Renal PKC-ε deficiency attenuates acute kidney injury and ischemic allograft injury via TNF-α-dependent inhibition of apoptosis and inflammation

Song Rong,1,3 Katja Hueper,6 Torsten Kirsch,1 Robert Greite,1 Christian Klemann,8 Michael Mengel,4 Matthias Meier,1 Jan Menne,1,2 Michael Leitges,1,5 Nathan Susnik,1 Martin Meier,7 Hermann Haller,1 Nelli Shushakova,1,2 and Faikah Gueler1

1Department of Nephrology, Hannover Medical School, Hannover, Germany; 2Phenos GmbH, Hannover, Germany; 3The Transplantation Center, Affiliated Hospital, Zunyi Medical College, Zunyi, China; 4Department of Laboratory Medicine and Pathology, University of Alberta, Edmonton, Canada; 5The Biotechnology Centre of Oslo, University of Oslo, Oslo, Norway; 6Institute for Diagnostic and Interventional Radiology, Medical School Hannover, Hannover, Germany; 7Imaging Center, Institute for Animal Science, Medical School Hannover, Hannover, Germany; and 8Centre for Paediatrics and Adolescent Medicine, Department of Pediatric Surgery, Hannover Medical School, Hannover, Germany

Submitted 5 July 2013; accepted in final form 12 July 2014

Rong S, Hueper K, Kirsch T, Greite R, Klemann C, Mengel M, Meier M, Menne J, Leitges M, Susnik N, Meier M, Haller H, Shushakova N, Gueler F. Renal PKC-ε deficiency attenuates acute kidney injury and ischemic allograft injury via TNF-α-dependent inhibition of apoptosis and inflammation. Am J Physiol Renal Physiol 307: F718–F726, 2014. First published July 23, 2014; doi:10.1152/ajprenal.00372.2013.—Acute kidney injury (AKI) increases the risk of morbidity and mortality after major surgery and transplantation. We investigated the effect of PKC-ε deficiency on AKI and ischemic allograft damage after kidney transplantation. PKC-ε-deficient and wild type (WT) control mice were subjected to 35 min of renal pedicle clamping to induce AKI. PKC-ε deficiency was associated with a marked improvement in survival and an attenuated loss of kidney function. Furthermore, functional MRI experiments revealed better renal perfusion in PKC-ε-deficient mice than in WT mice one day after IRI. Acute tubular necrosis and neutrophil infiltration were markedly reduced in PKC-ε-deficient mice. To determine whether this resistance to ischemia-reperfusion injury resulted from changes in local renal cells or infiltrating leukocytes, we studied a life-supporting renal transplant model of ischemic graft injury. We transplanted kidneys from H2b PKC-ε-deficient mice (129/SV) and their corresponding WT littermates into major histocompatibility complex-incompatible H11011 recipients (BALB/c) and induced ischemic graft injury by prolonged cold ischemia time. Recipients of WT allografts developed severe renal failure and died within 10 days of transplantation. Recipients of PKC-ε-deficient allografts had better renal function and survival; they had less generation of ROS and upregulation of proinflammatory proteins (i.e., ICAM-1, inducible nitric oxide synthase, and TNF-α) and showed less tubular epithelial cell apoptosis and inflammation in their allografts. These data suggest that local renal PKC-ε expression mediates proapoptotic and proinflammatory signaling and that an inhibitor of PKC-ε signaling could be used to prevent hypoxia-induced AKI.

protein kinase C-ε; ischemia-reperfusion injury; transplantation; allograft rejection; tumor necrosis factor-α; inflammation; functional magnetic resonance imaging

