Role of fibrinogen in acute ischemic kidney injury

I. Sörensen-Zender,1 S. Rong,1 N. Susnik,1 J. Lange,1 F. Gueler,1 J. L. Degen,2 A. Melk,3 H. Haller,1 and R. Schmitt1

1Department of Nephrology and Hypertension, Hannover Medical School, Hannover, Germany; 2Division of Pediatric Hematology/Oncology, Cincinnati Children’s Hospital Research Foundation and University of Cincinnati College of Medicine, Cincinnati, Ohio; and 3Department of Pediatric Nephrology, Hannover Medical School, Hannover, Germany

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Role of fibrinogen in acute ischemic kidney injury. Am J Physiol Renal Physiol 305: F777–F785, 2013. First published June 26, 2013; doi:10.1152/ajprenal.00418.2012.—Renal ischemia-reperfusion (I/R) is associated with activation of the coagulation system and accumulation of blood clotting factors in the kidney. The aim of the present study was to examine the functional impact of fibrinogen on renal inflammation, damage, and repair in the context of I/R injury. In this study, we found that I/R was associated with a significant increase in the renal deposition of circulating fibrinogen. In parallel, I/R stress induced the de novo expression of fibrinogen in tubular epithelial cells, as reflected by RT-PCR, immunofluorescence, and in situ hybridization. In vitro, fibrinogen expression was induced by oncostatin M and hyper-IL-6 reflected by RT-PCR, immunofluorescence, and in situ hybridization. In vitro, fibrinogen expression was induced by oncostatin M and hyper-IL-6 in primary tubular epithelial cells, and fibrinogen-containing medium had an inhibitory effect on tubular epithelial cell adhesion and migration. Fibrinogen+/− mice showed similar survival as wild-type mice but better preservation in early postischemic renal function. In fibrinogen−/− mice, renal function and survival were significantly worse than in fibrinogen+/− mice. Renal transplant experiments revealed reduced expression of tubular damage markers and attenuated proinflammatory cytokine expression but increased inflammatory cell infiltrates and transforming growth factor-β expression in fibrinogen−/− isografts. These data point to heterogeneous effects of fibrinogen in renal I/R injury. While a complete lack of fibrinogen may be detrimental, partial reduction of fibrinogen in heterozygous mice can improve renal function and overall outcome.

Fibrinogen; tubular expression; acute kidney injury; ischemia-reperfusion

ACUTE KIDNEY INJURY (AKI) remains a major clinical challenge that is still associated with unacceptably high mortality and morbidity (7). In the clinical setting, causes of AKI are variable, but ischemia-reperfusion (I/R) is one of the most common aetiologies (3). Renal I/R results from transient hypotension, as in sepsis or from cardiovascular surgery, and it is also an unavoidable consequence of renal transplantation. On the molecular level, renal I/R leads to a complex series of injurious interactions between the microvasculature, tubular epithelium, and infiltrating inflammatory cells. These processes are associated with cytokine release and oxidative stress, which further aggravate renal cell damage (3). Another consequence of I/R is activation of the coagulation system, which might extend I/R damage by disturbing blood flow or triggering proinflammatory immune processes (20, 37). Previous studies (1, 10, 13, 37) have shown that renal I/R can cause the accumulation of fibrinogen in the kidney. Fibrinogen is the main protein of the coagulation system and consists of two identical subunits that contain three polypeptide chains: Aα, Bβ, and γ (19). Besides its important function in coagulation, fibrinogen plays a critical role in inflammation, wound healing, and angiogenesis by interacting with blood cells, endothelial cells, and other cell types after leaking into the extravascular space (9, 14, 22). Although leakage of circulating fibrinogen into areas of acute damage may aggravate injury and trigger inflammation (12, 33), its presence might also be instrumental for normal regeneration (9). Circulating fibrinogen is predominantly synthesized by hepatocytes, but intestinal and pulmonary epithelial cells can also express fibrinogen (28, 30). More recently, fibrinogen gene transcripts have been found in rodent kidneys after I/R and in human renal transplant biopsies (21, 26). So far, there is no information regarding the potential functional role of fibrinogen in AKI. This study was designed to explore the expression pattern of fibrinogen in kidneys after I/R and to analyze its impact on renal function, injury, and repair.

