with minor modifications. Briefly, NADPH-d was assayed by measuring the reduction of 0.5 mM nitro blue tetrazolium with 1.0 mM NAPDH in 0.3 ml of 50 mM Tris chloride, pH 8.0, at 37°C for 15 min. The reaction was stopped with 0.7 ml of 100 mM sulfuric acid, and the absorbance of formazan product was determined at 585 nm. Diaphorase activity determined in the absence of NADPH was always subtracted from the NADPH-d activity.

Vascular responses to chemical stimuli. Renal resistance arteries and aortas were obtained from $Vim^{-/-}$ mice and from $Vim^{+/+}$ littermates. Arterial rings were prepared as previously described (23). Briefly, second order renal arteries were dissected, and segment rings (2 mm) were prepared and mounted on nylon filaments in a myograph for resistance arteries (24). Segments of thoracic aorta (3 mm) were dissected and mounted on stainless steel wires in a classic myograph (25). Isometric tension was recorded using a force displacement transducer (MP 100; BioPac Systems, Inc., La Jolla, CA). After equilibration, the tissue contractility was assessed by exposure to KCl (125 mM). Subsequently, the concentration–response curves to phenylephrine, serotonin, angiotensin II, ET-1, and phorbol dibutyrate were obtained by cumulative addition of the drug to the bath solution. For the relaxing agents (acetylcholine and sodium nitroprusside), these curves were conducted after preconstriction with a concentration of phenylephrine sufficient to reach 50% of the maximal response as determined with KCl (125 mM). EC_{50}, IC_{50}, and E_{MAX} were calculated individually for each concentration–response curve as previously described (23). Drugs were purchased from Sigma Chemical Co. (Saint Quentin Fallavier, France).

Vascular responses to mechanical stimuli. The responses of segments from renal resistance arteries to pressure and flow were determined as previously described (26). Briefly, second order renal arteries were dissected, and segments were cannulated at both ends and mounted in a video monitor perfusion system (Living System Instrumentation Inc., Burlington, VT). Arterial diameter was measured and recorded continuously. Pressure and flow rate could be changed independently. Arteries were submitted to different pressure steps (from 25 to 125 mmHg) with (100 μl/min) and without intraluminal flow and this was subsequently repeated after addition of either N^{φ}-nitro-L-arginine (L-NOARG, 10 μM; Sigma Chemical Co.), either alone or combined with indomethacin (10 μM; Sigma Chemical Co.). Active and passive diameters were determined as previously reported (27). Myogenic tone was calculated as percentage of passive diameter (measured diameter/passive diameter × 100), whereas flow-induced dilation was expressed as increases in diameter induced by flow.

Expression of data and statistical analysis. Data are expressed as means±SEM. Differences between the experimental groups were evaluated using one-way ANOVA, which was followed, when significant, by Bonferroni test. Two-way ANOVA for repeated measures was used to compare the concentration–response curves. Paired *t* test was used to compare the effect of surgery on blood pressure.

Results

All the experiments described hereafter were performed on animals from intercrosses between $Vim^{+/-}$ mice, which bear a mutated allele of vimentin gene from which bacterial β-galactosidase was expressed in place of vimentin.

Reduction of renal mass was lethal in vimentin-null mice. Reduction of renal mass resulted in 100% mortality of $Vim^{-/-}$ mice within 72 h, whereas no lethality was observed in $Vim^{+/+}$ littermates (Fig. 1). To investigate a possible role of anesthesia, renal bleeding, or lack of tissular compensatory growth in death of $Vim^{-/-}$ nephrectomized mice, we performed sham operation, hemorrhage, or two-thirds hepatectomy in vimentin-null mice. We observed no lethality in these three experimental conditions (data not shown).

To explore in detail the cause of death in $Vim^{-/-}$ mice subjected to subtotal nephrectomy, three approaches were conducted. First, biochemical data were recorded: they showed that plasma creatinine concentration was similar in normal nonoperated $Vim^{+/+}$ and $Vim^{-/-}$ mice (Table I) and increased 24 h after surgery to a value which was higher in $Vim^{-/-}$ animals than in $Vim^{+/+}$ littermates (Table I). At that time, creatinine concentration in $Vim^{-/-}$ mice was comparable to that obtained from wild-type animals in which both kidneys have been removed (201±2 μmol/liter). Plasma sodium, potassium, and chloride concentrations were not significantly different in

