Treatment with an Inhibitory Monoclonal Antibody to Mouse Factor B Protects Mice from Induction of Apoptosis and Renal Ischemia/Reperfusion Injury

Joshua M. Thurman,* Pamela A. Royer,* Danica Ljubanovic,† Belda Dursun,* Amanda M. Lenderink,* Charles L. Edelstein,* and V. Michael Holers*

*Department of Medicine, University of Colorado Health Sciences Center, Denver, Colorado; and †Department of Pathology, University Hospital Dubrava, Zagreb, Croatia

Complement activation in the kidney after ischemia/reperfusion (I/R) seems to occur primarily via the alternative complement pathway. The ability of an inhibitory mAb to mouse factor B, a necessary component of the alternative pathway, to protect mice from ischemic acute renal failure was tested. Treatment with the mAb prevented the deposition of C3b on the tubular epithelium and the generation of systemic C3a after renal I/R. Treated mice had significantly lower increases in serum urea nitrogen and developed significantly less morphologic injury of the kidney after I/R. For gaining insight into potential mechanisms of protection, the activity of caspases within the kidney also was measured, and it was found that caspases-2, -3, and -9 increased in a complement-dependent manner after renal I/R. Apoptotic cells were detected by terminal deoxynucleotidyl transferase catalyzed labeling of DNA fragments, and mice in which the alternative pathway was inhibited demonstrated significantly less apoptosis than control mice. Thus, use of an inhibitory mAb to mouse factor B effectively prevented activation of complement in the kidney after I/R and protected the mice from necrotic and apoptotic injury of the tubules.


The alternative pathway of complement is activated after ischemia/reperfusion (I/R) of the kidneys in rodents and humans (1,2), and complement deficiency protects mice from the full development of ischemic acute renal failure (ARF) (2,3). Complement activation by the classical and lectin pathways has been demonstrated after I/R of the heart (4), intestine (5,6), and skeletal muscle (7). Mannose-binding lectin is deposited in the postischemic kidney (8), perhaps indicating activation of this pathway. Complement activation after I/R of the kidney is critically dependent on an intact alternative pathway (2,3), however, and does not require the protein C4 (3). Selective inhibition of the alternative pathway, therefore, should confer protection against the development of ischemic ARF while leaving the classical and lectin pathways intact.

Renal I/R results in both epithelial cell necrosis and apoptosis (9). Although necrosis is often the more prominent morphologic finding, inhibition of apoptosis has been shown to protect mice from ischemic ARF (10). The caspases are a group of cysteine proteases that execute many of the processes that are necessary for cellular apoptosis (11). Studies have demonstrated that complement activation directly activates caspases within renal cells (12) and may also induce caspase activity within other tissues after I/R (13). Furthermore, the use of an anti-C5 antibody (which prevents formation of C5a and the membrane attack complex) has been shown to prevent the development of apoptosis after renal (14) and cardiac (13) I/R. Thus, there is reason to suspect that complement activation in the kidney after I/R mediates the development of apoptosis through the caspases and that this is an important mechanism of complement-mediated injury to the kidney.

We recently developed an inhibitory mAb to mouse factor B, designated 1379, that selectively blocks the alternative pathway of complement (15). This reagent may be of particular therapeutic benefit in injury states, such as renal I/R, that critically depend on an intact alternative pathway for complement activation to proceed. In the current study, we tested the ability of 1379 to prevent activation of complement in the kidney after I/R and to protect mice from ischemic ARF. Caspase activity and apoptosis within the kidneys were also measured to determine whether these are downstream effectors of complement-induced injury after renal I/R.

Materials and Methods

Protocol for Induction of Renal I/R

Male C57BL/6j mice (Jackson Laboratories, Bar Harbor, ME) were used for all experiments. Eight- to 10-wk-old mice that weighed 20 to 25 g received intraperitoneal injections of 1 mg of control mouse polyclonal IgG (Sigma-Aldrich, St. Louis, MO) or 1 mg of 1379 anti-mouse factor B (mouse IgG1,κ), which was generated as de-
scribed previously (15). Two hours later, the mice were anesthetized with 300 μl of 2,2,2-Tribromoethanol (Sigma) injected intraperitoneally. Additional anesthetic (50 μl) was administered as necessary to keep the mice sedated for the entire procedure. Mice were placed on a heating pad to maintain their body temperature during surgery. Laparotomies then were performed, and the renal pedicles were isolated by blunt dissection as described previously (2). The pedicles were clamped with surgical clips (Miltex Instrument Co., Inc., Bethpage, NY) for 24 min, and occlusion of blood flow was confirmed by visual inspection of the kidneys. The kidneys were observed for approximately 1 min to ensure blood reflow, then fascia and skin were sutured with 4-0 silk (United States Surgical, Norwalk, CT). Sham surgery was performed in an identical manner and for the same duration, except that the renal pedicles were not clamped. The mice were volume resuscitated with 0.5 ml of normal saline and kept in an incubator at 29°C to maintain body temperature. After 24 h, the mice were anesthetized, blood was obtained by cardiac puncture, and the kidneys were harvested. All animal procedures were in adherence to the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Serum and Plasma Measurements

