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Critical Protection from Renal Ischemia Reperfusion Injury by CD55 and CD59

Koel Yamada,* Takashi Miwa,* Jianuo Liu,* Masaomi Nangaku,† and Wen-Chao Song2*

Renal ischemia-reperfusion injury (IRI) is a feature of ischemic acute renal failure and it impacts both short- and long-term graft survival after kidney transplantation. Complement activation has been implicated in renal IRI, but its mechanism of action is uncertain and the determinants of complement activation during IRI remain poorly understood. We engineered mice deficient in two membrane complement regulatory proteins, CD55 and CD59, and used them to investigate the role of these endogenous complement inhibitors in renal IRI. CD55-deficient (CD55−/−), but not CD59-deficient (CD59−/−), mice exhibited increased renal IRI as indicated by significantly elevated blood urea nitrogen levels, histological scores, and neutrophil infiltration. Remarkably, although CD59 deficiency alone was inconsequential, CD55/CD59 double deficiency greatly exacerbated IRI. Severe IRI in CD55−/−/CD59−/− mice was accompanied by endothelial deposition of C3 and the membrane attack complex (MAC) and medullary capillary thrombosis. Complement depletion in CD55−/−/CD59−/− mice with cobra venom factor prevented these effects. Thus, CD55 and CD59 act synergistically to inhibit complement-mediated renal IRI, and abrogation of their function leads to MAC-induced microvascular injury and dysfunction that may exacerbate the initial ischemic assault. Our findings suggest a rationale for anti-complement therapies aimed at preventing microvascular injury during ischemia reperfusion, and the CD55−/−/CD59−/− mouse provides a useful animal model in this regard. The Journal of Immunology, 2004, 172: 3869–3875.

Acute renal failure is the most costly renal disease requiring hospitalization and ischemic acute renal failure, which is traditionally referred to as acute tubular necrosis (ATN),1 has an average mortality rate of 50% (1). There is no clinically accepted therapeutic regimen that will ameliorate or prevent cellular injury after ischemia to the kidneys (2–4). Although the pathogenesis of human ATN is not fully understood, a number of studies in animals have shown that the complement system is one of the important mediators of renal ischemia reperfusion injury (IRI) (5–8). Both anaphylatoxin (C3a, C5a)-dependent and membrane attack complex (MAC)-dependent mechanisms have been proposed for the complement role in animal models of renal IRI. For example, Zhou et al. (8) demonstrated that the primary effect of complement in a murine model of renal IRI was on the tubular epithelial cells rather than on the vascular endothelium and that this effect was mainly attributed to the formation of MAC on the tubules. Other studies have shown that C5a is critically involved in the pathogenesis of renal IRI in the mouse through its modulation of both neutrophil-dependent and independent pathways (6, 9).

In addition to experiments aimed at defining the complement mediators responsible for renal IRI, studies have also been conducted to elucidate the pathways by which complement is activated during ischemia reperfusion (IR). A recent study showed that mice deficient in the complement protein factor B were protected from renal IRI, suggesting that complement activation via the alternative pathway played an important role in this process (7). However, in other models of IRI, both classical pathway- and lectin pathway-dependent mechanisms have been implicated (10–13).

Currently, little is known about the determinants of complement activation during IR. Under normal circumstances, activation of complement on autologous tissues is restricted by soluble and cell surface-bound complement regulatory proteins (14, 15). However, the role of such complement regulatory proteins in the setting of IRI has not been investigated. In this study, we examined the function of two membrane complement regulators, CD55 (decay-accelerating factor) and CD59, in a murine model of renal IRI. Both CD55 and CD59 are GPI-anchored plasma membrane proteins (14–16). CD55 inhibits complement activation at the C3 and C5 convertase steps whereas CD59 prevents the assembly of MAC. We found that CD55−/−/CD59−/− mice were remarkably susceptible to complement-mediated renal IRI. Exacerbation of IRI in these mice was complement dependent and was associated with C3 and MAC deposition on microvascular endothelium and with evidence of peritubular capillary thrombosis. Our results suggest that complement-mediated microvascular injury, leading to an extension phase of ATN that compounds the initial ischemic assault (17), may be an important mechanism of complement injury during renal IRI. Thus, anti-complement therapies in the setting of IRI should be directed at blocking anaphylatoxin function as well as at preventing MAC-induced endothelial injury.