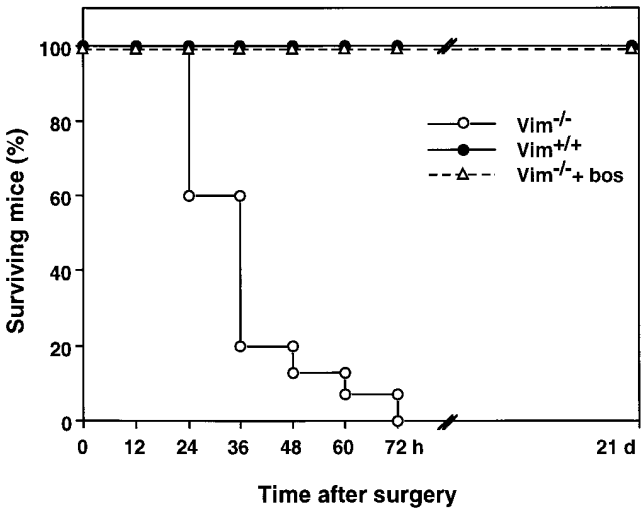

Figure 1. Survival rate of vimentin-null mice ($Vim^{-/-}$) and wild-type littermates ($Vim^{+/+}$) subjected to reduction of renal mass. Subtotal nephrectomy was lethal in 100% of mice lacking vimentin within 72 h, but not in $Vim^{+/+}$ littermates. Lethality in nephrectomized $Vim^{-/-}$ mice was totally prevented by treatment with bosentan, an ET receptor antagonist ($Vim^{-/-}$ + *bos*). Surviving animals were killed 21 d after surgery.

Table I. Effect of Lack of Vimentin on Cardiovascular and Renal Status in Mice Subjected to Reduction of Renal Mass

	$Vim^{+/+}$	$Vim^{-/-}$	$Vim^{-/-}$ + *bos*
MAP (mmHg)			
Before Nx	130±2	137±4	135±2
After Nx	128±3	116±4*	124±4
Heart weight (mg)	108±5	97±8	106±13
Lung weight (mg)			
Wet weight	146±9	151±13	134±15
Dry weight	29±1	31±1	30±2
Remnant kidney weight (mg)	138±5	116±7	123±11
Plasma creatinine (μmol/liter)			
Before Nx	29±5	30±4	ND
After Nx	100±15	181±11‡	84±4

All parameters were determined 24 h after subtotal nephrectomy (*Nx*) in vimentin-null mice treated ($Vim^{-/-}$ + *bos*) or not ($Vim^{-/-}$) with bosentan and in wild-type littermates ($Vim^{+/+}$) (n = 5 for each group). MAP was measured by tail-cuff method in conscious animals before and after surgery. Plasma creatinine before Nx was determined in five normal nonoperated $Vim^{+/+}$ and $Vim^{-/-}$ mice. *ND*, Not determined. No significant differences were observed in cardiovascular status and in compensatory growth of remnant kidney between the three experimental groups. By contrast, plasma creatinine was dramatically increased after subtotal nephrectomy in mice lacking vimentin as compared to wild-type littermates. This increment was totally prevented by treatment with bosentan. Data are means±SEM. ANOVA, followed by Bonferroni test: $Vim^{-/-}$ vs. $Vim^{+/+}$ and $Vim^{-/-}$ + *bos*; ‡$P < 0.005$; *significantly different from homologous value before Nx (paired *t* test: $P < 0.05$).

the two groups of animals (data not shown). Second, the cardiovascular status was evaluated: heart weight and wet and dry lung weight was comparable in $Vim^{-/-}$ and $Vim^{+/+}$ mice 24 h after surgery (Table I), indicating the absence of evident heart failure. MAP, which was recorded in conscious animals by the tail-cuff method, did not differ between $Vim^{-/-}$ and $Vim^{+/+}$ mice before surgery (Table I), and was slightly but significantly decreased only in $Vim^{-/-}$ animals 24 h after nephrectomy (Ta-