Serum urea nitrogen was determined for each mouse using a Beckman Autoanalyzer (Beckman, Fullerton, CA). Plasma C3adesArg (a stable degradation product of C3a) levels were measured by an ELISA according to the manufacturer’s instructions (Cedarlane Laboratories Ltd., ON, Canada). Alternative pathway activity in mouse serum was measured using an in vitro analysis of C3 deposition on zymosan A particles (Sigma-Aldrich) as described previously (15,16). Briefly, 10 μl of serum from each mouse to be tested was incubated with 10⁶ zymosan particles at 37°C for 30 min in a master mix that contained 5 mM MgCl₂ and 10 mM EGTA (to prevent classical pathway activation). C3 deposition on the particles was analyzed by flow cytometry.

Renal Morphology

Sagittal kidney sections were fixed and embedded in paraffin, and 4-µm sections were stained with periodic acid-Schiff (PAS). The sections were evaluated by a renal pathologist (D.L.) in a blinded manner. The cortex and the outer stripe of the outer medulla were assessed for epithelial necrosis, loss of brush border, tubular dilatation, and cast formation. At least 10 fields (×400) were reviewed for each slide, and the percentage of tubules that displayed these findings was determined. Kidney sections were scored as follows on the basis of the percentage of affected tubules: 0, none; 1, <10%; 2, 11 to 25%; 3, 26 to 45%; 4, 46 to 75%; and 5, >75% (2).

In Vitro Assay of Complement-Mediated Tubular Cell Injury

BUMPT cells, a mouse proximal tubular epithelial cell (PTEC) line (17), were provided by Dr. John Schwartz (Boston University, Boston, MA). Cells were grown to confluence in DMEM supplemented with 10% FBS, penicillin-streptomycin, and 0.2 U/ml IFN-γ (Peprotech, Rocky Hill, NJ). After the cells reached confluence, they were changed to 1:1 DMEM/Hams F12 supplemented with transferrin 5 mg/L (Invitrogen Corp., Carlsbad, CA), hydrocortisone 50 nM (Sigma), and insulin 5 mg/L for 2 d. Lactate dehydrogenase (LDH) was measured by the Cytotoxic-ONE assay (Promega, Madison, WI), and the results were read on a Wallac 1420 fluorescence plate reader with an excitation wavelength of 530 nm and an emission wavelength of 590 nm. To induce chemical hypoxia, we incubated cells for 2 h with 1 μM antimycin (Sigma) in DMEM without glucose (18). As a source of complement components, serum from C57BL/6J mice was added to a final concentration of 10%. Cells were treated for 15 min with serum that had been preincubated for 10 min with 40 μg of 1379 or control mouse IgG (Sigma) for each 100 μl of serum or with serum that had been heated to 55°C for 1 h to inactivate complement.

Figure 1. mAb 1379 inhibits complement activation in the kidney after ischemia/reperfusion (I/R). Deposition of C3 along the tubular basement membrane (TBM) was evaluated by immunofluorescence microscopy. Sham-treated mice that received control IgG (A) or 1379 (B) had intermittent deposition of C3 along TBM of tubules in the outer medulla. In mice that received control IgG before I/R (C), there was extensive C3 deposition along damaged tubules after 24 h. Mice that received 1379 before I/R (D) had only sparse patches of C3. (E) Western blot analysis using an antibody to C3 demonstrated that activation of the complement in mouse serum generated an approximately 40-kD activation fragment that was absent in C3−/− serum. This activation fragment of C3 was also present in lysates of kidneys subjected to I/R but was lower in mice treated with mAb 1379. Treatment with 1379 also prevented systemic rise in complement activation fragments. (F) Levels of C3adesArg were measured in plasma of mice after ischemia and 8 h of reperfusion. I/R resulted in a significant increase in systemic levels of C3adesArg. Treatment with 1379, however, prevented this rise in C3adesArg, and levels in the 1379-treated group were not significantly different from those in the sham-treated group. Representative images are shown (A through E); n = 4 for each experimental group. Magnification, ×200 in A through D.
and supernatant then was tested for LDH. Background LDH was measured in unmanipulated cells and serum to be added to the cells and also in cells after full lysis with 9% Triton X-100. The percentage of lysis of cells in each sample was calculated according to the equation:

