Preventing Renal Ischemia–Reperfusion Injury Using Small Interfering RNA by Targeting Complement 3 Gene


The complement system is one of the important mediators of renal ischemia–reperfusion injury (IRI). We hypothesized that efficient silencing of C3, which is the central component on which all complement activation pathways converge, could be achieved using small interfering RNA (siRNA), and that this would result in overall inhibition of complement activation, thereby preventing IRI in kidneys. A series of experiments was conducted, using a mouse model of IRI and vector-delivered C3-specific siRNA. We demonstrated the following: (1) renal expression of C3 increases as a result of IRI; (2) by incorporation into a pRNAT U6.1 vector, siRNA can be delivered to renal cells in vivo; (3) systemically delivered siRNA is effective in reducing the expression of C3 in an experimentally induced mouse kidney model of IRI; (4) similarly, siRNA reduces complement-mediated IRI-related effects, both in terms of renal injury (as evidenced by renal function and histopathology examination) and mouse mortality; and (5) silencing the production of C3 diminishes in vivo production of TNF-α. This study implies that siRNA represents a novel approach to preventing IRI in kidneys and might be used in a variety of clinical settings, including transplantation and acute tubular necrosis.

Key words: Complement 3, gene silencing, kidney, ischemia–reperfusion injury, siRNA

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Introduction

Renal ischemia–reperfusion injury (IRI) is the primary cause of acute renal failure, both in native kidneys and in allografts, and is of major importance in renal graft survival post-transplantation (1). As acute renal failure virtually always requires hospitalization, it represents a huge health care cost. In addition, ischemic acute renal failure, conventionally referred to as acute tubular necrosis (ATN) (2), has a high mortality rate, approaching an average of 50% in some series (3). Although the pathogenesis of human ATN is complex and incompletely understood, recent studies in animals have demonstrated a pivotal role for the complement system in mediating renal IRI (4–6). There appear to be two primary complement pathways in the development of IRI, the classical pathway and the alternative pathway, both dependent on complement activation (7–11). The kidney is unique in organ IRI and complement, since activation subsequent to IRI occurs only via the alternative pathway (12). Both anaphylatoxin (C3a, C5a)-dependent and membrane attack complex (MAC)-dependent mechanisms have been proposed as a means by which the complement cascade induces tissue injury in animal models of renal IRI (4–6, 13, 14). The kidney represents a major extra-hepatic site for the synthesis of the third complement component (C3), and therefore, has an important pro-inflammatory role in renal transplantation. Rejecting grafts have been shown to contribute substantially to circulating levels of C3, and renal C3 has been demonstrated to participate in T-cell priming and enhancement of rejection injury as well as IRI (15).

In animal models, renal IRI can be abrogated by treatment with complement inhibitors, such as anti-C5 antibodies and C5a receptor antagonists (16, 17), or by genetic manipulation of C3 in knockout mice (15). However, there is no clinically accepted therapy that will ameliorate or prevent cellular injury after renal ischemia (2, 18, 19).

Small interfering RNA (siRNA) is a powerful tool to silence gene expression in mammalian cells. We previously have used siRNA successfully to silence immune-associated genes, thereby inducing immune tolerance in the setting of transplantation (20–22). Recent studies showed that systemic administration of siRNA can prevent local gene expression (23). For example, systemic application of caspase-8 siRNA inhibits caspase-8 expression in mouse
liver and is capable of preventing Fas (CD95)-mediated apoptosis of hepatocytes (24). Intraperitoneal administration of siRNA caspase-8 and caspase-3 inhibits the expression of caspase-8 and caspase-3 and attenuates warm IRI to the liver (25). siRNA targeting Fas has been shown to protect mice against renal IRI (26). However, preventing renal IRI through silencing of complement genes using siRNA has not been reported.

In this study, we report for the first time that efficient silencing of C3, the central component of the complement activation cascade, can be achieved using siRNA; moreover, this results in the inhibition of complement activation and prevents renal IRI.

Materials and Methods

Mice
CD1 mice were purchased from The Jackson Laboratory (Bar Harbor, ME, USA). The mice were maintained under specific pathogen-free conditions. All mice were male and 6- to 10-week old. All experiments were performed in accordance with the Guide for the Care and Use of Animals Committee Guidelines.