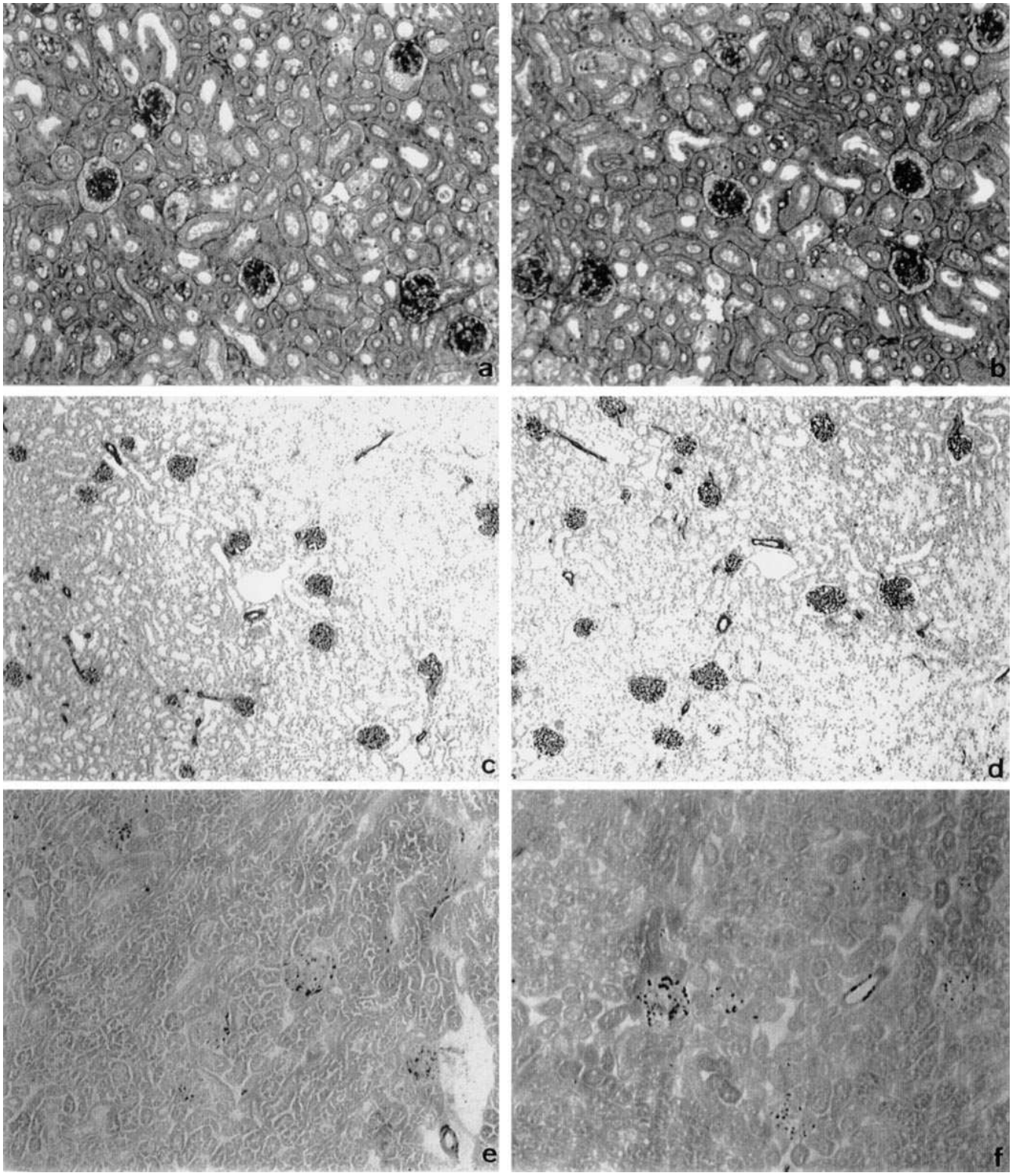

Figure 2. Renal morphology and vimentin expression in control nonoperated kidneys (*a*, *c*, and *e*) and in remnant kidneys (*b*, *d*, and *f*), 48 h after surgery. (*a* and *b*) Renal morphology of $Vim^{-/-}$ kidneys; (*c* and *d*) vimentin immunostaining of $Vim^{+/+}$ kidneys; (*e* and *f*) X-gal staining of $Vim^{-/-}$ kidneys (β-galactosidase activity was put under the control of vimentin promoter). Renal morphology and vimentin expression were not affected by reduction of renal mass. ×75.

ble I). Third, extensive histological analysis was performed. No evident hemorrhage or infarction was observed in heart, lung, intestine, or spleen. In regard to kidney size, the weight of the right kidney, removed at time of surgery, was comparable in $Vim^{+/+}$ (178±17 mg) and $Vim^{-/-}$ (170±22 mg) mice. As shown in Table I, compensatory growth of remnant kidney did not differ significantly between $Vim^{+/+}$ and $Vim^{-/-}$ animals, at least 24 h after surgery.

Morphology of the $Vim^{-/-}$ remnant kidneys was comparable to that of normal, nonoperated kidneys (Fig. 2, *a* and *b*). To assess whether reduction of renal mass modified vimentin expression in renal tissue, immunohistochemistry was performed on control and remnant kidneys from $Vim^{+/+}$ and $Vim^{-/-}$ animals. As expected from the original study (14) reporting the establishment of the vimentin-null mice strain, vimentin protein could not be detected by antivimentin antibodies in any structure of both control and remnant kidneys from $Vim^{-/-}$ mice (data not shown). In contrast, vimentin was revealed in glomeruli, vessels, and interstitial cells of kidneys from $Vim^{+/+}$ mice (Fig. 2 *c*). Vimentin labeling was comparable, in terms of distribution and intensity, in control and remnant kidneys, at least until 72 h after surgery (Fig. 2, *c* and *d*). In kidneys from $Vim^{-/-}$ mice, X-gal staining showed the same pattern of expression of the reporter gene β-galactosidase as that of vimentin in $Vim^{+/+}$ animals (Fig. 2, *e* and *c*). Nephron reduction did not affect X-gal staining in vimentin-null mice (Fig. 2, *e* and *f*).