Materials and Methods

Animals

CD55 knockout (CD55−/−) mice and CD59 knockout (CD59−/−) mice were generated as described previously (18, 19). These mice were deficient in the widely distributed GPI-decay-accelerating factor gene and CD59α.

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gene (20–22), respectively. The original CD55−/− and CD59−/− mice with a mixed C57BL/6-129J background were back-crossed with C57BL/6J mice for nine generations. C3 knockout (C3−/−) mice, back-crossed onto the C57BL/6J/129J parental strain for a total of seven generations, were initially purchased from The Jackson Laboratory (Bar Harbor, ME). CD55−/−CD59−/− (CD55−/−CD59−/−) mice were generated by cross-breeding CD55−/− and CD59−/− mice. Male WT and knockout mice weighing 25–30 g were used in all experiments. Mice were housed in a specific pathogen-free facility and were confirmed to be negative for common murine viral pathogens by routine sera analysis. Experiments were conducted by following established guidelines for animal care and all protocols were approved by the appropriate institutional committees.

**Induction of renal IRI**

Mice were anesthetized by i.p. administration of Avertin (Sigma-Aldrich, St. Louis, MO). Using a midline abdominal incision, the renal pedicles were occluded for 22 min with microaneurysm clamps. During the ischemic period, body temperature was maintained by placing the animals in a 39°C incubator. After removal of the clamps, the kidneys were observed for 1 min to see the color change indicative of blood reperfusion, and blood was collected before clamping and at 24 h after reperfusion. Mice were sacrificed at 24 h after reperfusion and kidneys were harvested for histologic analysis.

**Assessment of renal function**

Blood urea nitrogen (BUN) levels were determined using sera prepared from blood collected before and 24 h after IRI induction and with urea nitrogen reagents (Sigma-Aldrich) by following the manufacturer’s instructions.

**Renal morphology**

Kidneys were fixed in methyl Carnoy’s solution overnight and processed for paraffin embedding. Sections of 4 μm thickness were made and stained with the periodic acid-Schiff (PAS) reagent and counterstained with hematoxylin. Tubular injury was scored by estimating the percentage of tubules in the outer medulla and corticomedullary junction that showed epithelial necrosis or had necrotic debris or cast as follows: 0, none; 1+, <10%; 2+, 10–25%; 3+, 25–45%; 4+, 45–75%; 5+, >75%. Twenty viewing fields randomly selected from the outer medulla and corticomedullary junction on each slide section were examined at ×400 magnification. Kidney sections were also stained with Masson’s scarlet and blue (MSB), a method highly selective for fibrin, to detect capillary thrombosis (23). All evaluations were made on coded slides without knowledge of the experimental group to which the mice belonged.

**Immunohistochemistry**

Cryostat sections (4 μm) of frozen kidneys were stained for neutrophils and complement factors C9 (MAC), Brieﬁly, slides were dried and ﬁxed in methanol–acetone. They were then treated with a rabbit anti-mouse lactoferrin Ab (kindly provided by Dr. C. Teng, National Institute of Environmental Health Sciences, Research Triangle Park, NC) diluted 1/2000 with 1% BSA in PBS or with a rabbit anti-rat C9 Ab (kindly provided by Dr. P. Morgan, University of Wales College of Medicine, Cardiff, U.K.) which cross-reacts with mouse C9 (24), diluted at 1/500. Subsequently, the slides were treated with a biotinylated goat anti-rabbit IgG Ab, followed by incubation with a HRP avidin D (Vector Laboratories, Burlingame, CA). The slides were then developed by treating with 3,3’-diaminobenzidine (Sigma-Aldrich), followed by counterstaining with methyl green (Vector Laboratories, Burlingame, CA). Neutrophils were counted by examining 5–10 viewing ﬁelds randomly selected from the outer medulla and corticomedullary junction on each slide at ×400 magnification in a blinded manner. The number of neutrophils was averaged for each slide. The speciﬁcity of the anti-lactoferrin Ab and the anti-C9 Ab was assessed by the use of a nonimmune rabbit serum as the primary Ab.

