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B Cell Subsets Contribute to Renal Injury and Renal Protection after Ischemia/Reperfusion

Brandon Renner,* Derek Strassheim,* Claudia R. Amura,* Liudmila Kulik,* Danica Ljubanovic,† Magdalena J. Glogowska,* Kazue Takahashi,‡ Michael C. Carroll,§ V. Michael Holers,* and Joshua M. Thurman*

Ischemia/reperfusion (I/R) triggers a robust inflammatory response within the kidney. Numerous components of the immune system contribute to the resultant renal injury, including the complement system. We sought to identify whether natural Abs bind to the postischemic kidney and contribute to complement activation after I/R. We depleted peritoneal B cells in mice by hypotonic shock. Depletion of the peritoneal B cells prevented the deposition of IgM within the glomeruli after renal I/R and attenuated renal injury after I/R. We found that glomerular IgM activates the classical pathway of complement, but it does not cause substantial deposition of C3 within the kidney. Furthermore, mice deficient in classical pathway proteins were not protected from injury, indicating that glomerular IgM does not cause injury through activation of the classical pathway. We also subjected mice deficient in all mature B cells (μMT mice) to renal I/R and found that they sustained worse renal injury than wild-type controls. Serum IL-10 levels were lower in the μMT mice. Taken together, these results indicate that natural Ab produced by peritoneal B cells binds within the glomerulus after renal I/R and contributes to functional renal injury. However, nonperitoneal B cells attenuate renal injury after I/R, possibly through the production of IL-10.

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Ishmemia/reperfusion (I/R) is an important cause of acute kidney injury (AKI). Work in animal models has demonstrated a robust inflammatory response within the renal tubulointerstitium after I/R (1). These studies demonstrated that cellular (B cells, T cells, neutrophils, and macrophages) and soluble (complement, cytokines, and chemokines) components of the immune system contribute to tissue injury during reperfusion (2). Strategies that block various components of the immune response have proven protective in preclinical studies and hold promise as effective therapies for the prevention of AKI. However, the mechanisms by which the immune system is engaged after renal I/R are incompletely understood.

Several laboratories demonstrated that the complement system is activated within the kidney after I/R, and complement activation is an important cause of tissue injury. For example, mice deficient in factor B (B−/− mice; deficient in alternative pathway activity (3)) and mice deficient in C3 (C3−/− mice; deficient in complement activation through any of the activation pathways (4)) are protected from ischemic AKI. However, experiments using mice deficient in C4 (C4−/− mice; deficient in classical and mannose-binding lectin [MBL] activity) demonstrated that these mice were not protected from injury after renal I/R. Furthermore, C3 deposition was not observed in the kidneys of B−/− mice after I/R. These studies suggest that intrarenal complement activation after renal I/R occurs through the alternative pathway following the disruption of normal inhibitory protein expression (5) and does not require an intact classical pathway.

However, studies of ischemia in other organs demonstrated an important role for IgG and IgM in triggering complement activation and tissue injury. For example, natural Ab binds to neoantigens expressed within the intestine after I/R and causes tissue inflammation by activation of the classical and lectin pathways of complement (6–8). One study reported that a soluble product of B cells contributes to renal injury as well, although the renal injury did not seem to be mediated through the complement system (9). Work by another group using a similar model of renal I/R demonstrated that mice deficient in T and B cells were not protected from injury (10). These discrepancies may be due to the various functions that B cells can serve. They act as positive mediators of inflammation through their production of Ig. They also support T cell activation by acting as APCs, as well as through the production of cytokines, such as IL-4 and IL-6 (11). In contrast, some B cell subsets limit the immune response. Recent studies demonstrated that IL-10–producing B cells regulate the adaptive immune response and attenuate tissue injury in diseases, such as experimental autoimmune encephalitis and inflammatory bowel disease (11).

Given the growing role of therapies that target B cells, it is important to fully understand the pathologic and protective function of B cells in diseases, such as AKI. Because tubulointerstitial complement activation occurs primarily through the alternative pathway, it seemed unlikely that Ig is an important activator of the complement system after renal I/R. However, we hypothesized that other B cell functions, such as the production of IL-10, may modulate renal injury. To test this hypothesis, we depleted mice of their peritoneal B cells through hypotonic shock and subjected them to
renal I/R. The kidneys of these mice were evaluated to determine whether natural Ab produced by peritoneal B-1 cells contributes to complement activation and injury after renal I/R. We also subjected mice deficient in mature B cells or deficient in specific complement proteins to renal I/R to determine whether these immune factors are important in the development of renal injury after I/R.