Response to chemical and mechanical stimuli was altered in renal resistance arteries from vimentin-null mice. Since the results reported above were consistent with the possibility that renal failure in $Vim^{-/-}$ mice resulted from increased arterial vasoconstriction and/or reduced arterial vasodilation, we analyzed in vitro the ability of renal resistance arteries from mutant mice and from wild-type littermates to respond to chemical and mechanical stimuli. In isolated rings of second order renal arteries, arterial segment length, width, external diameter, internal diameter, wall thickness, applied tension, wall/lumen ratio, and contractile response to KCl-induced depolarization did not differ between $Vim^{+/+}$ and $Vim^{-/-}$ mice (data not shown). Contractile response to phenylephrine, serotonin, and angiotensin II was similar in arteries from $Vim^{+/+}$ and $Vim^{-/-}$ mice (Table II). By contrast, mutant mice showed an altered response to ET-1. Indeed, sensitivity to ET-1 was higher (lower EC_{50} values) in $Vim^{-/-}$ renal arterial rings than in $Vim^{+/+}$ controls (Table II) and maximal contraction, induced by pharmacological concentrations of ET-1, was reduced in $Vim^{-/-}$ renal rings (Table II). Similarly, sensitivity of renal arteries to phorbol dibutyrate was increased in mice lacking vimentin (Table II). Regarding the response to relaxing agents, the sensitivity of renal arteries to acetylcholine, an endothelium-dependent relaxing agent, which acts through the release of NO, was lower (higher IC_{50} values) in $Vim^{-/-}$ mice than in $Vim^{+/+}$ littermates (Table II). In contrast, the response to sodium nitroprusside, an NO donor, was comparable in renal arteries from $Vim^{-/-}$ and $Vim^{+/+}$ mice (Table II). To verify whether the abnormal responses to contractile and relaxing agents were specific to renal resistance arteries, we investigated the ability of aortas to respond to the same chemical stimuli. As shown in Table II, no difference was detected between $Vim^{-/-}$ and $Vim^{+/+}$ mice.

Table II. Effect of Lack of Vimentin on Vascular Responses to Contractile and Relaxing Agents in Renal Resistance Arteries and in Aortas

		Renal artery		Aorta	
		$Vim^{+/+}$	$Vim^{-/-}$	$Vim^{+/+}$	$Vim^{-/-}$
Contractile agents		$n = 10$	$n = 11$	$n = 6$	$n = 8$
Phenylephrine	EC_{50} (μM)	0.18±0.06	0.15±0.05	0.26±0.08	0.21±0.07
	Max (mN/mm)	0.72±0.09	0.73±0.11	2.30±0.31	2.10±0.07
Serotonin	EC_{50} (μM)	0.10±0.02	0.11±0.04	0.18±0.06	0.24±0.04
	Max (mN/mm)	0.80±0.13	0.70±0.12	1.80±0.10	1.50±0.06
Angiotensin II	EC_{50} (nM)	48±15	31±6	350±120	405±128
	Max (mN/mm)	0.39±0.12	0.32±0.12	0.21±0.05	0.33±0.06
ET-1	EC_{50} (nM)	12.1±3.1	5.5±1.2*	20.8±6.6	16.2±3.6
	Max (mN/mm)	0.65±0.05	0.43±0.05*	0.51±0.18	0.48±0.09
PDBu	EC_{50} (nM)	1.70±0.40	0.90±0.30*	2.7±0.08	3.2±0.60
	Max (mN/mm)	0.61±0.09	0.58±0.12	2.8±0.17	3.2±0.06
Relaxing agents		$n = 10$	$n = 9$	$n = 6$	$n = 8$
SNP	IC_{50} (nM)	18±6	13±4	21±8	14±3
	Max (%)	100±0	100±0	100±0	100±0
Acetylcholine	IC_{50} (μM)	0.31±0.17	1.7±0.5*	0.19±0.05	0.25±0.06
	Max (%)	68±3	60±6	72±8	62±6

The concentration required to obtain 50% of the maximal excitatory and inhibitory response (EC_{50} and IC_{50}) and the maximal response (Max) to contractile [phenylephrine, serotonin, angiotensin II, ET-1, and phorbol dibutyrate (*PDBu*)] and relaxing [sodium nitroprusside (SNP) and acetylcholine] agents were determined in segments of second order renal arteries and of aortas from vimentin-null mice ($Vim^{-/-}$) and from wild-type littermates ($Vim^{+/+}$). The lack of vimentin in renal arterial rings causes a higher sensitivity to ET-1 (lower EC_{50} value) and a lower sensitivity to acetylcholine (higher IC_{50} value), suggesting a tendency to increased vasoconstriction. It also results in a higher sensitivity to PDBu and in a reduction of maximal response to ET-1. By contrast, no significant differences were observed for all parameters between aortas from $Vim^{+/+}$ and those from $Vim^{-/-}$ mice. Data are means±SEM. ANOVA, followed by Bonferroni test: $Vim^{-/-}$ vs. $Vim^{+/+}$; $^*P < 0.01$.