**Immunofluorescence microscopy**

Cryostat sections (4 μm) of kidneys were ﬁxed with methanol/acetone and stained for complement factor C3, using a FITC-conjugated anti-mouse C3 Ab (Cappel Laboratories, Durham, NC) diluted 1/500. C3 deposition along the tubular basement membrane was scored according to the system used by Park et al. (25). At least 10 high power ﬁelds in the outer medulla and corticomedullary junction were assessed and scored as follows: 0, none; 1+, <3 tubules with <30% circumference stained in a discontinuous pattern; 2+, >3 tubules stained, of which at least one had >50% circumference stained in a continuous pattern; 3+, >60% tubules stained, of which the majority had >75% circumference stained in a continuous pattern; and 4+, >90% tubules stained, with the majority having >90% circumference stained. A group of C3 knockout mice served as negative controls for C3 staining.

**Complement depletion in vivo**

Cobra venom factor (CVF) (Quidel, San Diego, CA) was used to deplete complement in WT mice and CD55−/−CD59−/− mice. Mice were twice administered CVF (12 U/ml in 0.5 ml of PBS, i.p.), at 24 and 16 h before induction of renal IRI (26).

**Statistical analysis**

All values were presented as means ± SEM. Statistical comparisons were analyzed with the program StatView (Abacus Concepts, Berkeley, CA), using the ANOVA followed by the Bonferroni/Dunn method for multiple group comparisons and Student’s t test as appropriate. A p value of <0.05 was considered statistically significant.

**Results**

To assess the role of CD55 and CD59 in renal IRI, we subjected WT, CD55−/−, CD59−/−, and CD55−/−CD59−/− mice to a procedure of bilateral renal IRI. This procedure consisted of a 22-min ischemia followed by 24 h reperfusion. This treatment signiﬁcantly impaired renal function in WT mice as BUN levels increased from 24.7 ± 0.4 mg/dl before IR to 55.5 ± 5.0 mg/dl 24 h after reperfusion (Fig. 1). Deficiency of CD55 signiﬁcantly exacerbated renal IRI, as CD55−/− mice treated with the same procedure had a BUN value of 115.6 ± 8.7 mg/dl at 24 h post-reperfusion (p < 0.001, comparing WT and CD55−/− mice at 24 h) (Fig. 1). Remarkably, although deﬁciency of CD59 alone did not affect the sensitivity of the mice to renal IRI, CD59 deﬁciency on the background of CD55 deﬁciency signiﬁcantly increased IRI (Fig. 1). Average BUN level in CD59−/− mice at 24 h was 49.7 ± 14.0 mg/dl, which was comparable to that of WT mice, but BUN in CD55−/−CD59−/− mice increased to 185.1 ± 7.9 mg/dl (p < 0.001 comparing CD55−/− and CD55−/−CD59−/− mice at 24 h post-reperfusion).

Impairment of renal function, as assessed by BUN levels, was corroborated by histological evidence. On PAS staining, both WT and CD59−/− mice subjected to IRI had easily detectable, albeit

![FIGURE 1. Effect of renal ischemia and reperfusion on renal function in WT. CD55−/−, CD59−/−, and CD55−/−CD59−/− mice.](http://www.jimmunol.org/)
relatively mild, tubular injury (Figs. 2, A and B, and 4A) and limited neutrophil infiltration (Figs. 3, A and B, and 4B). More severe tubular damage and a great many more neutrophils were detected in the CD55−/CD59−/− mice when compared with WT, CD55−/−, and CD59−/− mice. Arrows indicate tubules with necrotic debris, and arrowheads indicate cast formation.