LONG COLD ISCHEMIA TIME increases the risk of delayed graft function after kidney transplantation, acute rejection, and progressive interstitial fibrosis (31). Members of the PKC family of serine/threonine kinases regulate many processes that contribute to renal damage and recovery during acute kidney injury, including adhesion, inflammation, apoptosis, migration, and proliferation (5, 8, 16). After lipopolysaccharide administration, PKC-ε inhibition markedly decreases renal macrophage activation and proinflammatory cytokine production (1, 4). The effects of PKC-ε depend on the type of cell and injury studied (36, 41). PKC-ε activation has been linked to protection from ischemic injury in the heart and other organs (3, 6, 45, 50, 51). In contrast, Nowak and coworkers (33, 34) showed that PKC-ε activation also has negative effects by inducing mitochondrial dysfunction and fragmentation in renal proximal tubular cells. Beneficial effects of PKC-ε inhibition with better cardiac outcome have been shown in animal models of cardiac hypertrophy and cardiac dysfunction (14, 22, 38). PKC-ε-deficient mice had less inflammation in sepsis models due to alterations of macrophage signaling (46). The aim of our present study was to assess the role of PKC-ε in renal ischemia-reperfusion (I/R) injury. First, we investigated PKC-ε-deficient mice in a model of renal I/R injury. Next, we examined the impact of local renal PKC-ε deficiency by investigating a life-supporting kidney transplant model of ischemia-induced renal allograft damage. PKC-ε deficiency resulted in reduced I/R injury with improved renal perfusion, renal function, and better survival. In recipients of PKC-ε-deficient allografts, attenuated TNF-α signaling was associated with a decrease in apoptosis and inflammation. Reduced expression of the adhesion molecule ICAM-1 and attenuated infiltration with monocytes/macrophages contributed to the superior allograft survival that we observed in PKC-ε-deficient allografts.

MATERIALS AND METHODS

Animals. PKC-ε-deficient mice were generated as previously described (25). Adult PKC-ε-deficient mice are healthy and do not show any overt abnormalities. Homozygous male PKC-ε-deficient mice (background: 129SV, H2b) and wild-type (WT) littersmates of the heterozygous cross were used in the renal I/R injury model. In addition, PKC-ε-deficient and WT mice served as kidney donors in the life-supporting kidney transplant model. BALB/c (H2b) mice were used as recipients and were supplied by Charles River (Sulzfeld, Germany). PCR genotyping verified PKC-ε deficiency. Mice weighing between 25 and 30 g (~12 wk of age) were used for all experiments. Animals were cared for in accordance with our institutional guidelines for experimental animals. They were on a 12:12-h
day-night cycle and had free access to food and drinking water. The local animal protection committee approved these experiments.

**Renal I/R injury.** Renal I/R injury was induced in homozygous male PKC-ε−/− mice and their corresponding WT littermate controls as previously described (18). Briefly, mice were anesthetized with isoflurane. After a median laparatomy, the renal pedicles were then bluntly dissected, and a nontraumatic vascular clamp was applied to both pedicles for 35 min. Survival and renal function were studied in n = 8 mice for 4 wk and histology for acute kidney injury was performed after 24 h after surgery in an additional n = 9 mice/group.

**Functional MRI.** MRI examinations were done 24 h after unilateral clamping of the right renal pedicle for 35 min using a 7-T small animal scanner (Bruker, Pharmascan) and a circular polarized volume coil (Bruker T10327V3). Animals were anesthetized by isoflurane inhalation, and respiration was monitored and kept between 30 and 50 breaths/min during the entire examination. For the visualization of renal morphology, respiratory-triggered, fat-saturated T2-weighted sequences were acquired in axial and coronal planes that covered both kidneys. The coronal plane was adjusted to the long axis of the kidney. Renal blood flow (RBF) was measured without administration of contrast agent using a fat-saturated abdominal aorta and vena cava recovery arterial spin labeling (ASL) sequence. Sequence parameters were as follows: repetition time/echo time = 10,000/16.4 ms, 13 inversion times (30, 100, 200, 300, 500, 700, 1,000, 1,200, 1,500, 2,000, 3,000, 5,000, and 8,000 ms), matrix = 128 × 128, field of view = 35 × 35 mm², slice thickness = 2 mm, and number of slices = 1. Parameter maps of RBF were then calculated on a pixel-by-pixel basis.