MATERIALS AND METHODS

Mice. Mice used in this study were either wild-type male C57BL/6J mice (10–16 wk; Janvier, France) or previously described C57BL/6J mice with a genetic deletion of the fibrinogen Aα-chain lacking all components of fibrinogen in the circulation (39, 40). Heterozygous mice have no overt phenotypic differences, but their levels of circulating fibrinogen are reduced to 70% (39). Genotyping was done by PCR of tail biopsy genomic DNA, as previously described (39). All experimental procedures were in agreement with institutional and legislative regulations and were approved by the local authorities.

I/R. Renal I/R injury was induced through unilateral and bilateral clamping of the renal pedicles as previously described (38). Male fibrinogen+/+, fibrinogen+/−, and fibrinogen−/− mice were anesthetized with isoflurane. After a median laparotomy, renal pedicles were dissected, and a nontraumatic vascular clamp was applied for 27 min (n = 16 for fibrinogen+/+ and fibrinogen+/− mice and 12 for fibrinogen−/− mice). For functional and survival experiments, blood samples were taken on days 0, 1, 3, 5, and 7, and renal function was analyzed by serum creatinine and urea measurements using an automated method (Beckman Analyzer; Beckman Instruments, Munich, Germany). Mice were killed on day 7. Unilaterally clamped animals were killed after 24 h, and kidneys were harvested for further examination (n = 6).

Isograft transplantation. In vitro, fibrinogen−/−, fibrinogen+/−, and fibrinogen−/− mice were anesthetized with isoflurane, and the donor kidney and ureter were harvested en bloc including the renal artery and ureter. These ischemia times induce reliable I/R injury in this model. After a left nephrectomy of the recipient, vascular cuffs were anastomosed to the recipient renal segment, and grafts were harvested for further examination. Following transplantation, these isografts showed better preservation of renal function than in wild-type mice (38). Histological examination (Giemsa, hematoxylin and eosin, and Masson trichrome) revealed better preservation of tubular structure in isografts than wild-type grafts (38). As previously described (38), the rats were anesthetized with pentobarbital, and their kidneys were harvested for further evaluation. All animal procedures were performed in accordance with the guidelines of the German government for the use of experimental animals.
Histology, immunostaining, and TUNEL. After kidneys had been harvested, a representative part of each kidney was fixed immediately in PBS-buffered 4% paraformaldehyde (PFA) and embedded in paraffin. Four-micrometer sections were used for immunostaining and for hematoxylin and eosin (H&E) staining to evaluate histological damage. Damage scoring was performed by a blinded observer using H&E-stained sections according to the protocol previously published by Broekema et al. (4). In brief, for each representative section, damage scores were averaged from 10 high-power fields of outer medulla that were ranked from 0 to 4 according to tubular dilatation, intraluminal casts, cell flattening, and loss of the brush-border membrane. Immunostaining was performed using the following primary antibodies: polyclonal goat anti-mouse fibrinogen (Nordic Immunology); monoclonal rat anti-mouse CD45 (BD Pharmingen, BD Biosciences), monoclonal rabbit anti-mouse Ki67 (Thermo Scientific), rabbit anti-megalin (a kind gift of Dr. Biemesderfer, Yale University School of Medicine), rabbit anti-NaCl cotransporter (NCC; Millipore), goat anti-Tamm Horsfall protein (Santa Cruz Biotechnology), and rabbit anti-aquaporin-2 (Abcam). Deparaffinized kidney sections were boiled in citrate buffer for antigen retrieval, blocked with 5% milk, and incubated overnight at 4°C with primary antibodies. This was followed by antibody visualization using Alexa 488/Alexa 547 secondary antibodies (Molecular Probes/Invitrogen, Carlsbad, CA). FITC-labeled Lotus tetragonolobus lectin (LTL) was used as a brush-border marker (Vector Laboratories). Quantification of CD45- and Ki67-expressing cells was done by counting of positive cells in 10 randomly chosen, nonoverlapping fields (×400 magnification) in the outer medulla. A fluorescein in situ death detection kit was used according to the manufacturer’s instructions for TUNEL assay (Roche Applied Science). TUNEL-positive tubular cells and total 4’,6-diamidino-2-phenylindole-positive tubular cells were counted in 10 nonoverlapping fields of the outer medulla in each sample (×400 magnification). Data are presented as percent ratios of TUNEL-positive epithelial cells versus total 4’,6-diamidino-2-phenylindole-positive epithelial cells.