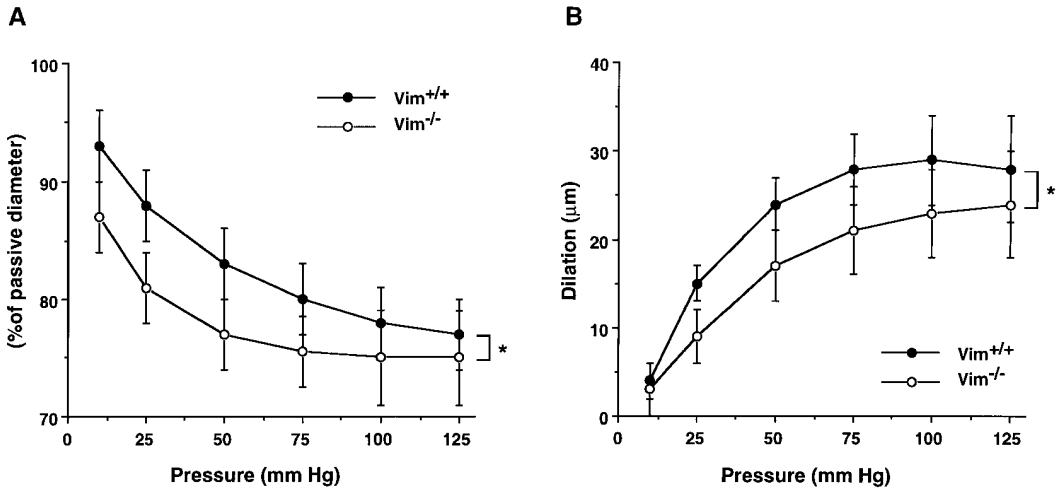

Figure 3. Pressure-induced tone (myogenic tone) (*A*) and flow-induced dilation (shear stress) (*B*) in resistance renal arteries from vimentin-null mice ($Vim^{-/-}$) and wild-type littermates ($Vim^{+/+}$). The curves show that, in the absence of vimentin, the myogenic tone, expressed as percent passive diameter, was increased, whereas the flow-induced dilation, expressed as change in diameter, was decreased in second order renal arteries. Data are means±SEM. ANOVA, followed by Bonferroni test: $Vim^{-/-}$ vs. $Vim^{+/+}$. $^{*}P < 0.05$.

We next evaluated the pressure (myogenic)-induced tone and the flow (shear stress)-induced dilation in second order renal arteries. As shown in Fig. 3 *A*, myogenic tone (active tone in the absence of flow) was higher in renal resistance arteries from $Vim^{-/-}$ mice than in those from $Vim^{+/+}$ littermates. Addition of L-NOARG (10 μM), an NO synthase inhibitor, and indomethacin (10 μM) did not affect the pressure-induced tone in both groups (data not shown). Flow-induced dilation was decreased in $Vim^{-/-}$ arteries as compared with those from $Vim^{+/+}$ mice (Fig. 3 *B*). Addition of L-NOARG (10 μM) to the perfusate and to the superfusate significantly reduced flow-induced dilation in both groups (Fig. 4). Addition of indomethacin (10 μM) together with L-NOARG further reduced significantly flow-induced dilation exclusively in $Vim^{-/-}$ renal arteries (Fig. 4).

Renal synthesis of ET and NO was modified in vimentin-null mice. Since in vitro studies evidenced a link between vimentin and ET-1 and NO, two pivotal factors in control of renal blood flow (28), we next investigated the ability of kidneys to synthesize these agents in response to increased blood flow in our model of renal mass reduction. For this purpose, we measured the renal ET-1 mRNA and protein content as well as the renal NADPH-d activity, a marker of NO synthase (18, 19), in normal nonoperated kidneys and in remnant kidneys 24 h after surgery. ET-1 protein was more abundant in nonoperated kidneys from $Vim^{-/-}$ mice compared with those from $Vim^{+/+}$ mice (Fig. 5 *B*), while ET-1 mRNA was undetectable in kidneys from either animal (Fig. 5 *A*). ET-1 mRNA and protein increased in remnant kidneys of both $Vim^{+/+}$ and $Vim^{-/-}$ animals. However, this increase was larger in vimentin-