Regions of interest were placed manually into the renal cortex of both kidneys on RBF maps by one reader who was blinded to the animal group identity, and mean RBF values were determined separately for the right kidney with I/R injury and the other kidney without I/R injury.

**Kidney transplantation.** Kidney transplantation from PKC-ε-deficient mice and their WT littermates to BALB/c mice was performed as previously described (17, 18). In brief, animals were anesthetized with isoflurane, and the left donor kidney attached to a cuff of the aorta and the renal vein with a small caval cuff and the ureter was removed en bloc. After left nephrectomy of the recipient, the vascular cuffs were anastomosed to the recipient abdominal aorta and vena cava, respectively, below the level of the native renal vessels. The ureter was directly anastomosed to the bladder (19). Prolonged ischemia times were used to induce ischemic allograft damage (60 min of ischemia time).

Kidneys were harvested 24 h after I/R injury and 6 days after transplantation. Half of each allograft was immediately fixed in buffered formalin and embedded in paraffin, and the other half was shock frozen in liquid nitrogen. Paraffin sections (3 μm) were cut and stained with periodic acid-Schiff. Evaluation of acute tubular necrosis (ATN) was performed using a semiquantitative grading system, where 0 = no ATN, 1 = focal ATN with <10% of tubuli of the cortex affected, 2 = moderate ATN with 10–25% of tubuli affected, 3 = severe ATN with 25–50% of tubuli affected, and 4 = very severe ATN with >50% of the tubuli affected. Analysis was done by a nephropathologist without knowledge of the animal group identity. Cryosections were immunostained using the following primary antibodies: rat anti-mouse granulocyte receptor-1 (Serotec, Oxford, UK), monoclonal rat anti-mouse ICAM-1 (Serotec), rat anti-mouse monocyte/macrophage (F4/80, Serotec), polyclonal goat anti-mouse TNF-α (Santa Cruz Biotechnology, Santa Cruz, CA), and polyclonal rabbit anti-mouse active caspase 3 (BD Pharmingen, Heidelberg, Germany). For indirect immunofluorescence, nonspecific binding sites were blocked with 10% normal donkey serum (Jackson ImmunoResearch) for 30 min. Thereafter, cryosections were incubated with the primary antibody for 1 h in a humid chamber at room temperature. For fluorescence visualization of both primary antibodies, sections were further incubated with Cy3-conjugated secondary antibodies (Jackson ImmunoResearch) for 1 h. Sections were analyzed using a Zeiss Axioplan-2 imaging microscope with AxioVision 4.3 (Zeiss, Jena, Germany). The analysis was performed without knowledge of the animal assignment. To assess leukocyte cell infiltration, 10 different view fields/renal section were analyzed in each kidney. For granulocyte infiltration, cell numbers were counted. For inflammation and monocyte/macrophage infiltration, a semiquantitative grading system was used, where 0 = <5% leukocytes/view field, 1 = 5–25% leukocytes/view field, 2 = 26–50% leukocytes/view field, 3 = 51–75% leukocytes/view field, and 4 = marked infiltration with >75% of the tubulo-interstitial area affected. For ICAM-1 and inducible nitric oxide (NO) synthase (iNOS) evaluation, semiquantitative scoring was used, where 0 = no expression, 1 = mild expression, 2 = moderate expression, 3 = marked expression, and 4 = intense staining >75% of the glomeruli affected.

**Generation of ROS.** The redox-sensitive fluorophore dihydroethidium (DHE) was used to evaluate O₂⁻ production in the kidney in situ (19). Cryosections (6 μm thick) of frozen tissue were incubated with 0.1 mM DHE dissolved in Hepes-Tyrode buffer solution (132 mM NaCl, 4 mM KCl, 1 mM CaCl₂, 0.5 mM MgCl₂, 9.5 mM HEPES, and 5 mM glucose) for 12 min at room temperature. After incubation, images were obtained with the use of the Leica IM 500 imaging system (excitation: 520 nm and emission: 605 nm).