Materials and Methods

Animals

Male mice aged 8–12 wk were used for all experiments. C57BL/6 mice were used for experiments in which peritoneal B cells were depleted and as control animals for other experiments. B cell-deficient μMT mice were obtained from The Jackson Laboratory (Bar Harbor, ME). Mice deficient in C4, C1q, and MBL A/C were generated as previously described (12–14), and were each backcrossed for >10 generations onto a C57BL/6 background. Animal care before and during the experimental procedures was conducted in accordance with the policies of the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All animal procedures were approved by the University of Colorado Denver – Institutional Animal Care and Use Committee.

IgM purification

Serum from C57BL/6 mice was buffer exchanged with 20 mM NaPO₄ and run over a Protein G column to remove IgG. Polyethylene glycol 4000 (Sigma-Aldrich, St, Louis, MO) was added to the flow-through at a final concentration of 7%, mixed by inversion, and incubated at room temperature for 3 h. The mixture was centrifuged at 2000 × g for 30 min. The pellet was resuspended in PBS, and the IgM-containing fraction was further purified by size-exclusion chromatography using a Sephadex 26/60 Superdex 200 column (GE Amersham, Piscataway, NJ). The purity of the isolated IgM fraction was assessed by Coomassie staining and Western blot analysis.

Depletion of peritoneal B cells by hypotonic shock

Peritoneal B cells were depleted, as previously described (15). Mice received i.p. injections of distilled water (0.5 ml) 12 and 6 d prior to undergoing renal I/R. Control mice were injected with 0.5 ml PBS. Depletion of peritoneal B-1 cells after injection with water was confirmed by FACS analysis. Peritoneal cells were obtained by lavage and stained with Abs to B220 (Southern Biotechnology Associates, Birmingham, AL), CD19 (BD Pharmingen, San Diego, CA), and CD5 (BD Pharmingen). Cells were then washed and resuspended in 500 μl PBS, run on a FACSCalibur machine (BD Biosciences, San Jose, CA), and analyzed with CellQuest Pro software (BD Biosciences).

Renal I/R protocol

Mice were anesthetized with 300 μl 2,2,2-Tribromoethanol (Sigma-Aldrich) injected i.p., and they were placed on a heating pad to maintain their body temperature during surgery. Laparotomies were performed, and the renal pedicles were located and isolated by blunt dissection. The pedicles were clamped with surgical clips (Miltex Instrument, Bethpage, NY), and occlusion of blood flow was confirmed by visual inspection of the kidneys. The clamps were left in place for 24 min and then released. The kidneys were observed for ∼1 min to ensure blood reflow, and then fascia and skin were sutured with 4-0 silk (U.S. Surgical, Norwalk, CT). Sham surgery was performed in an identical fashion, except that the renal pedicles were not clamped. The mice were volume resuscitated with 0.5 ml normal saline injected s.c. and kept in an incubator at 29˚C to maintain body temperature until the time of sacrifice. After 8, 24, 48, or 72 h of reperfusion, the mice were anesthetized, and blood was obtained by cardiac puncture. Laparotomy was performed, and the kidneys were harvested.

Histology, TUNEL staining, and immunofluorescence microscopy

For light microscopy, sagittal sections were fixed, embedded in paraffin, and 4-μm sections were cut and stained with periodic acid-Schiff (PAS). The

**FIGURE 1.** IgM is deposited in mouse glomeruli. Immunofluorescence microscopy revealed that IgM was present in the mesangium of mice 24 h after sham treatment (A, C) or renal I/R (B, D). E, Quantitative analysis confirmed that mesangial IgM deposition was increased after ischemia. F, Western blot analysis under reducing conditions of lysates made from kidneys subjected to sham treatment or I/R also demonstrates IgM. Arrowheads indicate glomeruli. A and B, Original magnification ×400; C and D, original magnification ×100.
sections were evaluated by a renal pathologist (D.L.) in a blinded fashion. The cortex and outer stripe of the outer medulla were assessed for epithelial necrosis, loss of brush border, tubular dilatation, and cast formation. At least ten fields (×400) were reviewed for each slide, and the percentage of tubules displaying these findings was determined. The kidney sections were scored as follows based on the percentage of affected tubules: 0, none; 1, <10%; 2, 11–25%; 3, 26–45%; 4, 46–75%; and 5, >75%.