\[
\% \text{ lysis} = \frac{\text{OD}_{\text{sample}} - \text{OD}_{\text{serum}} - \text{OD}_{\text{background}}}{\text{OD}_{\text{full lysis}} - \text{OD}_{\text{background}}} \times 100
\]

**Immunofluorescence and Detection of Apoptotic Cells**

Kidneys were snap-frozen in OCT compound (Sakura Finetek, U.S.A., Inc., Torrance, CA). Four-micrometer sections were cut with a cryostat and stored at -70°C. The slides later were fixed with acetone and stained with FITC-conjugated anti-mouse C3 (Cappel, Durham, NC) or FITC-conjugated F(ab')2 anti-mouse IgG (Cappel) diluted 1:150 and counterstained with hematoxylin (Vector Laboratories, Inc., Burlingame, CA). Detection of apoptotic cells was performed using a Fluorescein-FragEL DNA fragmentation detection kit (Calbiochem, San Diego, CA) in which deoxynucleotidyl transferase binds to the 3'-OH ends of DNA exposed by apoptotic endonucleases, and sections were counterstained with hematoxylin (Vector Laboratories). Six to 10 high-power fields in the outer stripe of the outer medulla were examined. The area of positive labeling for each field was determined using SlideBook software 4.0 (Intelligent Imaging Innovations, Denver, CO), and the results for each sample were averaged.

**Western Blot Analysis**

Renal tissue was homogenized in RIPA lysis buffer as described elsewhere (19). Homogenates were centrifuged at 14,000 rpm for 15 min at 4°C, and the supernatant was collected. Purified factor B (15), 100 μg of protein lysates, or 2.5 μl of serum from C57BL/6, fB−/− or C3−/− mice (20) were run on 10% Bis-Tris gels, transferred to nitrocellulose membranes, and probed with horseradish peroxidase–conjugated anti-mouse C3 (Cappel) or with anti-mouse factor B (clone 1379). For blots that were probed with the 1379 antibody, the protein then was detected with an horseradish peroxidase–conjugated sheep anti-mouse IgG (Amersham Biosciences, Piscataway, NJ). Because this antibody identifies proteins in mouse serum, fB−/− serum was used to confirm specificity of the identified protein. The proteins were detected with Western Lightening chemiluminescence reagent (PerkinElmer Life Sciences, Boston, MA).

**Caspase Measurement**

The activity of caspases-2, -3, -8, and -9 were determined by using fluorescence substrates as described previously (21,22). Briefly, whole kidney was mixed with lysis buffer and homogenized with 10 strokes in a glass-Teflon homogenizer. The lysate then was centrifuged at 4°C at 100,000 g in a Beckman Ti70 rotor for 1 h. The resultant supernatants (cytosolic extracts) immediately were aliquoted, frozen in liquid N2 and stored at -70°C. Lysate protein was measured by the Bradford method with BSA as standards. Next, 200 μg of protein extract (20 to 50 ml of lysate) was mixed with 10 μl of the substrate (final concentration, 50 μM). The assay volume was made up to 200 μl with assay buffer, and the reaction was initiated by addition of substrate. Peptide cleavage was measured over 1 h at 30°C using a Cytofluor 4000 series fluorescence plate reader (Perceptive Biosystems, Framingham, MA) at an excitation wavelength...
of 380 nm and an emission wavelength of 460 nm. A 7-amido-4-methyl coumarin (AMC) standard curve was determined for each experiment. Caspase activity was expressed in nanomoles of AMC released per minute of incubation time per milligram of lysate protein.

**Statistical Analyses**

Multiple group comparisons were performed using ANOVA with post test according to Student-Newman-Keuls. Comparison between the control- and 1379-treated groups was performed with unpaired t testing. P < 0.05 was considered statistically significant. Results are reported as mean ± SEM.