**RNA extraction and real-time quantitative PCR.** Frozen kidneys were ground to a coarse powder in liquid nitrogen, and total RNA was extracted using TRIzol reagent (Invitrogen). For quantitative PCR, 1 μg DNase-treated total RNA was reverse transcribed using SuperScript II reverse transcriptase (Invitrogen), and quantitative PCR was performed on an SDS 7700 system (Applied Biosystems) using Rox dye (Invitrogen), FastStart Taq polymerase (Roche Diagnostics) and gene-specific primers, and Fam-Tamra-labeled TaqMan probes (BioTez). PCR amplification was initiated at 96°C (10 min) followed by 40 cycles for 10 s at 95°C and 1 min at 60°C. For normalization, we used the β-actin signal. The sequences of the TaqMan reaction set as follows: β-actin, Fam-AGGCTATGCTTCCCTACGGCAT- Tamra, forward 5'-TAACCCACACTGTGGCACC-3', and reverse 5'-AGCCAGGTCCAGACGCAG-3'; and TNF-α, Fam-ACTGAC- CTTCTGTCCTCACCAGG-TAMRA, forward 5'-GTGACAGCGCT- GTGCTACA-3' and reverse 5'-AGGGCAATTACGTACCGGC-3'. Quantification was carried out using qgene software (20).

**Statistical analysis.** After verifying normal distribution by the Khomogorov-Smirnov test, we compared treatment groups by ANOVA and a post hoc Scheffé test. Differences were considered as significant at P < 0.05. Relative RBF measured by MRI was compared between PKC-ε-deficient mice and WT mice using unpaired t-tests. Data are presented as means ± SE. For statistical evaluation, SPSS 12.01 software was used.

**RESULTS**

PKC-ε deficiency attenuates I/R injury. PKC isoforms are participants in a variety of signaling cascades that mediate I/R injury. To elucidate the role of PKC-ε, we performed bilateral renal pedicle clamping in PKC-ε−/− mice and their corresponding WT controls. All WT control mice died within 3 days of I/R injury. In contrast, 60% of PKC-ε-deficient mice lived longer than 28 days postischemia (Fig. 1A). Furthermore, the loss of renal function in PKC-ε-deficient mice was significantly
PKC-ε deficiency markedly improved survival to 60% survival over 28 days. A: all wild-type (WT) mice died within 3 days after IRI. PKC-ε deficiency markedly increased survival to 60% survival over 28 days. IRI caused severe renal dysfunction with elevation of serum (s-)creatinine levels in WT mice, which was markedly attenuated by PKC-ε deficiency (P < 0.05 vs. WT mice). B: blastocidin-induced ATN (Fig. 2, A and C) and inflammatory cell infiltration with granulocytes (granulocyte receptor-1-positive cells; Fig. 2, B and D) were significantly reduced in PKC-ε-deficient mice compared with WT control mice. Renal perfusion of the unclipped kidney of PKC-ε-deficient mice was lower than in WT mice.

**ROS generation and apoptosis were markedly reduced in PKC-ε-deficient allografts.** The formation of ROS is an early event in I/R injury, and it drives apoptosis of renal cells and acute tissue damage. Therefore, we looked for ROS by DHE staining in renal allografts (Fig. 4, A and D). Within 4 h after transplantation, WT allografts exhibited substantial ROS generation in the tubulointerstitium (Fig. 4A) and glomeruli (data not shown). Markedly less ROS generation was detected in PKC-ε-deficient allografts (Fig. 4D). Since ROS generation due to severe hypoxia induces apoptosis and, consequently, ATN, we examined periodic acid-Schiff-stained renal tissue 1 day after transplantation. PKC-ε-deficient allografts had significantly less ATN and inflammation than allografts from WT control allografts (Figs. 3A and 4G). Expression of active caspase-3, an apoptosis marker, was elevated in WT allografts in the cytoplasm of damaged proximal tubuli mainly in the outer stripe of the outer medulla, an area that is very sensitive to hypoxic damage due to the corticomedullary O2 gradient (Fig. 4, B and E) (2). PKC-ε-deficient allografts had reduced active caspase-3 staining (Fig. 4E).