mRNA in situ hybridization. Nonradioactive in situ hybridization was performed according to standard procedures (34). In brief, paraffin sections of PFA-fixed tissue were rehydrated. Tissue was digested with 10 µg/ml proteinase K in 0.1 M Tris (pH 7.5) for 10 min at 37°C. The digestion was stopped with 0.2% glycine. After being washed, slides were postfixed with 4% PFA-0.2% glutaraldehyde for 20 min, washed, and incubated with 2 N HCl. After another wash step, slides were incubated in 0.1 M triethanolamine (pH 8) for acetylation. For prehybridization, the tissue was incubated with prehybridization buffer for 2–4 h at 65°C. Next, the appropriate DIG-labeled riboprobe was diluted in hybridization buffer and incubated with the tissue overnight at 65°C. After several washing steps in 2× SSC and being blocked, the tissue was incubated with anti-DIG antibody (Roche) for 2 h at room temperature. After samples were washed in PBS-Tween (PBST) and NTMT, the RNA signal was developed with BM purple (Roche) in the dark for 2–48 h. After postfixation in 4% PFA, slides were mounted.

Cell culture. Primary renal tubular epithelial cells (PTECs) were isolated as previously described (35) and grown in REGM2 (PromoCell, Heidelberg, Germany). PTECs and mouse proximal tubular (mPT) cells (kindly provided by Dr. Cantley, Yale University School of Medicine) were stimulated with 25 ng/ml hyper-IL6 (kindly provided by Dr. Rose-John, Christian-Albrechts University, Kiel, Germany) or 25 ng/ml oncostatin M (Sigma-Aldrich, St. Louis, MO). Cells were lysed 24 h after stimulation for RNA isolation. PTEC colony growth was analyzed in the presence of the indicated concentrations of fibrinogen (Dunn Labotechnik) or equivalent concentrations of BSA (Sigma-Aldrich) as previously described (35). The total surface area of outgrowing colonies was measured at 6 days of culture using a standard cell culture microscope camera and Imaged software [National Institutes of Health (NIH)]. For cell adhesion assays, mPT cells were trypsinized and washed with DMEM containing 10% FCS (GIBCO-Invitrogen). After two washes with non-FCS-containing DMEM, equal numbers of cells were plated on uncoated or collagen type I-coated 24-well plates in the presence of the indicated concentrations of fibrinogen or albumin as a control protein diluted in DMEM for 2 h [protein concentrations were chosen according to our previous study (39)]. Cells were washed three times with PBS, and the total number of adherent cells was counted. For wound healing, a confluent monolayer of mPT cells was washed with non-FCS-containing DMEM. Cells were incubated in DMEM containing fibrinogen or BSA and scraped with a sterile 20-μl pipette tip, and wounds were photographed at 8 and 24 h. The percentage of wound closure in the presence of fibrinogen or albumin was calculated using ImageJ software (NIH).

Western blot analysis. Western blot analysis was performed as previously described (39). In brief, a representative part of each cell was homogenized and extracted with a standard cell culture microscope camera and ImageJ software (NIH).

Fig. 1. Intrarenal fibrinogen (Fbg) after ischemia-reperfusion (I/R). A: quantification of intrarenal Fbg by Western blot analysis. Immunofluorescence showed Fbg exclusively in the intravascular spaces of contralateral control kidneys (B), whereas the staining pattern at 24 h after I/R revealed massive deposition throughout the peritubular interstitium (C and D) with maximal intensity in the outer medullary region (E). Fbg reactivity was also found in the luminal space of damaged tubules (F and G), where it sometimes colocalized with sloughed epithelial cells (H). Original magnification: ×400 in B, C, and F and ×630 in D, E, and G.