**Results**

**Treatment with 1379 Prevents Activation of Complement in the Kidney after I/R**

Mice were treated with 1 mg of control mouse IgG or 1379 and then subjected to renal I/R. Similar to previous studies (2), sham-treated mice (Figure 1, A and B) demonstrated intermittent deposition of C3 along the tubular basement membrane (TBM), and mice that were subjected to I/R demonstrated extensive deposition of C3 along the TBM of tubules in the outer medulla (Figure 1C). We did not see C3 deposition in the large vessels or peritubular capillaries. Pretreatment with 1379, however, prevented the deposition of C3 along the TBM after I/R (Figure 1D). Western blot analysis demonstrated that activation of complement in serum using zymosan generated an approximately 40-kD C3 fragment that was minimally present in serum that contained 10 mM EDTA (which prevents complement activation) and absent in serum from C3−/− mice (Figure 1E). In kidney lysates, the approximately 40-kD C3 degradation fragment was present at baseline and increased after renal I/R. Treatment with 1379 prevented formation of this fragment, however, further demonstrating that 1379 prevented complement activation within the kidney after I/R.

Renal I/R resulted in a significant increase in the level of C3adesArg in plasma after 8 h of reperfusion (874 ± 343 versus 45 ± 13 ng/ml in sham-treated animals; P < 0.05; Figure 1F). By 24 h of reperfusion, the plasma C3adesArg levels had returned to baseline (data not shown). Treatment with 1379, however, prevented the rise in plasma C3adesArg at 8 h of reperfusion, and the levels were not significantly different from those in sham-treated animals (96 ± 37 versus 45 ± 13 ng/ml in sham-treated animals; NS).

**Treatment with 1379 Protects Mice from Ischemic ARF**

Treatment with 1379 attenuated the functional and morphologic injury of the kidney after I/R. The normal serum urea nitrogen (SUN) for unmanipulated C57BL/6J mice is 19 ± 1 ng/ml in sham-treated animals. After ischemia and 24 h of reperfusion, the 1379 treated animals had significantly lower SUN than mice that had received control IgG (78 ± 16 versus 119 ± 15 mg/dl, P < 0.05; Figure 2), although SUN in this group still were significantly higher than in sham-treated animals (22.5 ± 2.6 for the sham group that received control IgG; P < 0.05). Treatment with 1379 also ameliorated the morphologic damage seen after renal I/R (Figure 3). We previously found that this duration of ischemia causes reversible renal injury that peaks after 24 h of reperfusion and that mice recover within 2 to 3 d (19). When graded after 24 h of reperfusion by a pathologist in a blinded manner, the control-treated mice demonstrated extensive tubular injury in the outer stripe of the outer medulla (Figure 3D), and the damage was significantly milder in mice that had received 1379.

**Alternative Pathway Was Fully Inhibited in Serum of Mice that Received 1379**

Kidney sections from mice that were treated with control IgG or 1379 were examined for bound IgG by immunofluorescence. In mice that received 1379 before sham surgery (Figure 4B) and renal I/R (Figure 4C), IgG was detectable in the glomeruli but could not be seen in the tubulointerstitium. In unmanipulated mice that received 1379, the alternative pathway was measured using an in vitro assay of alternative pathway activity (16) and found to be inhibited for at least 24 h after injection (data not shown), and activity was fully suppressed in mice that received 1379 before I/R (Figure 4D). Control mice that were subjected to I/R had reduced alternative pathway activity compared with serum from unmanipulated mice (42 ± 6 versus 100 ± 4%; P < 0.001). This
likely is due to consumption of alternative pathway components in the kidney (as evidenced by increased C3a in the plasma), and previous studies have shown decreased hemolytic activity in the serum of mice after I/R (14). Factor B levels in the serum of 1379-treated mice did not detectably decrease by Western blot analysis (Figure 4E), however, indicating that 1379 inhibits factor B but is nondepleting.

Treatment with 1379 Protects PTEC from Complement-Mediated Cytotoxicity after Chemical Hypoxia

Complement activation directly can cause cytotoxicity, or it indirectly can contribute to injury through inflammatory or vasoactive effects. Immortalized murine PTEC were grown in culture and exposed to whole mouse serum, and LDH release into the supernatant was measured as a marker of cell injury. The LDH readings were corrected for background levels that already were present in the medium and serum that were added to the cells. We have found that the addition of serum to the cells does not cause a significant release of LDH by the cells (after correcting for the LDH present in the serum). When the cells were exposed to antimycin A to induce chemical hypoxia before incubation with serum, however, LDH release rose significantly (Figure 5). This increase was significantly attenuated when the serum was treated with 1379 before incubation with the cells. We believe that hypoxia may alter PTEC expression of the complement inhibitor Crry, rendering the cells susceptible to alternative pathway–mediated injury. Thus, chemical hypoxia and the alternative pathway act synergistically to injure epithelial cells. Furthermore, the cytotoxicity is a direct result of alternative pathway activation and can occur independent of inflammatory cells or altered hemodynamics.