TUNEL staining was performed using TACS-XL Blue Label Kit with Nuclear Fast Red counterstain (Trevigen, Gaithersburg, MD), according the manufacturer’s instructions. To assess the prevalence of apoptotic cells, 25 glomeruli and 25 fields in the cortex and outer medulla were examined in a blinded fashion. The number of TUNEL+ bodies per field was counted, and the results for each kidney section were averaged.

For immunofluorescence microscopy, sagittal sections of the kidneys were snap-frozen in OCT compound (Sakura Finetek, Torrance, CA). Four-micrometer sections were cut with a cryostat and stored at −70°C. At the time of staining, the slides were fixed with acetone and incubated with primary Ab. The Abs used include FITC-conjugated anti-mouse C3 (MP Biomedicals, Solon, OH), FITC-conjugated Abs to mouse IgG and IgM (Jackson ImmunoResearch Laboratories, West Grove, PA), and anti-mouse C4 (Hycult Biotech, Uden, The Netherlands). Secondary Abs were obtained from Jackson ImmunoResearch Laboratories. Sections were counterstained with hematoxylin (Vector Laboratories, Burlingame, CA). Staining was visualized using a Nikon T2000 inverted fluorescent microscope and SlideBook software (Intelligent Imaging Innovations, Denver, CO). For all immunofluorescence studies, at least three kidneys were examined per group, and >10 high-powered fields were examined per kidney. For quantitative analysis of glomerular IgM deposition, images of 14–30 glomeruli were obtained per section. The fluorescence intensity was determined using ImageJ software, and the mean value for each treatment group was determined. For the low-powered view of glomerular IgM (Fig. 1E), the brightness and contrast were adjusted to improve the visibility of the staining.

Western blot analysis
Renal tissue was homogenized in radioimmunoprecipitation buffer lysis buffer containing 1% Triton X-100, 0.5% deoxycholic acid, 150 mM NaCl, 20 mM β-glycerophosphate, 20 mM Tris-HCl (pH 8), 5 mM EGTA, 3 mM...
MgCl₂, 0.1% SDS, 1 mM Na₂VO₄, and one tablet of complete, EDTA-free, protease inhibitor mixture (Roche Applied Science, Indianapolis, IN). Homogenates were centrifuged at 14,000 rpm for 15 min at 4°C, and the supernatant was collected. Fifty micrograms of protein for each sample was resolved by electrophoresis with a 10% Bis-Tris polyacrylamide gel (Invitrogen) and transferred to a nitrocellulose membrane. The membrane was probed with an HRP-conjugated Ab to mouse IgM (Southern Biotechnology Associates) and was visualized using a chemiluminescence detection kit (Amersham Biosciences, Piscataway, NJ).

**Serum urea nitrogen measurements**

Serum urea nitrogen (SUN) was measured using an Alfa Wasserman ACE Chemistry Analyzer.

**ELISAs**

Serum IgM was measured by ELISA. Briefly, ELISA plates (Corning Glass, Corning, NY) were coated with 100 ng anti-mouse IgM (Southern Biotechnology Associates). After the plates were washed and blocked with 1% BSA (Sigma-Aldrich) for 2 h, serum samples were diluted 1:50 and added to the wells. Standard curves were generated using serial dilutions of purified IgM (7.8125–500 ng/ml). The plates were then washed, and IgM was detected with an HRP-conjugated anti-mouse IgM Ab (Southern Biotechnology Associates). The plates were then developed with ABTS (Sigma-Aldrich) and read on a Spectramax PLUS plate reader (Molecular Devices, Sunnyvale, CA).

**Statistical analysis**

Data were graphed and analyzed using Prism software (GraphPad, San Diego, CA). ANOVA with a Tukey–Kramer posttest was used for multiple-group comparisons; t tests were used for experiments involving two groups. A p value <0.05 was considered significant.

**Results**

**Glomerular IgM is seen in wild-type mice**

We examined sham-treated mice and mice subjected to renal I/R for tissue deposition of IgG and IgM. IgG was not seen in any of the kidneys, but IgM was seen in the mesangium of sham-treated kidneys (Fig. 1). The level of mesangial IgM increased after renal I/R (Fig. 1E, 1F), suggesting that circulating IgM binds to the mesangium during reperfusion. IgM was not seen along the tubular basement membrane.