**TNF-α expression was significantly reduced in PKC-ε-deficient allografts.** TNF-α, an important mediator of apoptotic cell death due to I/R injury (10), has been shown to be regulated by some PKC isoforms (24). To investigate whether the antiapoptotic phenotype of PKC-ε-deficient allografts is associated with altered TNF-α signaling, we performed immunohistochemistry and quantitative PCR for TNF-α. We local-
ized TNF-α protein expression to the media of the vessel wall (Fig. 4, C and F) and to the tubules of the outer stripe of the outer medulla (data not shown). PKC-ε-deficient allografts showed less TNF-α protein expression than WT allografts (Fig. 4F). Furthermore, we found significantly lower levels of TNF-α mRNA in PKC-ε-deficient allografts than in WT allografts (P < 0.05; Fig. 4H).

PKC-ε deficiency of the allograft reduced inflammation. Ischemic allograft injury leads to acute inflammation with monocyte/macrophage infiltration that results from activation of adhesion molecules and upregulation of iNOS. Therefore, we asked whether PKC-ε deficiency alters inflammation after ischemic allograft injury. To answer this question, we stained monocytes/macrophages with F4/80 6 days posttransplantation (Fig. 5). Many F4/80-positive monocytes/macrophages were present in the perivascular and tubulointerstitial compartment in WT allografts (Fig. 5A). Monocyte/macrophage infiltration into PKC-ε-deficient allografts was much lower (Fig. 5D). Since adhesion molecules are important mediators of cell infiltration, we investigated ICAM-1 expression. We found intense staining of ICAM-1 in the glomeruli and intima of the vessel wall as well as in the tubulo-interstitium in WT allografts (Fig. 5B); in PKC-ε-deficient allografts (Fig. 5E), ICAM-1 upregulation was less pronounced than in WT allografts.

Another marker of inflammation and rejection is iNOS. It has been shown that PKC-ε affects iNOS expression in macrophages (9). In addition, data from cardiac transplantation using iNOS-deficient allografts showed that NO derived from iNOS contributed to the inflammatory response during acute cardiac allograft rejection (30). We found intense staining for iNOS 6 days after transplantation in the glomeruli and vessel walls of rejecting WT allografts (Fig. 5C); the iNOS signal was reduced in PKC-ε-deficient mice (Fig. 5F).
DISCUSSION