Treatment with 1379 Attenuates Activity of Caspases-2, -3, and -9 in the Kidney after I/R

We next assessed the effect of alternative pathway inhibition on caspase activity within the kidney. I/R induced an increase in the activity of caspases-2, -3, and -9 (Figure 6), and the activity of these caspases was significantly lower in the group that received 1379 compared with the group that received control IgG. Caspase-8 activity did not increase after I/R (Figure 6), and complement inhibition did not have an effect on caspase-1 activity (data not shown). To determine whether treatment with 1379 altered the development of apoptosis after I/R, kidney sections from mice that were subjected to I/R was evaluated by transferase-mediated

Figure 4. 1379 inhibits circulating factor B but is not detectable in the tubulointerstitium. Immunofluorescence for mouse IgG was performed using kidneys of sham-treated mice that received control IgG (A) and 1379 (B) and mice that received 1379 before undergoing I/R (C). Mice that received 1379 demonstrated IgG in the glomeruli (arrowheads), but IgG was not detectable in the tubulointerstitium. (D) Alternative pathway activity was measured in the serum of mice after ischemia (or sham surgery) and 24 h of reperfusion. Alternative pathway activity was significantly lower in mice subjected to I/R than in sham-treated controls. Mice that received 1379 had nearly undetectable alternative pathway activity, significantly lower than in either other group. (E) Western blot analysis for factor B in the serum of mice treated with 1379 showed that the levels of circulating factor B (approximately 90 kD) did not decrease during the period of inhibition. Purified factor B and serum from an fB−/− mouse were used to confirm that the middle band shown for the serum samples was factor B. Representative images are shown (A through C); n = 4 for each experimental group. Serum from two mice was tested at each time point for E, as shown.
Figure 5. 1379 protects proximal tubular epithelial cells (PTEC) from autologous injury by the alternative pathway after chemical hypoxia. Cells were subjected to chemical hypoxia with 1 μM antimycin A and exposed to mouse serum with or without the mAb 1379 or to heat-inactivated serum (HIS). Lactate dehydrogenase was measured as a marker of cell lysis. The percentage of cells lysed in each group was calculated in relation to full lysis of the cells using Triton X-100 (see Materials and Methods). Exposure to hypoxia and mouse serum resulted in a significantly greater percentage of lysed cells than exposure to serum alone. Treatment with 1379 or HIS, however, resulted in significantly less cell lysis than treatment with control IgG.

dUTP nick-end labeling (TUNEL) staining. Apoptosis was not a prominent finding in either group after 8 h of reperfusion. After 24 h of reperfusion, however, TUNEL-positive nuclei could be seen in both groups (Figure 7), but apoptosis was significantly more extensive in the control group than in the group that had received 1379. Activation of the alternative complement pathway after renal I/R, therefore, seems to mediate caspase activation and the development of epithelial apoptosis.

Discussion

We have demonstrated that an inhibitory mAb to mouse factor B prevents activation of the alternative pathway in the kidney after I/R. Treatment with 1379 before I/R almost completely prevented the deposition of C3 along the TBM and generation of systemic C3adesArg (which may mediate systemic effects after local injury). This antibody therefore is capable of preventing the generation of local and systemic complement activation fragments after renal I/R.

Treatment of mice with 1379 ameliorated the functional and morphologic renal injury that is caused by I/R. Treatment with 1379 significantly attenuated the rise in SUN and morphologic injury seen at 24 h, although the protection was only partial. Although complement antagonists have been shown previously to ameliorate ischemic ARF (14,23,24), those agents nonselectively block the function of downstream activation fragments that are generated by any activation pathway. The mAb used in our study is a specific and potent inhibitor of the alternative complement pathway, and its effects can be presumed to be due to inhibition of this pathway.