**Depletion of peritoneal B cells protects mice from renal I/R**

We depleted the peritoneal B cells in wild-type mice for 2 wk by injecting distilled water into the peritoneum, and control mice were

**Deficiency of the classical and MBL complement pathways does not protect mice from injury after I/R.** Mice with deficiency in C4, MBL-A and MBL-C, and C1q were subjected to renal I/R. SUN levels in all three strains were comparable to wild-type animals that were concurrently subjected to renal I/R.

Serum IL-10 and CXCL1 were measured using ELISAs obtained from ELISATech, Aurora, CO, according to the manufacturer’s protocol.

**FIGURE 3.** Depletion of peritoneal B cells protects mice from renal I/R. A, Depletion of the peritoneal B cells attenuated the peak level of SUN seen after renal I/R. Kidney sections from control (B) and B cell-depleted (C) mice were stained with PAS. Despite functional protection after peritoneal B cell depletion, widespread tubular necrosis was seen in the outer medullas of both groups. B and C, Original magnification ×400.

**FIGURE 4.** Deficiency of the classical and MBL complement pathways does not protect mice from injury after I/R. Mice with deficiency in C4, MBL-A and MBL-C, and C1q were subjected to renal I/R. SUN levels in all three strains were comparable to wild-type animals that were concurrently subjected to renal I/R.

**FIGURE 5.** The classical and alternative pathways of complement are activated in distinct locations within the kidney after renal ischemia/reperfusion. Immunofluorescence microscopy was performed on kidneys from sham-treated wild-type animals (A) and wild-type (B), β²⁻⁻ (C), and C4⁻⁻ (D) animals subjected to renal I/R. Mesangial C4 (red) was prominent in the glomeruli of wild-type and β²⁻⁻ mice, but it was not seen in the tubulointerstitium. C3 (green) was seen in the tubulointerstitium of wild-type and C4⁻⁻ mice, but it was not seen in the kidneys of β²⁻⁻ mice. Original magnification ×400.

**FIGURE 4.** Deficiency of the classical and MBL complement pathways does not protect mice from injury after I/R. Mice with deficiency in C4, MBL-A and MBL-C, and C1q were subjected to renal I/R. SUN levels in all three strains were comparable to wild-type animals that were concurrently subjected to renal I/R.
injected with an equal volume of PBS. Depletion of the B-1 population was confirmed by flow-cytometry analysis of B220, CD5, and CD19 (Fig. 2). This protocol was reported to reduce peritoneal B-1 cells, thereby preventing tissue injury by pathogenic natural IgM Ab (15). Lysis of the peritoneal B-1 cells did not alter the overall levels of circulating IgM (Fig. 2C), but it did reduce levels of mesangial IgM after sham treatment and after renal I/R compared with control mice (Fig. 2D–H). Depletion of peritoneal B-1 cells was also associated with a significantly attenuated increase in SUN after 24 h of reperfusion (the peak of injury; Fig. 3A). SUN levels were not significantly different from control mice by 48 h of reperfusion. Although the decrease in mesangial IgM was associated with protection of renal function, mice that underwent peritoneal B cell depletion still demonstrated tubular necrosis comparable to that seen in wild-type mice (Fig. 3B, 3C).

Mice deficient in the classical or MBL pathway of complement are not protected from renal I/R

IgM bound to Ag is a potent activator of the classical pathway of complement, and it can activate the MBL pathway (7). To assess whether activation of these pathways by IgM contributes to renal dysfunction after I/R, we subjected C4−/−, C1q−/−, and MBL−/− mice to renal I/R. None of these strains showed functional protection from renal injury in our model (Fig. 4). This suggests that injury caused by glomerular IgM is not mediated through activation of the classical or lectin pathways of complement.

The classical and alternative pathways of complement are activated in distinct compartments of the kidney after I/R

To further characterize the mechanisms of complement activation in the kidney after I/R, we performed dual-staining immunofluorescence for C3 and C4. C4 was present in the mesangium of sham-treated mice (Fig. 5A) and appeared to increase after I/R (Fig. 5B), consistent with the mesangial deposition of IgM. The pattern of mesangial C4 was not notably different in fB−/− mice subjected to I/R (Fig. 5C), as one would expect because these mice are only deficient in the alternative pathway. Mesangial C4 was not seen in C4−/− mice.