C3-siRNA design
Three target sequences of C3 gene were selected. The oligonucleotides containing sequences specific for C3 (5′-GATCCGGCTTTTTAGGAAGTTTGGACCATGACAGG-3′ and 5′-AGCTTTGGAACAAACATCTTTAGGAAGTTTGACTTCCTTGAACAGTGGACAGAGGTGTCAGTGACTTCCT-3′ and 5′-GGACCATAGAAGAGTTGAATTCTTCAAGAGACTGTGCAAGACTTCCTAAAGATTTTTTCCAAA-3′) were synthesized and annealed. A C3-siRNA expression vector that expresses hairpin siRNAs under the control of the mouse U6 promoter and cGFP genes was constructed by inserting pairs of annealed DNA oligonucleotides into a pRNAT-U6.1/Neo siRNA expression vector that had been digested with BamHI and HindIII (Genescript, Piscataway, NJ, USA).

In vitro silencing of the C3 gene
Macrophage cells were transfected with C3-siRNA using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). The vehicle alone and scrambled (non-sense) siRNA were used as negative controls. Briefly, cells were plated into 24-well plates (1 × 10⁵ cells per well) and allowed to grow overnight, to reach 90% confluency. Cells were transfected with 2 μg C3-siRNA or negative control siRNA plasmids in serum-reduced medium for 5 h, then incubated in complete medium for 24 h. All RNAs were prepared for subsequent analysis.

Renal IRI model
CD1 mice, aged 6-8 weeks, were anesthetized with an intraperitoneal injection of ketamine (100 mg/kg) and xylazine (20 mg/kg) and placed on a heating pad to maintain their body temperature during surgery. Following abdominal incisions, renal pedicles were bluntly dissected, and a microvascular clamp (Roboz Surgical Instrument, Garthersburg, MD, USA) was placed on the left renal pedicle for 25 or 30 min. During the procedure, animals were kept well hydrated with warm saline and at a constant temperature (37°C). After ischemia, the clamps were removed. The right kidney was removed. Thereafter, incisions were sutured, and the animals were allowed to recover, with free access to food and water. Blood was collected and the left kidney was recovered for analysis, 24 h after reperfusion.

Assessment of renal function
Blood samples were obtained from the inferior vena cava at 24 h post-ischemia. Serum creatinine levels were measured by the core laboratory at the London Health Sciences Center to monitor renal function. Blood urea nitrogen (BUN) also was measured.

Histology and neutrophil detection
At 24 h post-ischemia, kidneys were dissected from mice and tissue slices were fixed in 10% Formalin and processed for histology examination using standard techniques. Formalin tissue was embedded in paraffin and 5-μm sections were stained with H&E. These sections were examined in a blinded fashion by a pathologist. The percentage of histology changes in the cortex and medulla were scored using a semi-quantitative scale designed to evaluate the degree of infarction, tubular vacuolization and cast formation on a 5-point scale based on injury area of involvement as follows: 0, <10%; 1, 10–25%; 2, 25–50%; 3, 50–75% and 4, 75–100%. A pathologist quantitatively assessed neutrophil infiltration by counting the number of neutrophils per high-powered field (400×) over five fields, then averaging neutrophil numbers.

Immunohistochemistry
Kidneys were snap-frozen in OCT compound (Sakura Finetek, Torrance, CA, USA) and stored at –80°C. Five-micrometer frozen sections were cut with cryostat. Sections were fixed in cold acetone for 10 min and allowed to air-dry. Sections then were blocked with 10% normal horse serum in PBS. The primary antibody rabbit anti-mouse C3 (Hycult Biotechnology, Uden, The Netherlands), rabbit anti-rat C9 (kindly gifted by Dr. P. Morgan, University of Wales College of Medicine, Cardiff, UK), which cross-reacts with mouse C9 (27) or rabbit anti-mouse TNF-α (Santa Cruz Biotechnology, CA, USA) was added to the sections for 1 h at room temperature. Sections were then rinsed with PBS and treated with a ready-to-use peroxidase blocking solution (DakoCytomation, Carpinteria, CA, USA). After washing with PBS, the slides were incubated with rabbit EnVision+ HRP (DakoCytomation) for 30 min. Sections were washed and developed with ready-to-use DAB for 1–3 min and counterstained with hematoxylin (DakoCytomation). The semi-quantitative method was applied to measure the C3-positive staining. Iso-type sera and omitting primary antibody were used as negative controls.