To investigate whether severely exacerbated renal IRI in the CD55−/CD59−/− mice was complement dependency, we depleted plasma C3 by treating WT and CD55−/−CD59−/− mice with CVF before IRI induction. Absence of C3 did not significantly improve renal IRI in WT mice as assessed by BUN, tubular injury score or neutrophil infiltration (Fig. 5). In contrast, depletion of C3 ameliorated renal IRI in CD55−/−CD59−/− mice as indicated by marked reductions in BUN and tubular injury scores (Fig. 5). No significant difference was observed between WT and complement-depleted CD55−/−CD59−/− mice in their BUN levels and tubular injury scores. Unexpectedly, although complement depletion significantly reduced neutrophil infiltration in CD55−/−CD59−/− mice (p < 0.05, comparing CVF-treated and nontreated CD55−/−CD59−/− mice), the extent of neutrophil infiltration in CVF-treated

**FIGURE 2.** Light microscopy showing representative outer medulla sections of the kidney from WT (A), CD59−/− (B), CD55−/− (C), and CD55−/−CD59−/− mice (D) 24 h after reperfusion (PAS, ×200). The most severe tubular injuries were observed in CD55−/−CD59−/− mice when compared with WT, CD55−/−, and CD59−/− mice. Arrows indicate tubules with necrotic debris, and arrowheads indicate cast formation.

**FIGURE 3.** Immunohistochemical staining showing different degrees of neutrophil infiltration in the outer medulla of WT (A), CD59−/− (B), CD55−/− (C), and CD55−/−CD59−/− (D) mouse kidneys 24 h after reperfusion (×200). CD55−/− and CD55−/−CD59−/− mouse kidneys had significantly more abundant neutrophil infiltrates than WT or CD59−/− mice.
CD55⁻/-CD59⁻/- mouse kidneys was still significantly greater than that of CVF-treated WT mice (Fig. 5C).

We next examined C3 and MAC depositions using immunofluorescence and immunohistochemistry, respectively, in the kidneys of mice subjected to IRI or sham-operation. Positive C3 staining was observed along the tubular basement membrane of WT, CD55⁻/-, CD59⁻/-, and CD55⁻/-CD59⁻/- mouse kidneys 24 h after reperfusion. Tubular injury and neutrophil infiltration were most prominent in CD55⁻/-CD59⁻/- mice. However, CD55⁻/- mice also incurred significantly exacerbated tubular injury and neutrophil infiltration. Sham-operated WT mouse kidneys had no tubular injury and very few neutrophils. Data shown are mean ± SEM with the number of mice per group indicated as N beneath each column. * p < 0.001 vs WT mice; ** p < 0.001 vs CD59⁻/- mice; *** p < 0.01 vs CD55⁻/- mice.

FIGURE 4. Semiquantitative analysis of tubular damage (A) and neutrophil infiltration (B) in WT, CD59⁻/-, CD55⁻/-, and CD55⁻/-CD59⁻/- mouse kidneys 24 h after reperfusion. Tubular injury and neutrophil infiltration were most prominent in CD55⁻/-CD59⁻/- mice. However, CD55⁻/- mice also incurred significantly exacerbated tubular injury and neutrophil infiltration. Sham-operated WT mouse kidneys had no tubular injury and very few neutrophils. Data shown are mean ± SEM with the number of mice per group indicated as N beneath each column. * p < 0.001 vs WT mice; ** p < 0.001 vs CD59⁻/- mice; *** p < 0.01 vs CD55⁻/- mice.

C3 depletion by CVF ameliorated renal IRI in CD55⁻/-CD59⁻/- mice. Pretreatment with CVF significantly improved renal function in CD55⁻/-CD59⁻/- mice (Fig. 5A) before ischemia; •, 24 h after reperfusion. Thus, BUN levels in CVF-treated CD55⁻/-CD59⁻/- mice 24 h after IR were not significantly different from that of WT mice. C3 depletion also reduced tubular injury in CD55⁻/-CD59⁻/- mice to the level incurred by WT mice (A) but did not completely prevent the increase in neutrophil infiltration (C). Data shown are mean ± SEM with the number of mice per group indicated as N beneath each column. * p < 0.001 vs WT mice.