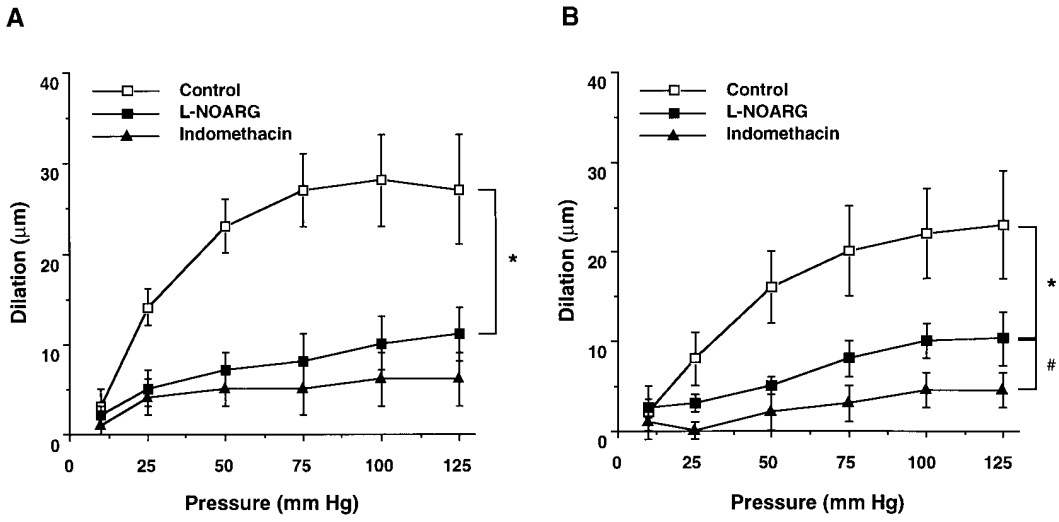

Figure 4. Flow-induced dilation (shear stress) in resistance renal arteries from $Vim^{+/+}$ (*A*) and $Vim^{-/-}$ (*B*) mice. In each group, artery segments were treated with L-NOARG (10 μM), either alone or combined with indomethacin (10 μM), and with the vehicle of indomethacin (ethanol 0.1%) as control. The curves show that addition of L-NOARG reduced flow-induced dilation in both $Vim^{+/+}$ and $Vim^{-/-}$ arteries, whereas addition of indomethacin induced a further reduction in flow-induced dilation, exclusively in $Vim^{-/-}$ arteries. Data are means±SEM. ANOVA, followed by Bonferroni test: control vs. L-NOARG $^{*}P < 0.05$; L-NOARG vs. indomethacin $^{\#}P < 0.05$.

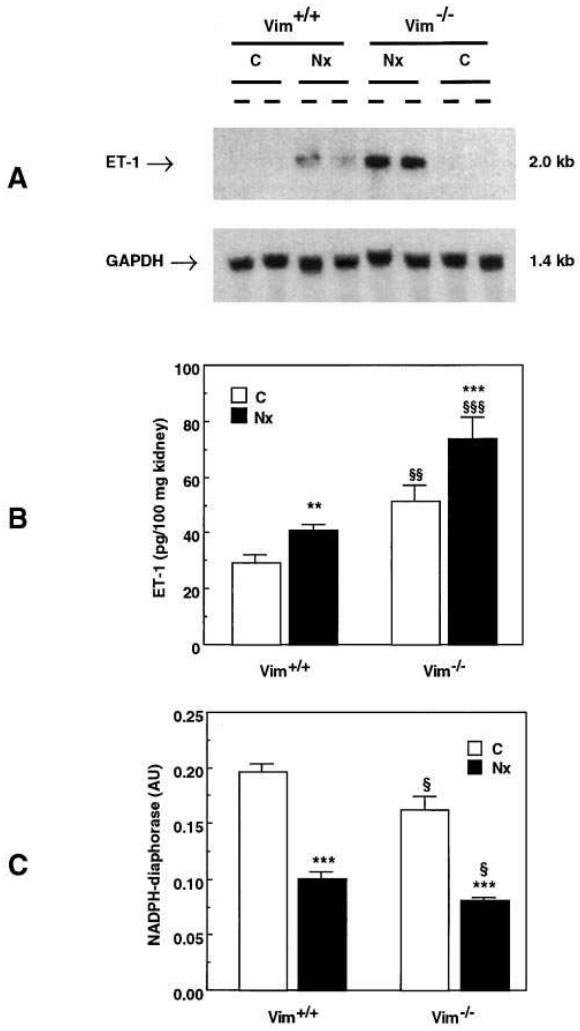

Figure 5. ET-1 mRNA and protein and NADPH-d activity in nonoperated control kidneys (*C*) and in remnant kidneys (*Nx*) from $Vim^{-/-}$ and $Vim^{+/+}$ mice, 24 h after nephrectomy. (*A*) Northern blot analysis shows that the ET-1 mRNA, undetectable in the kidneys of both $Vim^{+/+}$ and $Vim^{-/-}$ control mice, increased markedly after nephrectomy. The increase was greater in $Vim^{-/-}$ mice (threefold, $P < 0.001$). These results were confirmed in two additional independent experiments. (*B*) The changes of ET-1 mRNA correlate with those of the protein, which increased after nephron reduction, particularly in $Vim^{-/-}$ mice ($P < 0.001$). (*C*) NADPH-d activity, an NO synthase isozyme-independent marker, decreased after nephrectomy. The decrease was greater in $Vim^{-/-}$ mice ($P < 0.05$). Data are means±SEM. ANOVA, followed by Bonferroni test: C vs. Nx: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$; $Vim^{-/-}$ vs. $Vim^{+/+}$: $^{§}P < 0.05$, $^{§§}P < 0.01$, $^{§§§}P < 0.001$.

null animals (Fig. 5, *A* and *B*). NO generation, as judged from NADPH-d activity, was decreased in remnant kidneys of $Vim^{+/+}$ and $Vim^{-/-}$ mice, but the reduction was greater in $Vim^{-/-}$ mice (Fig. 5 *C*). NADPH-d activity was lower in nonoperated kidneys from $Vim^{-/-}$ mice compared with those from $Vim^{+/+}$ mice (Fig. 5 *C*).