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kidney was frozen in liquid nitrogen immediately after being harvested. Tissue was homogenized, and protein electrophoresis was performed as previously described (39). Proteins were transferred to polyvinylidene difluoride membranes, blocked with 5% milk in PBST, and probed overnight at 4°C with polyclonal goat anti-mouse fibrinogen (Nordic Immunology). Membranes were then washed and incubated with secondary antibody conjugated with horseradish peroxidase diluted in 5% milk and Tris-buffered saline-Tween. Antibody

Fig. 2. Tubular Fbg expression after I/R. A: immunofluorescence stainings for Fbg were positive in the cytoplasm of epithelial cells of a subset of tubuli at 24 h after I/R. B–D: double stains for Fbg and proximal tubular megalin and the brush-border marker Lotus tetragonolobus lectin (LTL) showed no colocalization. E: immunostaining of Tamm-Horsfall protein (THP) in the thick ascending limb of loop of Henle revealed no overlap with Fbg. F: partial overlap of the Fbg immunosignal was found with the thiazide-sensitive NaCl cotransporter (NCC) in the late part of the distal convoluted tubule. G: mRNA in situ hybridization for the Fbg Aα-subunit revealed expression in the cortical tubules of postischemic kidneys. H: significant renal upregulation of Fbg Aα-, Bβ-, and γ-subunits was also shown by quantitative PCR at 24 h post-I/R (n = 6). DAPI, 4’,6-diamidino-2-phenylindole. Original magnification: ×400. Values represent means ± SE. *P < 0.05; **P < 0.001.

Fig. 3. In vitro expression of Fbg in primary renal tubular epithelial cells (PTECs). A–C: low baseline expression of Fbg subunits was found in murine PTECs. Expression of all subunits was significantly increased after exposure to hyper-IL-6 (H-IL6; 25 ng/ml), which is a fusion protein of IL-6 and the soluble form of the IL-6 receptor, as shown by quantitative PCR. Similarly, oncostatin M (OSM) increased the expression of Fbg-Aα and Fbg-Bβ (D and E), but no effect was seen on Fbg-γ (F). Values represent means ± SE. **P < 0.001.
binding was visualized by chemiluminescence (SuperSignal West Pico Chemiluminescent, Thermo Scientific). Rabbit anti-mouse GAPDH (Sigma-Aldrich) was used as an internal loading control and for normalization of protein quantification. Immunoblots were scanned and quantified using ImageJ densitometry software.

Quantitative RT-PCR. RNA was isolated from frozen kidney tissue using the RNeasy Mini kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions. Reverse transcription was performed with Moloney murine leukemia virus reverse transcriptase (Promega) and random primers (Promega). Amplified cDNA was used as a template for quantitative PCR. Levels of mRNA expression were determined by quantitative real-time PCR using a Roche Lightcycler 480 System with SYBR green master mix and the following specific primers: fibrinogen-γ, 5'-ACTACAACCCAGACCAACC-3' (forward) and 5'-TTGTCTCGTGGGTAAACAGC-3' (reverse); transforming growth factor (TGF)-β, 5'-CACAATTCCTGGGTACCTGG-3' (forward) and 5'-CAACAATTCCTGGGTACCTTGG-3' (reverse); monocyte chemotactic protein (MCP)-1, 5'-CTACCTACTCATTTGGGATCATC-3' (forward) and 5'-AGCCATGTACGTAGCCATCC-3' (reverse); actin-β, 5'-AGCCATGTACGTAGCCATCC-3' (forward) and 5'-CTCTCAGCTGTGGTGGTAA-3' (reverse). For fibrinogen-A and fibrinogen-B, a QuantiTect Primer Assay (Qiagen) was used. Melting curves were examined to verify that a single product was amplified. For quantitative analysis, relative mRNA levels were calculated according to the 2^(-ΔΔCt) method (where Ct is threshold cycle). All samples were normalized to β-actin gene expression.

Statistical analysis. Results are expressed as means ± SE. Statistical significance was assessed by one-way ANOVA or two-way ANOVA with Bonferroni post hoc test or by an unpaired t-test or by log rank tests performed on Kaplan-Meier survival curves (GraphPad Software, San Diego, CA). P values of <0.05 were considered to be statistically significant.

RESULTS

I/R is associated with the intrarenal deposition of fibrinogen. C57Bl/6 mice underwent 27 min of I/R by unilateral clamping of the renal pedicle. Postischemic kidneys were harvested 24 h later and were compared with contralateral control kidneys. Western blot analysis revealed massive renal fibrinogen accumulation after I/R (Fig. 1A). The intrarenal abundance of fibrinogen-A, -B, and -γ was more than 10 times greater in postischemic kidneys compared with control kidneys. Immunostaining using a polyclonal antibody that recognizes both fibrin and fibrinogen revealed strong immunoreactivity throughout the tubulointerstitial space in postischemic kidneys, whereas control kidneys displayed a strictly intravascular staining pattern (Fig. 1, B–E). Staining intensity was most pronounced in the peritubular compartment of the outer medulla, where tubular dam-

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age is maximal in I/R injury. Fibrinogen staining was also detected in the intraluminal spaces of damaged tubules, where it colocalized with detached epithelial cells (Fig. 1, F–H). Secondary antibody alone was used as a negative control and showed no signal (data not shown).