FIGURE 5. C3 depletion by CVF ameliorated renal IRI in CD55⁻/-CD59⁻/- mice. Pretreatment with CVF significantly improved renal function in CD55⁻/-CD59⁻/- mice (A) before ischemia; •, 24 h after reperfusion. Thus, BUN levels in CVF-treated CD55⁻/-CD59⁻/- mice 24 h after IR were not significantly different from that of WT mice. C3 depletion also reduced tubular injury in CD55⁻/-CD59⁻/- mice to the level incurred by WT mice (A) but did not completely prevent the increase in neutrophil infiltration (C). Data shown are mean ± SEM with the number of mice per group indicated as N beneath each column. * p < 0.001 vs WT mice.

In contrast with the lack of increased tubular C3 staining in CD55⁻/- and CD55⁻/-CD59⁻/- mice, consistent C3 staining in peritubular capillaries in the outer medulla was detectable only in CD55⁻/- and CD55⁻/-CD59⁻/- mice (Fig. 6, E and F). Likewise, positive staining of C9 was detected only on medullary peritubular capillaries of IRI-challenged CD55⁻/-CD59⁻/- mice (Fig. 7, A and B). The specific deposition of C3 and C9 in the peritubular capillaries of CD55⁻/-CD59⁻/- mice suggested that MAC-induced microvascular injury and dysfunction may have contributed to the exacerbation of renal IRI in these animals. To test this hypothesis, we stained kidney sections of IRI-challenged mice with MSB. After staining, prominent erythrocyte congestion in the peritubular microvascular vessels and fibrin formation, indicative of capillary thrombosis, were observed in IRI-challenged
CD55−/−CD59−/− mice but not in similarly challenged WT, CD55−/−, or CD59−/− mice (Fig. 7, C and D).

Discussion

Currently, evidence is accumulating for a pathological role of the complement system in renal and other types of IRI (5, 7–9). Why the complement system tends to be activated under the setting of IR and what are the factors that influence complement activation during this process are interesting questions that remain to be fully addressed. Reperfusion injury of ischemic skeletal muscle and intestinal epithelia has been found to invoke the classical pathway of complement in a natural Ab-dependent manner (10–12). In contrast, renal IRI has been shown to depend on the alternative pathway of complement, as factor B-deficient, but not C4-deficient mice, were protected from renal IRI (7, 8). Though the evidence of complement participation in IRI is compelling, the role of membrane complement regulatory proteins in this process remains to be characterized. Under normal homeostatic conditions, host cells are protected from complement attack by a number of membrane-anchored complement regulatory proteins (14, 15). In this study, we set out to investigate the function of two key membrane complement regulatory proteins, CD55 and CD59, in a murine model of IRI. Our data suggest that CD55 and CD59 play a critical and synergistic role in preventing renal IRI.

By applying a bilateral renal IR protocol, we evaluated the role of CD55 and CD59 in renal IRI through the use of genetically engineered mice that are deficient in either CD55, CD59, or both. We found that CD55−/−, but not CD59−/−, mice were significantly more susceptible to renal IRI compared with WT mice. Interestingly, although CD59-deficiency alone was inconsequential, inactivation of CD59 in CD55−/− mice further exacerbated renal IRI in CD55−/− mice. These results are consistent with our previous demonstration of a synergistic role of CD55 and CD59 in protecting RBC from complement lysis (19), and they suggest that CD55 plays a key role in preventing the initiation of the complement cascade after IR. We further demonstrated that C3 depletion by the administration of CVF ameliorated renal IRI in CD55−/−CD59−/− mice, thus providing direct evidence for the conclusion that exacerbated renal IRI in CD55−/−CD59−/− mice resulted from increased complement injury. Notably, CVF treatment did not significantly reduce renal IRI in WT mice (Fig. 5), suggesting that under our experimental protocol (22-min ischemia) the complement system did not play a critical role in renal IRI in WT mice. This conclusion was in accord with the results of Park et al. (25) who found that administration of a recombinant mouse C3 inhibitory protein, Crry-Ig, failed to attenuate renal IRI induced by 20- to 30-min ischemia. The lack of complement participation in normal mice reaffirms the effectiveness of complement regulatory proteins in inhibiting complement activation during IR. However, our data do not exclude the possibility that prolonged ischemia in WT mice may lead to complement activation that could overwhelm the protection of CD55 and CD59. Further studies in WT mice are needed to define the kinetics of complement activation in relation to the degree of the ischemic assault. Such studies will facilitate the development of rational anti-complement therapies for human IRI conditions.