Several PKC isoforms are expressed in renal tissue, and some of them are upregulated after I/R injury. PKC-ε is among the upregulated isoforms (37). However, the exact role of PKC-ε in I/R injury is still debated. In cardiac preconditioning experiments, upregulation of PKC-ε has been shown to be protective (7, 35). On the other hand, PKC inhibition in a model of acute heart allograft rejection improved survival (26), and, in other animal models of cardiac hypertrophy, PKC-ε inhibition has been shown to attenuate cardiac dysfunction (14, 22). In the present study, we showed that PKC-ε deficiency resulted in improved kidney function and survival after renal I/R injury. In PKC-ε-deficient kidneys, there was less renal perfusion impairment after I/R injury than in WT kidneys as measured by ASL with noninvasive functional MRI (20). Renal perfusion of the unclipped kidney of PKC-ε-deficient mice was lower in than WT mice; the reason for this is not known and needs further investigation. In previous studies, we showed that the renal perfusion impairment correlates with the severity of I/R injury in mice and rats (53) and with inflammation and the progression to chronic kidney disease (21). In healthy volunteers, ASL has been shown to be a reproducible and robust way to assess renal perfusion (15). Due to the quality of RBF assessment by ASL, we think that our results with better renal perfusion stress the protective effect of PKC-ε deficiency after I/R injury. Renal microcirculatory dysfunction causes alterations in renal tissue oxygenation, NO bioavailability, and oxygen radical homeostasis (13). It has been shown that
preservation of RBF by protecting peritubular capillaries with cartilage oligomeric matrix protein-angiopoietin 1 treatment improved outcomes after renal I/R injury and reduced neutrophile infiltration (23). Similarly, we found that tissue damage and inflammatory cell infiltration with granulocytes were reduced in PKC-ε-deficient mice. This is in line with a previous study (4), which showed that PKC-ε deficiency leads to compromised host defense against bacterial infection due to a significant reduction in the generation of TNF-α, NO, and IL-1β. Nowak and coworkers (34) showed that PKC-ε activation mediated mitochondrial dysfunction and fragmentation in proximal tubular epithelial cells (34). Furthermore, their data showed that PKC-ε activation after oxidant injury in proximal tubular epithelial cells decreases mitochondrial function and active Na⁺ transport and that inhibition of PKC-ε activation improves these functions (33). To determine if the beneficial effects of PKC-ε deficiency is due to PKC-ε deficiency of local renal cells or circulating leukocytes, we investigated PKC-ε deficiency in a model of ischemia-induced allograft damage after kidney transplantation. This allowed us to compare PKC-ε deficient and WT allografts. Recipients in all experiments were BalbC mice with functional PKC-ε genes and, therefore, normal leukocyte function.

We found that PKC-ε-deficient allografts had improved survival and less loss of renal function. One of the mechanisms by which PKC-ε may improve survival is by attenuating the generation of ROS, which contribute to apoptosis of kidney cells. We observed that PKC-ε deficiency resulted in reduced ROS generation in allografts after transplantation. These results are in line with previous data of Li and coworkers (28) showing that ROS generation in vascular smooth muscle cells was PKC-ε and PKC-α dependent. They also demonstrated that downregulation of PKC reduces the cleavage of caspase-3 in vascular smooth muscle cells. In line with this observation, we found that PKC-ε deficiency of the renal allograft resulted in diminished active caspase-3 expression in tubular epithelial cells of the allograft after ischemic injury.

Depending on the stress model and cell type, PKC-ε regulates different pro- and antiapoptotic signaling pathways. Pro-apoptotic enhancement of peripherin aggregation by PKC-ε activation has been observed in neuroblastoma cells (48). In contrast, PKC-ε activation had antiapoptotic effects resulting from inhibition of TNF-α-related apoptosis-inducing ligand (42, 43) and prevention of activation and translocation of Bax to the mitochondria (29) in MCF-7 breast cancer cells. In the present study, PKC-ε deficiency had antiapoptotic effects in renal tubular epithelial cells, which exhibited less acute tubular necrosis and attenuated caspase-3 activation. Since TNF-α has been shown to be an important mediator of tubular epithelial cell apoptosis in a model of unilateral ureter obstruction (32), we suspected that PKC-ε deficiency might affect TNF-α activation in renal allografts as well. Indeed, we found signifi-

---

**Fig. 4.** PKC-ε deficiency reduced ROS generation and apoptosis. A and D: within 4 h after ischemic transplant injury, increased ROS generation in the tubulointerstitial compartment of WT allografts was detected (A); in contrast, PKC-ε-deficient allografts showed markedly reduced generation of ROS (D). Magnification: ×200. B and E: upregulation of cleaved caspase-3 activation as a marker of apoptosis was reduced in tubular epithelial cells (E) in PKC-ε-deficient allografts compared with WT allografts (B). C and F: TNF-α protein expression was upregulated in WT allografts (C) but not in PKC-ε-deficient allografts (F). Bars = 50 μm. G: ATN was significantly reduced in PKC-ε-deficient allografts compared with WT allografts. **p < 0.01. H: TNF-α mRNA expression was significantly reduced in PKC-ε-deficient allografts compared with WT allografts. *p < 0.05.
cantly reduced TNF-α mRNA and protein expression in PKC-ε-deficient allografts, but the exact mechanism of the TNF-α and PKC-ε interaction remains to be explored.