C3 did not colocalize with mesangial C4, indicating that, although the classical pathway is engaged at this location, mesangial complement activation is well controlled by endogenous regulators (16). As has been previously described, C3 is deposited along the tubules of wild-type mice after I/R (Fig. 5B) but was not seen in fB−/− mice. Tubular C3 was seen along the tubules of C4−/− mice (Fig. 5D), indicating that tubulointerstitial complement activation

**FIGURE 6.** B cell-deficient mice develop more severe injury than wild-type controls after renal I/R. Wild-type and μMT mice were subjected to renal I/R and 8–72 h of reperfusion. A, After 24 h of reperfusion, the SUN levels in μMT mice were higher than in wild-type animals that were concurrently subjected to renal I/R. B, PAS-stained sections of kidneys reperfused for 24 h (the peak of injury) were examined, and the extent of tubular necrosis was graded by a pathologist. Widespread tubular injury was seen in wild-type (C) and μMT (D) mice. C, TUNEL staining was performed on tissue sections from both strains. The prevalence of TUNEL+ cells was greater in the cortex and outer medulla of μMT mice. No difference was seen in the prevalence of TUNEL+ cells in the glomeruli. Examples of TUNEL+ cells in a glomerulus and in the outer medulla are shown in the insets and are indicated with arrows. PAS stain was used in C and D. C–E, Original magnification ×400. *p < 0.05, **p < 0.01.
requires the alternative pathway but does not require the classical or MBL pathway.

Mice lacking mature B cells sustain more severe injury than wild-type controls

We also subjected μMT mice to renal I/R. Contrary to the mice with depletion of only the peritoneal B cells, we found that the μMT mice sustained more severe renal dysfunction than wild-type mice, as assessed by SUN levels after 24 h of reperfusion (the peak of injury; Fig. 6A). Although the mean SUN value for the μMT mice remained higher than that for wild-type mice out to 72 h of reperfusion, the difference was no longer significant after 24 h. Significant necrosis was seen in the tubulointerstitium of μMT and wild-type mice (Fig. 6B–D). TUNEL staining was used to assess the prevalence of apoptotic cells in the glomeruli and tubulointerstitium (Figs. 6E), and a greater number of apoptotic cells were seen in the tubulointerstitium of μMT mice than in wild-type controls. Few apoptotic cells were seen in the glomeruli of either strain, and differences between the strains were not significant. After 8 h of reperfusion, serum levels of IL-10 were undetectable in μMT mice.

Reconstitution of mice with IgM does not restore protection from renal injury after I/R

IgM was purified from the serum of wild-type mice by polyethylene glycol precipitation. μMT mice were injected with IgM or with vehicle and were subjected to renal I/R. The reconstituted μMT mice had levels of IgM comparable to wild-type mice (Fig. 8A). However, reconstitution of circulating IgM did not restore the mesangial IgM deposits (Fig. 8B). Renal injury was no different from that seen in control μMT mice (Fig. 8C). These results indicate that the purification procedure we used did not restore IgM with specificity for mesangial Ags and suggest that the effect of IgM on renal function may be mediated by specific clones of IgM. It is unclear why the purified IgM failed to restore the mesangial deposits. Possible explanations are that the mesangial-specific IgM circulates at very low concentrations because most of it is bound up in tissue or that the IgM-purification process that we used depletes the mesangial-specific clone(s).

Discussion

These studies demonstrated that B cells play a complex role in the development of injury after renal I/R. Natural Ab IgM was present within the mesangium of mice at baseline, and it increased during reperfusion. Depletion of peritoneal B-1 cells by hypotonic shock reduced glomerular IgM. This treatment attenuated the degree of renal dysfunction after I/R only slightly, and reduction of glomerular IgM did not prevent tubular injury. Reconstitution of B cell-deficient mice with purified IgM failed to restore mesangial IgM, highlighting that the interaction of IgM with mesangial structures is a specific finding and is not due to passive trapping of circulating IgM.
Our results also demonstrate that the complement system is activated in the glomeruli and the tubulointerstitium by distinct mechanisms after I/R. The classical pathway was engaged by IgM deposits in the mesangium, leading to C4 deposition, but this activation was well controlled and did not proceed to the level of C3 cleavage. Although the deposition of IgM in the mesangium contributed to renal dysfunction during reperfusion, this effect was not mediated by the complement system. In contrast, the classical and MBL pathways were not required for complement activation in the renal tubulointerstitium after I/R, and complement activation at this location required only an intact alternative pathway. Tubulointerstitial alternative pathway complement activation proceeded through the cleavage of C3. Previous work also showed that tubulointerstitial complement activation generates C5b-9 and contributes to renal injury (3, 4).