**I/R is associated with the intrarenal de novo expression of fibrinogen.** The strong peritubular immunosignal for fibrinogen was accompanied by a weaker but clearly discernable cytoplasmic signal in a subset of tubules (Fig. 2A). Positively marked tubules were not observed in noninjured kidneys. Double staining of fibrinogen with megalin or LTL, both markers of proximal tubular cells, showed no overlap (Fig. 2, B–D). There was also no overlap with the loop of Henle marker Tamm Horsfall protein (Fig. 2E) nor with thiazide-sensitive NCC in the early part of the distal convoluted tubule. However, in the late part of the distal convoluted tubule, the luminal signal for NCC often overlapped with the cytoplasmic fibrinogen signal (Fig. 2F). Fibrinogen immunoreactivity ended at the transition from the late part of the distal convoluted tubule to the connecting segment, as marked by aquaporin-2-positive cells (data not shown). To test whether the tubular fibrinogen signal reflected local synthesis or epithelial uptake, fibrinogen mRNA in situ hybridization for the Aα-subunit was performed. In accordance with the immunofluorescence data, a similar distribution pattern of riboprobe labeling was found in the cortical tubules of posts ischemic kidneys (Fig. 2D), whereas control kidneys lacked any signal (data not shown). Quantitative PCR of whole kidney confirmed minimal baseline expression of fibrinogen Aα-, Bβ-, and γ-subunits in control kidneys. A robust increase in all three subunits was observed at 24 h after I/R (Fig. 2E). These data indicate that injured renal tubules can initiate local fibrinogen synthesis.

**Fibrinogen expression by renal tubular epithelial cells in vitro.** To better characterize the expression of fibrinogen by renal cells, we used PTECs. PTECs showed no significant expression of fibrinogen under baseline conditions. Similarly, no significant expression was observed after stimulation with IL-6, a major inducer of hepatic fibrinogen expression (36). This was not unexpected since renal cells lack IL-6 receptors (IL-6Rs) (29). However, during AKI, IL-6 has been shown to stimulate renal cells together with a soluble form of the IL-6R (sIL-6R) in a process termed trans-signaling (29). Indeed, hyper-IL-6, which is a fusion protein of IL-6 and sIL-6R (11), induced robust expression of all three fibrinogen subunits in PTECs (Fig. 3, A–C). Except for the γ-subunit, similar results were observed when tubular cells were stimulated with oncostatin M, a pleiotropic IL-6 family cytokine that is highly expressed in stressed kidneys (26) (Fig. 3, D–F).

**Fibrinogen has direct effects on tubular epithelial cell function in vitro.** A study (5) in other organ systems has shown that proregenerative cellular functions such as proliferation, cell attachment, and migration can be modified by the presence of fibrinogen. While we found no significant impact of fibrinogen on PTECs or mPT cell proliferation in bromodeoxyuridine uptake experiments (data not shown), we observed that the addition of fibrinogen in serum-free medium inhibited the normal expansion of outgrowing PTEC colonies (Fig. 4, A and B). Reduced expansion of PTEC colonies was paralleled by an inhibitory effect of fibrinogen on epithelial cell adhesion. The presence of fibrinogen significantly reduced the cellular attachment capacity of PTECs to uncoated or collagen type I coated culture dishes (Fig. 4C). Similarly, fibrinogen reduced the cell migration capacity after wound scratching in mPT monolayer cultures (Fig. 4, D and E).