Both anaphylatoxin- and MAC-mediated tubular injuries have been proposed as potential mechanisms for complement-dependent pathogenesis of renal IRI (6, 8, 9). Although we demonstrated that exacerbated renal IRI in CD55−/−CD59−/− mice was complement dependent, the mediator(s) responsible for the increased renal injury remains to be defined. Nevertheless, the fact that CD55−/−CD59−/− mice incurred more renal IRI than CD55−/− mice suggests that renal IRI in the double knockout mice was at least partially dependent on MAC as a consequence of CD59 deficiency. In a preliminary experiment, administration of a C5a receptor antagonist (Ref. 28; kindly provided by Dr. J. Lambris, University of Pennsylvania, Philadelphia, PA) to CD55−/− mice did not significantly ameliorate renal IRI (data not shown), raising the possibility that MAC-induced injury may play a part in IRI of CD55−/− mice as well.

Notwithstanding the marked impairment in renal function and histological evidence of tubular injury, we detected no significant increase in C3 deposition on renal tubules of CD55−/− or CD55−/−CD59−/− mice. Indeed, there was prominent peritubular C3 staining in sham-operated WT mice and such staining was actually reduced in IRI-challenged CD55−/− and CD55−/−CD59−/− mice. Because similar peritubular staining was not observed in IRI-challenged C3-deficient mice, the staining must be interpreted as specific and could possibly reflect local C3 synthesis. Given the severe tubular injury

Table I. C3 staining scores

<table>
<thead>
<tr>
<th>Mice</th>
<th>Number</th>
<th>Score</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT (sham)</td>
<td>7</td>
<td>2.1 ± 0.1</td>
<td>NS</td>
</tr>
<tr>
<td>WT</td>
<td>9</td>
<td>1.9 ± 0.1</td>
<td>NS</td>
</tr>
<tr>
<td>CD59−/−</td>
<td>8</td>
<td>1.8 ± 0.1</td>
<td>NS</td>
</tr>
<tr>
<td>CD55−/−</td>
<td>11</td>
<td>1.4 ± 0.1</td>
<td>0.0218</td>
</tr>
<tr>
<td>CD55−/−CD59−/−</td>
<td>7</td>
<td>0.7 ± 0.0</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

*Values shown are mean ± SEM. Values of p are for comparisons between mutant and WT mice.