Measurement of renal C3 mRNA levels by quantitative real-time PCR
Total RNA was extracted from kidneys and cells using Trizol (Invitrogen). Total RNA was reverse-transcribed using oligo-dT primer and reverse transcriptase (Invitrogen). Real-time PCR reactions were performed to examine gene expression in Stratagene MX 4000 multiplex quantitative PCR system using SYBR Green PCR Master mix (Stratagene, La Jolla, CA, USA) and 100 nM of gene-specific forward and reverse primers. Primers used for the amplification of murine C3, TNF-α and GAPDH were as follows: C3, 5′-CCCTGCGCCCTTACCCCTTCATCCTGTTGATGACATCAAGAAGGTGGTGAA-3′ (forward) and 5′-ATCACCCCGAAGTTCAGTAGACAG-3′ (reverse); TNF-α, 5′-CTCCCTCCCGAAAGACACATTATGTGAGGGAGAT-3′ (forward) and 5′-GATCCGGCTTTTTAGGAAGTTTGGACCATGACAGG-3′ (reverse); and GAPDH, 5′-TGATGACATCAAGAAGGTGGTGAA-3′ (forward) and 5′-TGATGACATCAAGAAGGTGGTGAA-3′ (reverse). The reaction conditions were 10 min at 95°C, 15 s at 95°C, 1 min at 58°C and 1 min at 72°C (40 cycles). Samples were normalized using the housekeeping gene GAPDH, and a comparative CT method was used for the analysis.

In vivo siRNA administration and siRNA tracking
Fifty micrograms of C3-siRNA plasmid DNA were diluted in 1000 μL of PBS and injected into mice by tail vein. Forty-eight hours after siRNA
injection, tissues were collected, frozen in OCT compound (Sakura Finetek) and stored at \(-80^\circ \text{C}\). Kidney tissue sections (5 \( \mu \text{m} \)) were prepared on a cryostat and examined for GFP gene expression under a fluorescent microscope.

Statistical analysis
Data are expressed as means \( \pm \) SEM. Statistical comparisons between groups were performed using Student’s t-test. Histopathology data were analyzed using chi-square. Mortality was plotted on a standard survival curve and group differences compared using Fisher’s exact test. Statistical significance was determined as \( p < 0.05 \).

Results

The C3 is widely expressed in the kidney and upregulated after IRI
As proof of principle, we first detected the expression of C3 in a kidney IRI model. Renal expression of C3 was determined both at the level of mRNA, using quantitative real-time PCR, and at the protein level by immunohistochemical staining. In the kidneys of healthy control mice, high expression of C3 transcript was detected. After IRI, C3 mRNA levels were significantly elevated up to 8.4 \( \pm \) 1.2-fold over baseline levels (Figure 1A). Immunohistochemical staining for C3 in healthy kidney tissue revealed faint expression on tubular epithelial cells with more intense staining of mesangial cells (Figure 1B). It was apparent that, 24 h after reperfusion following IRI, C3 expression in ischemic kidneys was upregulated on tubular epithelial cells, with C3 localized to cellular debris and injured tubular epithelial cells. These data are in agreement with the previous reports, in which C3 was shown to be upregulated in kidney after suffering a longer period of IRI (14).

Silencing C3 in vitro using siRNA
We have successfully silenced other genes using synthesized siRNA duplicates (20). In this study, we designed siRNA expression vectors to provide robust expression for in vivo studies within kidneys. To determine whether C3 hairpin siRNA could inhibit the C3 expression, we first transfected C3-siRNA into a macrophage line, which constitutively express high levels of C3, with three different sequences of C3-specific siRNAs. The silencing efficiency was assessed at the level of mRNA using real-time PCR. Figure 2 demonstrates the results of three different sequences of siRNA (sequences 1–3) and their effects on C3 expression in C3-siRNA-treated versus empty-vector-treated macrophages. Results show that sequence 1 of C3-siRNA displayed the most potent silencing efficacy.