Treatment with an ET receptor antagonist prevented lethality in nephrectomized vimentin-null mice. Finally, we tested the hypothesis that the increased ET-1/NO ratio could be responsible for impaired vascular response and, therefore, for lethality after the reduction of renal mass in mice lacking vimentin. To address this issue, bosentan, an ET-A/ET-B receptor antagonist (29), was infused in $Vim^{-/-}$ mice undergoing reduction of renal mass. Infusion of bosentan via osmotic minipumps resulted in 100% survival of these mice up to time of killing, which was performed at 21 d after surgery (Fig. 1). In $Vim^{-/-}$ nephrectomized mice treated with bosentan, plasma creatinine concentration, evaluated 24 h after surgery, was significantly lower than in untreated mice and was comparable to the value obtained in $Vim^{+/+}$ nephrectomized animals (Table I). As shown in Table I, no cardiovascular parameters were affected by this treatment.

Discussion

In this work we showed that, in mice lacking vimentin: (*a*) vascular response to chemical and mechanical stimuli was impaired; (*b*) reduction of renal mass, a pathological situation requiring vascular adaptation to survival, was lethal in 100% of mice; and (*c*) infusion of bosentan, an ET receptor antagonist, prevented lethality after reduction of renal mass. Furthermore, we provided evidence that ET-1 and NO production was altered in mutant mice. Altogether, these results indicate that vimentin plays a key role in the modulation of vascular tone, possible via the tuning of ET-1/NO balance.

Vimentin, the most widely distributed intermediate filament, is expressed in all mesenchyme-derived cells, including vascular endothelial and smooth muscle cells (11). The lack of an obvious phenotype in mice lacking vimentin, recently reported by Colucci-Guyon et al. (14), was enigmatic and raised the question of the evolutionary advantages of high conservation of vimentin sequence. Our present observations shed light on this apparent paradox by showing that vimentin plays a key role under pathological conditions which require vascular adaptation, such as reduction of renal mass, a situation which induces an immediate and sustained vasodilation in order to maintain renal function.

Regarding the cause of lethality in mice lacking vimentin subjected to renal ablation, the 100% survival observed after sham operation, hemorrhage, or two-thirds hepatectomy in vimentin-null mice rules out a major role for anesthesia, renal bleeding, or lack of tissular compensatory growth in death of these animals. According to these data, compensatory growth was comparable in $Vim^{-/-}$ and $Vim^{+/+}$ remnant kidneys, at least 24 h after nephron reduction. Along the same line, MAP was not different in vimentin-null mice as compared with wild-type littermates, and was not increased after surgery. Furthermore, the absence of cardiac hypertrophy, as judged from heart weight, and/or of lung edema, as judged from lung water content, argues against a major cardiovascular dysfunction. By contrast, the severe alteration of renal function associated with a normal renal morphology suggests that death was the consequence of a lack of vascular adaptation to nephron reduction, resulting in end-stage renal failure. Indeed, it is noteworthy that plasma creatinine concentration and survival time were comparable to those observed in $Vim^{+/+}$ binephrectomized animals. Furthermore, treatment with bosentan, which totally prevented lethality in $Vim^{-/-}$ mice, restored renal function in a range comparable to that observed in wild-type littermates. Altogether, these results were consistent with the possibility that renal failure in $Vim^{-/-}$ mice resulted from increased arterial vasoconstriction and/or reduced arterial vasodilation. This

hypothesis was confirmed by the observation that pressure-induced tone was enhanced while flow-induced dilation was reduced in renal resistance arteries lacking vimentin.

ET-1 and NO are two pivotal factors in the control of renal blood flow (28) and the imbalance between these factors is likely to result in hemodynamic abnormalities. The enhanced production of ET-1, a potent renal vasoconstricting agent (30), in kidneys from $Vim^{-/-}$ mice together with the observation that infusion of bosentan, an ET-1 receptor antagonist, prevented lethality in 100% of $Vim^{-/-}$ nephrectomized mice strongly supports the functional involvement of ET-1 increment in death of mutant mice. On the other hand, the reduction of the maximal contraction induced by pharmacological concentrations of ET-1 in renal resistance arteries may be accounted for by a downregulation of ET-1 receptors and argues in favor of a role for increased ET-1 concentrations in $Vim^{-/-}$ tissues. The enhanced sensitivity of renal arteries to phorbol dibutyrate in $Vim^{-/-}$ mice suggests that protein kinase C may participate in the downregulation of ET-1 receptors, as previously reported in other systems (31, 32). Along the same line, increased sensitivity to phorbol esters in $Vim^{-/-}$ arteries is consistent with the observation that myogenic tone, which depends, in part, upon activation of protein kinase C (33), was increased in renal arteries from vimentin-null mice.