**Fibrinogen deficiency is associated with higher mortality after renal I/R injury.** To test the biological effects of fibrinogen in vivo, we used fibrinogen-deficient mice that underwent 27 min of bilateral renal ischemia and compared them with homozygous and heterozygous littermates. Animal survival and serum creatinine and serum urea levels were monitored for 7 days post-I/R. The overall survival of fibrinogen−/− mice was significantly reduced compared with fibrinogen+/− and fibrinogen+/+ mice (Fig. 5A). However, fibrinogen+/+ mice had the highest creatinine and urea values, whereas fibrinogen−/− mice showed intermediate levels and fibrinogen+/− mice had the lowest values on postischemic day 1 (Fig. 5, B–D). Fibrinogen deficiency is associated with higher mortality after renal I/R injury. To test the biological effects of fibrinogen in vivo, we used fibrinogen-deficient mice that underwent 27 min of bilateral renal ischemia and compared them with homozygous and heterozygous littermates. Animal survival after bilateral I/R. Fbg+/+, Fbg+/−, and Fbg−/− littermates underwent 27 min of bilateral I/R. Animal survival (A) and serum levels of creatinine (B) and urea (C) were monitored for 7 days. n = 16 Fbg+/+, and Fbg+/− mice and 12 Fbg−/− mice. *P < 0.05; **P < 0.001.
and C). No significant differences were found on successive days, with renal function gradually normalizing in all surviving mice (Fig. 5, B and C). Additional mice were subjected to transient unilateral renal I/R, and kidneys were harvested at 24 h. While histological examination of I/R kidneys showed similar degrees of tubular epithelial damage (Fig. 6, A–C), there were differences in the expression of the tubular injury markers Kim1 and neutrophil gelatinase-associated lipocalin (NGAL), which were highest in fibrinogen+/+ mice, lower in fibrinogen+/− mice, and lowest in fibrinogen−/− kidneys (Fig. 6, E and F). No significant differences were found in tubular cell apoptosis (TUNEL), cell proliferation (immunostaining for Ki67), or infiltration of leukocytes (immunostaining for CD45; data not shown). Kidneys of fibrinogen+/+ mice showed a trend for lower expression levels of the proinflammatory cytokines IL-1β and MCP-1, whereas expression of anti-inflammatory TGF-β was significantly higher than in fibrinogen+/− kidneys (Fig. 6, G–I).

To focus on the impact of intrarenal fibrinogen expression, fibrinogen+/+, fibrinogen+/−, and fibrinogen−/− mice were used as donors and fibrinogen+/− mice as recipients in renal transplant experiments. I/R damage was induced by prolonged warm and cold ischemia times (30 and 60 min) while the right recipient kidney stayed in situ providing sufficient renal function for animal survival. Postischemic grafts were analyzed at 24 h after transplantation. As expected, a strong upregulation of fibrinogen-α mRNA was found in transplants from fibrinogen+/+ and fibrinogen−/− donors, whereas no induction was found in fibrinogen−/− kidneys (Fig. 7A). Similar to our findings in I/R kidneys, microscopic damage scoring was not significantly different between groups, but there was less Kim1 expression in fibrinogen−/− grafts (Fig. 7, B–D). Quantification by TUNEL staining revealed no significant differences in apoptosis, but there was increased tubular cell proliferation in fibrinogen−/− grafts (Fig. 7E). This was paralleled by significantly more intrarenal CD45-positive cells and F4/80-positive cells in fibrinogen−/− grafts (Fig. 7, F and G). Similar to I/R kidneys, expression of IL-1β and MCP-1 was lower in fibrinogen−/− grafts (Fig. 7, H and I).

**DISCUSSION**

Fibrinogen has been associated with a variety of important functions in acute and chronic injury models (9, 12, 14, 22, 33). Here, we show that fibrinogen massively accumulates in the kidney after I/R in two different mouse models and that this accumulation is not only a consequence of fibrinogen leakage from the circulation but also of local expression by stressed renal tubular cells. Knockout of fibrinogen leads to reduced...
survival after I/R, indicating the importance of extrarenal factors since kidney function was not worse compared with wild-type mice. Heterozygous mice have equal survival rates as wild-type mice but significantly better early renal function after I/R. Postischemic kidneys of fibrinogen−/− mice show a reduction in tubular injury markers and a phenotypic shift of inflammatory infiltration and cytokine expression. In agreement with a recent publication by Ajay et al. (1), these data indicate that renal function, inflammation, and survival critically depend on the amount of available fibrinogen.