Despite the fact that natural IgM contributes to renal injury after I/R, mice wholly deficient in mature B cells developed more severe injury than wild-type control mice. These mice had lower levels of serum IL-10 than wild-type mice during reperfusion. IL-10 is an anti-inflammatory cytokine that was demonstrated to play an important role in protecting mice from ischemic AKI (17). Several recent studies demonstrated that a population of IL-10–producing regulatory B cells helps to regulate the adaptive immune response in various diseases (11), and our results suggest that IL-10–producing B cells may also control the inflammatory response in this acute injury model. In contrast to the glomerular location of the pathogenic IgM, the protective effect of B cells manifested as reduced tissue injury in the tubulointerstitium (Fig. 6).

A previous study reported that RAG-1–deficient mice (which lack mature B cells and mature T cells) are not protected from renal I/R (10). In contrast, a study by Burne-Taney et al. (9) found that a soluble factor produced by B cells contributes to renal injury after I/R and that this B cell mediated injury is not mediated through complement activation. It is noteworthy that we used identical mice and a similar protocol as that used in this latter study, but the B cell-deficient mice were protected in the study by Burne-Taney et al. (9). However, the pathogenic factor B cell factor was found to be present in serum, and transfer of splenic lymph node B cells into the μMT mice was not pathogenic. These results are consistent with the divergent B cell effects that we have described: production of a pathogenic soluble factor but cell-mediated protection of the kidneys. A full understanding of the role of B cells in renal I/R will require further experiments to dissect the different roles of the different B cell subsets.

Our results indicate that, as with the intestine, natural Ab binds to Ags expressed in the postischemic kidney. However, in the case of renal I/R, the IgM-mediated injury does not seem to be mediated through activation of the classical complement pathway. The decline in renal function associated with mesangial IgM deposition may be due to direct effects of IgM on the mesangial structures. A previous study also showed that MBL contributes to tubular complement activation after renal I/R (18). It is possible that MBL contributes to the initiation of complement activation, and the alternative pathway then amplifies the activation. It is also possible that the longer ischemic time used in that study (45 min) generated more endogenous ligand for MBL, and, therefore, the MBL pathway played a greater role in that model.

The above studies do not conclusively demonstrate that protection of the kidney by B cells is directly mediated by production of IL-10. However, such a hypothesis is consistent with the protective role of IL-10 in ischemic AKI and with the recent work describing a population of IL-10–producing regulatory B cells (11). Future studies that specifically deplete or restore the CD1d<sup>+</sup>CD5<sup>+</sup> regulatory B cell population may help to confirm a direct role for regulatory B cells in protecting the kidney after I/R. It is also worth noting that the contribution of IgM to renal injury in our model was modest. This is not surprising given that renal ischemia primarily causes injury of tubules in the outer medulla not glomeruli. Nevertheless, the effect of natural IgM on the increase in SUN after renal I/R seemed to be mediated by a specific clone of IgM with specificity for mesangial structures. This interaction may also have pathologic effects on renal function in other models of injury, particularly those that primarily cause severe mesangial injury.

B cell-depleting Abs have emerged as a promising therapy for several renal diseases, including lupus nephritis, membranous disease, and focal segmental glomerulosclerosis. However, it has become clear that B cells have a complex role in modifying disease. Our results have several implications. First, they demonstrate that B cells can dampen renal injury after I/R and that therapies that nonspecifically target B cells may exacerbate AKI. Second, our results demonstrate that natural Ab IgM may bind to the injured kidney and reduce renal function. Therefore, the optimal approach to treating renal diseases may require the development of agents that can target the discrete B cell populations or agents that interfere with binding of Ig to specific renal Ags. Such an approach may also improve the treatment of nonrenal autoimmune diseases in which B cells might play a similarly complex role.

In summary, our results demonstrate that B cells can have protective and pathogenic functions during the development of AKI. The pathogenic role seems to be mediated by natural Ab IgM, which binds to the mesangium after I/R. Depletion of peritoneal B-1 cells reduced mesangial IgM and protected mice from injury after I/R. B cells also play a role in protecting the kidneys after I/R, probably by production of the anti-inflammatory cytokine IL-10.

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Disclosures

J.M.T. is a consultant for and stockholder in and V.M.H. is chief scientific officer for and is a stockholder in Taligen Therapeutics, Inc.

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