In regard to NO, a potent renal vasodilating agent (34), our observations that its synthesis is decreased after subtotal nephrectomy are in agreement with a recent study performed in the rat, which evidenced, by various biochemical and immunohistochemical approaches, a dramatic impairment of NO generation in the remnant kidney from 7 d after nephron reduction (35). In this work, we provide evidence that (*a*) the decrease of NO synthesis is detected as early as 24 h after surgery and is even more pronounced in kidney tissue and in renal resistance arteries lacking vimentin and (*b*) this decrease resulted in impaired vasodilation. Indeed, both acetylcholine- and flow-induced vasodilation, which depend mainly on endothelial NO synthesis (1, 36, 37), were reduced in mice lacking vimentin. In contrast, the response of renal arteries to sodium nitroprusside, an NO donor, was similar between $Vim^{-/-}$ and $Vim^{+/+}$ mice, suggesting that vimentin disruption impaired endothelial NO generation but did not affect the vasodilatory effect of NO on vascular smooth muscle cells.

From our observations of an impaired ET-1/NO ratio in $Vim^{-/-}$ mice, we reasoned that pharmacologic interventions aiming to minimize this imbalance should either limit ET-1 synthesis or action, or provide NO, which is lacking in mutant mice. Because infusion of NO donors raised difficulties in terms of pharmacokinetics and bioavailability of the drugs, we chose to infuse animals with a stable, well-known, nonspecific ET-A/ET-B receptor antagonist (29). The efficiency of this treatment, which suppressed lethality and restored the ability of $Vim^{-/-}$ kidneys to adapt to nephron loss, illustrates the extent to which ET-1/NO ratio is crucial in maintenance of renal function.

The fact that the enhanced ET-1/NO ratio in normal tissues (nonoperated kidneys) did not lead to a pathological phenotype in $Vim^{-/-}$ mice under physiological conditions is intriguing. Further investigations are required to investigate whether morphology of renal arteries and/or synthesis of other vasoactive molecules, which could compensate for the ET-1/NO imbalance, were modified in vimentin-null mice. It must be noted that in $Vim^{-/-}$ mice, at variance with $Vim^{+/+}$ littermates, a component of flow-induced dilation in renal arteries depended upon prostaglandin production, since it was significantly reduced by indomethacin.

The molecular mechanism(s) responsible for vascular dysfunction in mice lacking vimentin remain speculative. Cytoskeletal proteins are thought to participate in the mechanotransduction of both flow-induced dilation (shear stress) and pressure-induced tone (myogenic tone) into a biochemical signal (1–3, 38). Both inhibition of microtubule polymerization by nocodazole (1) and stabilization of F-actin with phalloidin (39) abolished the flow-mediated upregulation of NO synthase. Data on shear stress-induced ET-1 modifications (1, 40, 41) remain contradictory, likely because of differences in intensity of shear stress from one study to another. Davies has reported recently that depolymerization of microtubules with nocodazole or colchicine prevented the shear stress–induced ET-1 downregulation (1). Along the same line, actin filament disruption resulted in changes of stretch-activated ion channel activity (42). The involvement of vimentin in mechanotransduction has not been investigated previously, although a rearrangement of vimentin filaments in endothelial cells subjected to mechanical stress (5), including shear stress (5, 12), has been reported. In this study, we demonstrated that vimentin is involved in the mechanotransmission and/or mechanotransduction of shear and tensile stress. Since Remuzzi (43) has provided evidence recently for a flow-induced dilation of renal arteries after reduction of renal mass, we propose that, in mutant mice, the lack of vimentin network impaired the nephrectomy-induced shear stress transmission and therefore induced the ET-1/NO imbalance.

In conclusion, our study provides the first evidence that vimentin is essential for (*a*) vascular response to contractile and relaxing agents and to mechanotransduction of shear stress and myogenic tone in renal resistance arteries; and (*b*) survival after reduction of renal mass, a pathological condition characterized by dramatic adaptive vasodilation. These results strongly suggest that vimentin is a key element in vasomotor adaptation, and that it seems to be necessary for both endothelial and smooth muscle cell function. The observation that the lack of vimentin differentially affects the vascular response to several contractile agents argues against a constitutive defect in the smooth muscle contractile apparatus in $Vim^{-/-}$ cells. Rather, in view of recent reports (8–10, 44) showing a relationship between cytoskeleton elements and signal transduction, it is likely that the lack of vimentin impairs the transduction of chemical and mechanical stimuli in the vessel wall. The phenotype described in this report raises the possibility that vimentin might be involved in human vascular disorders.

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