While controlled inflammation represents a beneficial response in many types of injury, overwhelming inflammation in renal I/R contributes to tissue destruction, scarring, and fibrosis (3). Tubular cells secrete proinflammatory cytokines such as TNF-α, MCP-1, IL-8, IL-6, and IL-1β, which to attract and activate inflammatory cells (3). In parallel, the tubular epithelium initiates a local expression program that is reminiscent of the hepatic acute phase response (26). In this context, genes that are typically expressed in liver cells, such as α1-antitrypsin, lipopolysaccharide-binding protein, and, as confirmed by our study, fibrinogen, are transcribed (26). Luyckx and colleagues (26) have hypothesized that local generation of acute phase reactants in the kidney might be crucial for a rapid coordinated inflammatory response to injury. Our present results might support this hypothesis because lack of fibrinogen was associated with a more anti-inflammatory phenotype, including less MCP-1 and IL-1β and more TGF-β. However, these changes were relatively minor considering the overall massive inflammatory renal response induced by I/R. Interestingly, there was an increased inflammatory infiltrate in fibrinogen−/− grafts in transplantation experiments, whereas all groups had equal amounts of intrarenal infiltrate in the I/R model. These data suggest that leukocyte tissue infiltration is not only dependent on the binding of circulating fibrinogen to
endothelial cells (1, 20, 21, 38) but also affected by the presence of fibrinogen within the tissue.

Our in vitro results showed a strong antiadhesive effect of fibrinogen on renal epithelial cells. We therefore anticipated that fibrinogen might disturb cell functions that are considered instrumental in stress resistance and tubular repair. Antiadhesive effects of fibrinogen have previously been described in other cell types and have mainly been interpreted as a mechanism to limit uncontrolled thrombus growth (23, 24). In vivo, we could not find any significant changes in adhesion-dependent injury and repair processes such as tubular cell detachment or epithelial repopulation of denuded basement membranes. However, it is possible that the presence of fibrinogen that we observed in renal tubules might have contributed to the better outcome in heterozygous mice. Through its integrin-binding domains, fibrinogen might diminish the adhesion of desquamated epithelial cells that would otherwise form casts (16). In this case, the antiadhesive properties of fibrinogen would have a beneficial effect by helping to avoid tubular obstruction.

All differences that we found in postischemic kidneys of different fibrinogen genotypes were relatively subtle considering the substantially higher death rate of knockout animals. A straightforward explanation for this discrepancy could be that the lack of fibrinogen leads to inadequate haemostasis after surgery. Increased surgery-related death rates of fibrinogen-deficient mice have been described in other surgical models (18). On the other hand, we did not find clinical signs of increased haemorrhage or blood loss. Fibrinogen-deficient mice only died after bilateral but not unilateral I/R surgery. Although this argues against an important impact of enhanced bleeding, we cannot formally exclude that microhemorrhagic events contributed to the increased mortality in fibrinogen-deficient mice. Another factor might be a potential difference in remote organ damage. Remote organ damage describes a phenomenon in which renal I/R injury acts as an instigator and multiplier of cardiac, pulmonary, and hepatic damage (8, 15, 17, 31). Current knowledge about the underlying pathophysiology is still limited, and there are no data on the potential involvement of components of the coagulation system. Since the lack of fibrinogen can increase acute liver and lung injury (2, 27, 41), aggravation of post-I/R remote organ injury is not unlikely. In this case, systemic, multiorgan deterioration might have contributed to systemic illness and increased animal death.

The decreased functional outcome of fibrinogen-deficient mice is in line with earlier failed attempts to improve renal I/R by pharmacological defibrination (13, 25). Consistent with those studies, our results argue against aggressive fibrinogen depletion as an adequate treatment option, while a moderate reduction might be beneficial in renal I/R injury. Alternatively, it might be a valid strategy to target specific domains of the molecule since many biological functions identified for fibrinogen depend on distinct nonoverlapping epitopes (6). Along these lines, we and others (21, 32, 38, 42) have previously demonstrated that the fibrinogen breakdown product, Bβ15-42, has beneficial effects in renal I/R injury. Other domains of fibrinogen might be equally important, and their deletion might aggravate or attenuate I/R injury and inflammation independently of fibrinogen’s coagulant function. Consistently, it has been recently suggested that fibrinogen binding to ICAM-1 might aggravate postischemic renal damage (1). A more detailed understanding of specific fibrinogen domains and their complex interactions with different molecular binding partners will therefore be an important goal for further research.

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DISCLOSURES

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

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