Using an in vitro model of chemical hypoxia, we also showed that an intact alternative pathway acts synergistically to amplify the hypoxic injury to tubular epithelial cells. Thus, in the setting of hypoxia, an intact alternative pathway is harmful directly to renal tubular epithelial cells and does not require inflammatory cells to cause injury. Renal tubular epithelial cells have membrane-bound complement inhibitors on their surface. We recently showed that I/R alters the polarized expression of these inhibitors and permits alternative pathway activation to occur (19). The in vitro studies presented here further support the hypothesis that I/R alters the mechanisms whereby renal tubular epithelial cells protect themselves from autologous alternative pathway activation. These results are also consistent with previous studies that demonstrated that injury to renal tubular epithelial cells caused them to become complement activating (25,26).

Several studies have shown that the caspases are important mediators of ischemic ARF (10,22,27–29), and the complement system has been shown previously to induce apoptosis of rat mesangial cells via caspase-3 (12). Previous studies, however, have not determined specifically whether proapoptotic caspase activity is a mechanism of complement-mediated injury to the kidney after I/R. We measured the activity of several caspases in the kidneys of mice, and caspases-2, -3, and -9 increased in a complement-dependent manner in postischemic kidneys.

Two pathways of caspase-3–mediated apoptosis have been described. The “death receptor” pathway involves signaling through cell surface receptors. The mitochondrial pathway, in contrast, is initiated by caspase-2–mediated permeabilization of mitochondria and the release of cytochrome C or by changes in the Bcl-1 family proteins (30). This pathway is activated in response to intracellular stress, including oxidative injury, and catalyzes the conversion of pro–caspase-9 to its active form (9). Treatment with 1379 also significantly reduced the extent of TUNEL-positive staining seen in the outer medulla after I/R. This method of detection may not always discriminate apoptotic cells from necrotic or sublethally injured cells (9). Nevertheless, the reduction in caspase-2, -3, and -9 activity and the decreased TUNEL-positive staining that was seen in mice that received 1379 suggest that inhibition of the alternative pathway prevented apoptosis, perhaps via the mitochondrial pathway.

A previous study in which mice were treated with soluble Crry-Ig (a complement inhibitor that works at the level of the C3 and C5 convertases) did not show significant protection with use of the inhibitor (31). Both groups of mice in that
study received heparin, however perhaps diluting any benefit conferred by the Crry-Ig because heparin is an effective complement inhibitor (32). C3−/− mice were protected compared with C3+/+ controls, suggesting that despite the heparin, some of the injury was complement mediated. It is possible that the Crry-Ig, which is a fairly large molecule, was restricted to the intravascular space. Because Crry must be present at the location of the C3 and C5 convertases, tissue penetration is critical to its function. The mAb 1379 may bind factor B intravascularly, however, and thereby deprive the tubulointerstitium of this factor even without penetration of the tissue. Nevertheless, treatment with 1379 did not seem to confer protection equivalent to that seen previously in fB−/− mice in which the increase in SUN and the acute tubular necrosis scores were approximately half those seen in wild-type mice (2). This suggests that the antibody did not prevent all of the complement-mediated injury, perhaps as a result of its inability to penetrate the tubulointerstitium and inhibit locally produced factor B (Figure 4). Furthermore, given the incomplete protection seen in both of these studies, there likely are complement-independent pathways of injury. Determining which pathways of injury are independent of the alternative pathway may help in devising combinations of agents that can be expected to confer additive protection.

**Conclusion**

Complement inhibitors have proved protective in models of I/R injury to the heart (4) and intestine (5). Because complement activation after renal I/R critically depends on the function of factor B (2), we tested an inhibitory mAb to factor B in a model of ischemic ARF. Pretreatment with this agent prevented complement activation and protected mice from the development of functional and morphologic renal injury after I/R. Alternative pathway inhibition reduced the necrotic injury of hypoxic PTEC *in vivo* and *in vitro*. It is interesting that alternative pathway inhibition also reduced apoptotic injury of the tubules, and complement-induced apoptosis seems to involve the mitochondrial pathway. Alternative pathway activation is an early event after renal I/R, and treatment with 1379 may be an effective therapeutic agent for the prevention of ischemic ARF.

**Acknowledgments**

This work was supported by National Institutes of Health grants AI31105 (V.M.H.), DK064790-02 (J.M.T.), and DK56851 (C.L.E.), as well as a postdoctoral fellowship from the International Society of Nephrology (D.L.).

This work was presented in part in abstract form at the annual meeting of the American Society of Nephrology; St. Louis, MO; October 27 to November 1, 2003.
References


14. Quigg RJ, Kozono Y, Berthiaume D, Lim A, Salant DJ, Weinfield A, Griffin P, Kremmer E, Holers VM: Blockade of antibody-induced glomerulonephritis with Crry-Ig, a...


