ALTERED RENAL TUBULAR EXPRESSION OF THE COMPLEMENT INHIBITOR CRRY PERMITS COMPLEMENT ACTIVATION AFTER ISCHEMIA/REPERFUSION

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Complement activation has been shown to be an important event in the development of ischemic acute renal failure (ARF) in mice. Studies in complement-deficient mice have shown that these mice are protected from renal failure after ischemia/reperfusion (I/R). Complement activation after renal I/R occurs via the alternative pathway and is independent of natural antibody. The alternative pathway is ordinarily activated at low levels via an enzymatic process, called “tickover,” which results in continuous low-level production of C3b. Such activation of the alternative pathway does not usually cause injury to self cells due to the presence of membranebound and fluid-phase complement inhibitors. Although several inhibitors of complement activation are present within the mouse kidney, only complement receptor 1–related protein y (Crry) is present on mouse tubular epithelial cells, the cells primarily injured during I/R. We hypothesized that altered expression or localization of Crry by tubular epithelial cells might contribute to the activation of complement after I/R. Therefore, we set out to study the effects of renal ischemia upon Crry and the relation of changes in Crry expression and complement activation. We found that altered tubular expression of Crry proceeds, and appears to permit, complement activation along the basolateral aspect of the tubules.

Mice were subjected to 24 minutes of bilateral renal ischemia and were sacrificed after 0, 2, 6, 24, and 48 hours of reperfusion. Serum was collected for serum urea nitrogen (SUN) measurements, and kidneys were evaluated for tubular injury and complement C3 deposition. A significant increase in the percentage of tubules showing complement deposition along the tubular basement membrane was seen by 6 hours of reperfusion (Figure 1A). The degree of tubules showing complement deposition peaked at 24 hours of reperfusion, involving 46–75% of the tubules, and returned to baseline by 48 hours of reperfusion. The acute tubular necrosis (ATN) grade did not significantly increase until after 24 hours of reperfusion and returned to baseline by 48 hours of reperfusion (Figure 2B). Thus, complement activation is an early event that precedes severe morphological injury after I/R.

At baseline, Crry was heavily polarized to the basolateral aspect of the renal tubules, abutting the site of occasional focal C3 deposition along the tubular basement membrane (Figure 2A). In kidneys subjected to 24 minutes of ischemia this polarity was lost before reperfusion, and Crry could be seen within the tubule lumen (Figure 2B). As shown in Figure 1, the tubules showed signs of injury at these early time points but did not yet show necrosis. The altered Crry expression is therefore a biochemical change of non-necrotic tubules. After 6 and 24 hours of reperfusion individual tubules demonstrated little remaining Crry (Figure 2, C and D). Those tubules that no longer demonstrated Crry expression had C3 deposited along their circumference.

To confirm that the loss of inhibition by Crry is an important event in the pathogenesis of ischemic ARF and complement activation, we subjected Crry-deficient and wild-type mice to a milder renal insult. When subjected to 20 minutes of ischemia and 24 hours of reperfusion, Crry+/- mice had significantly greater SUNs (111 ± 18 versus 52 ± 8 mg/dl; P < 0.01) and
significantly greater morphological injury (average ATN score, 3.9 ± 0.3 versus 2.56 ± 0.6; P < 0.05) than did wild-type controls.

Figure 1 The kinetics of complement activation after I/R. (A) The extent of C3 deposition was significantly increased by 6 hours of reperfusion and peaked at 24 hours. (B) Tubular damage as assessed by light microscopy and (C) SUN peaked at 24 hours of reperfusion and had returned to baseline by 48 hours of reperfusion. Epithelial cell swelling and disruption of the brush border (arrow) were evident by 6 hours, and necrosis (arrowhead) was evident by 24 hours.

*P < 0.05 versus baseline

Figure 2 The polarity of Crry is lost after I/R. (A–D) Dual staining for Crry (green) and C3 (red) was performed. (A) At baseline Crry was polarized to the basolateral aspect of the tubules in the kidney and scant C3 deposition was seen. (B) After 24 minutes of ischemia, the polarity of Crry expressed by proximal tubules was lost. Crry was seen more diffusely throughout the cells and within the tubule lumen (arrow). After 6 (C) and 24 hours (D) of reperfusion, an increasing percentage of tubules in the outer medulla ceased to express Crry. Those tubules that no longer expressed Crry showed extensive C3 deposition along their tubular basement membranes.