FIGURE 6. Immunofluorescent staining for C3 in the renal outer medulla of sham-operated WT (A), IR-challenged C3−/− (B), WT (C), CD59−/− (D), CD55−/− (E), and CD55−/−CD59−/− (F) mice 24 h after reperfusion (×200). C3 deposition along the tubular basement membrane was observed in all mice except the C3-deficient mice, which served as a negative control for the specificity of C3 staining. Peritubular C3 staining in CD55−/−CD59−/− mice was significantly reduced compared with that in WT, CD55−/−, and CD59−/− mice (see also Table I). In contrast, deposition of C3 in peritubular capillaries was detectable only in CD55−/− (inset, E, ×400) and CD55−/−CD59−/− mice (inset, F, ×400).
incurred by CD55<sup>−/−</sup> and CD55<sup>−/−</sup>CD59<sup>−/−</sup> mice, peritubular C3 synthesis might be expected to be impaired and this could well explain the reduction in peritubular C3 staining in these mice (Fig. 6 and Table I). In contrast with the lack of increased tubular C3 deposition, increased C3 staining was observed on outer medulla capillary blood vessels of CD55<sup>−/−</sup> and CD55<sup>−/−</sup>CD59<sup>−/−</sup> mice, and increased capillary C9 deposition was observed in CD55<sup>−/−</sup>CD59<sup>−/−</sup> mice. Thus, capillary blood vessel, but not tubular, C3 or MAC deposition correlated with impaired renal function and exacerbated tubular injury in CD55<sup>−/−</sup> and CD55<sup>−/−</sup>CD59<sup>−/−</sup> mice. These findings are consistent with the known tissue distribution patterns of CD55 and CD59 in the mouse kidney. Both proteins are highly expressed on vascular endothelial cells and, although they were detected by immunohistochemistry in the mouse glomeruli, CD55 and CD59 were minimally expressed, if at all, on proximal tubules of the mouse (29, 30). Endothelial expression of CD55 is also known to be induced by inflammatory stimuli (31).

Collectively, our data support the hypothesis that complement-mediated microvascular injury is the primary event leading to exacerbated renal IRI in CD55<sup>−/−</sup>CD59<sup>−/−</sup> mice. In further support of this hypothesis, MSB staining showed conspicuous capillary congestion and thrombosis in the kidneys of CD55<sup>−/−</sup>CD59<sup>−/−</sup> mice, but not that of other groups of mice. Although we did not detect fibrin formation in the kidneys of IRI-challenged CD55<sup>−/−</sup> mice, it is still possible that microvascular injury also occurred in these mice. Both anaphylatoxins and MAC may have contributed to the endothelial injury and activation after IR in the CD55<sup>−/−</sup>CD59<sup>−/−</sup> mice. Apart from direct loss of function associated with necrotic injury, activated endothelial cells may increase adhesion molecule expression and produce inflammatory cytokines and lipid mediators (32, 33). Such events may create medullary vascular congestion and accumulation of intravascular leukocytes. Additionally, activation and injury of endothelial cells may induce a procoagulant response (34), leading to the formation of thrombus in the microvasculature. This congestion and thrombosis may in turn impair oxygen delivery to the outer medulla, resulting in an “extension phase” of ischemia which compounds the initial IR insult (17).

Although increased neutrophil infiltration was observed in both CD55<sup>−/−</sup> and CD55<sup>−/−</sup>CD59<sup>−/−</sup> mice, it remains to be determined whether neutrophils were the ultimate mediators of exacerbated renal IRI in these mice. In a previous study, de Vries et al. (6) showed that C5a-mediated renal IRI in the mouse was independent of neutrophils. In this regard, it is of interest to note a dissociation between renal IRI and neutrophil infiltration in CVF-treated CD55<sup>−/−</sup>CD59<sup>−/−</sup> mice. Although CVF treatment reduced renal IRI in CD55<sup>−/−</sup>CD59<sup>−/−</sup> mice to the level incurred by WT mice (Fig. 5, A and B), it did not completely reverse the increase in neutrophil infiltration in these mice (Fig. 5C). The latter result may reflect a separate role of CD55 in regulating neutrophil migration as recently demonstrated by Lawrence et al. (35).

In summary, in this study, we have shown that CD55<sup>−/−</sup>CD59<sup>−/−</sup> mice were highly susceptible to renal IRI compared with WT controls and that this susceptibility was complement dependent. Our data suggest that CD55 and CD59 function synergistically to inhibit renal IRI and that CD55 plays a key role in preventing the initiation of the complement cascade after IR. Exacerbation of renal IRI in CD55<sup>−/−</sup>CD59<sup>−/−</sup> mice appeared to stem from MAC-mediated endothelial injury and dysfunction in the renal outer medulla, resulting in an extension phase of ischemia. The CD55<sup>−/−</sup>CD59<sup>−/−</sup> mouse should provide a useful animal model for assessing anti-complement therapies aimed at preventing microvascular injuries during IR.

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References