Hypoxic renal allograft damage is characterized by inflammatory cell infiltration with neutrophils and later macrophages and lymphocytes. Macrophages constitute 40–60% of infiltrating cells during acute allograft rejection. In patients with acute rejection, interstitial macrophage infiltration was significantly higher than in nonrejecting patients (47). Furthermore, macrophage infiltration 3 mo after transplantation correlated inversely with graft survival (44). In the present study, we identified markedly reduced interstitial inflammation by monocytes/macrophages in PKC-ε-deficient allografts. Monocyte/macrophage infiltration is mediated by upregulation of adhesion molecules, such as ICAM-1. Previously, we (12) have shown that blockade of ICAM-1 upregulation by antisense RNA improved renal isograft survival, attenuated I/R injury, and enhanced immediate graft function. In previous studies, we also observed improved allograft survival in urokinase-type plasminogen activator receptor-deficient mice (18) and mice in which complement activation had been blocked (17). In both models, ICAM-1 levels were lower than in WT or untreated animals. In line with these results, we observed decreased ICAM-1 expression in PKC-ε-deficient allografts. This should contribute to a decrease in monocyte/macrophage infiltration and improved allograft survival. I/R injury, as well as acute renal allograft rejection, has been shown to be associated with increased expression of proinflammatory iNOS (49, 52). iNOS expression has also been linked to transplant rejection in a rat model of allogenic kidney transplantation, where macrophage depletion resulted in reduced acute rejection and iNOS expression. However, the effect of iNOS inhibition is contradictory and might be organ and cell type specific. In hypoxic lung injury, selective iNOS inhibition has been shown to attenuate hypoxia-induced lung injury (39), whereas in cardiac I/R injury, the same iNOS inhibitor exacerbated peroxidative and apoptotic damage (39, 40). In the present study, the diminished renal iNOS expression in PKC-ε-deficient allografts was accompanied by protective effects with less inflammation and apoptosis.

I/R injury initiates and induces alloimmune responses, leading to acute and chronic allograft rejection (11, 27). Here, we identified PKC-ε as an important mediator of transplant-induced I/R injury that results from upregulation of ICAM-1 and inflammatory cell infiltration. Our findings are at odds with data published by Tanaka and colleagues (50), who showed that treatment with a PKC-ε activator plus a PKC-δ inhibitor reduced cardiac injury and reduced graft coronary artery dis-
ease in a murine model of allogenic heart transplantation. In their study, however, the different effects of the PKC-ε activator and PKC-δ inhibitor could not be distinguished. In contrast, in another study (26), treatment with a PKC-ε inhibitor caused improved survival in experimental heart transplantation by reducing inflammatory cell infiltration. The latter result supports the findings of our study, which showed that PKC-ε deficiency resulted in better allograft survival and reduced macrophage infiltration.

Taken together, we delineated the distinct role of local renal PKC-ε expression in ischemia-induced allograft damage. The fact that PKC-ε allograft deficiency attenuates I/R-induced apoptosis and blunts the generation of proinflammatory agents, including TNF-α, ICAM-1, and iNOS, suggests that PKC-ε antagonists could prove useful in preventing ischemia-induced graft injury.

ACKNOWLEDGMENTS

The authors thank Yvonne Nikolai, Herle Chlebusch, and Kerstin Bankes for excellent technical assistance.

DISCLOSURES

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

AUTHOR CONTRIBUTIONS


REFERENCES


PKC-ε AND ACUTE KIDNEY INJURY