Figure 1: Expression of C3 in murine (CD1) kidney tissue. (A) C3 expression detected by quantitative real-time PCR. Left kidney was subjected to clamping, as described in Materials and Methods. Twenty-four hours after clamping, kidney was recovered and total RNA was extracted. Transcriptions were amplified using primers specific to C3 and GAPDH genes. Healthy kidneys were used as normal controls. Data are expressed as mean \( \pm \) SEM; statistical significance when compared with normal control, \( *p < 0.05 \). (B) C3 expression detected by immunohistochemical staining. Kidney tissues of clamped and healthy (normal control) mice were frozen and sectioned. Samples were stained with anti-C3 Ab. Data shown represent experiments performed on six animals per group.
Cell lines were cultured in vitro and were transfected with three different sequences of C3-specific siRNA using Lipofectamine 2000. Twenty-four hours after siRNA transfection, cells were recovered to extract total RNA. Transcripts of C3 and GAPDH from silenced and nonsilenced cells were determined using quantitative real-time PCR. Vector was as calibrator sample, C3 gene was normalized by GAPDH, a housekeeper gene. Relative quantity of C3 mRNA was expressed as mean ± SEM. Statistical significance when compared with sequence 1 versus vector (*) was denoted at p < 0.05.

Silencing C3 in vivo using siRNA

In vivo gene silencing in kidney had been successfully achieved by a ‘hydrodynamic’ injection (28). We accordingly delivered C3-siRNA to kidneys using the same method. To track the distribution of C3-siRNA, we cloned hairpin siRNA into a GFP expression vector, pRNAT U6.1. When the vectors were transduced into renal cells, the GFP reporter gene could be tracked by its green fluorescence, so as to assess the efficiency of systemically administered siRNA delivery to renal cells. Forty-eight hours after C3-siRNA vector injection, we examined kidneys for siRNA delivery and distribution by screening for green fluorescence. Compared to control kidneys, green fluorescence was clearly enhanced in tubular cells of siRNA-treated kidneys (Figure 3A). In contrast, green fluorescence was not observed in PBS-injected control mice, demonstrating that siRNA in this vector can gain access to renal cells.

Upon confirmation that siRNA can be delivered to kidney tissue through systemic administration of C3-siRNA, we next investigated in vivo silencing efficacy. Mice (6 mice for each group) were treated with 50 μg C3-siRNA, PBS or vector, 48 h prior to IRI induction. The expression of C3 in kidneys was detected by real-time PCR and was markedly suppressed after treatment with C3-siRNA (p < 0.05 vs. PBS-treated, vector-treated groups) (Figure 3B). Inhibition of C3 also was observed by immunohistochemical staining with the reduction of C3 deposition in kidneys from siRNA-injected mice (Figure 3C).

To confirm that silencing C3 would attenuate the complement activation cascade, we examined the presence of the C9 component. C9 deposition in kidney was detected using immunohistochemistry (27). As shown in Figure 3D, C9 deposition in C3-siRNA-treated kidneys was significantly decreased when compared to controls.

Blocking the C3 pathway attenuates IRI-induced renal dysfunction

Given that siRNA appeared to block the expression of C3 and, hence, the complement pathways in mice exposed to IRI, we hypothesized that siRNA also would prevent renal injury caused by complement in ischemia–reperfusion (IR). In siRNA-treated versus empty-vector-treated or PBS-treated mice (n = 8 per group), we measured BUN and serum creatinine levels 24 h after reperfusion, to assess the degree of renal dysfunction. We first tested siRNA protection in a reversible model of IRI, which minimizes vascular thrombosis and avoids animal mortality. After clamping for 25 min, the levels of both BUN (Figure 4A) and creatinine (Figure 4B) significantly increased when compared to unclamped controls. However, treatment with C3-siRNA, prior to inducing IRI, significantly prevented increase of BUN and serum creatinine values 24 h after IRI injury.

We next tested the capacity of C3-siRNA in protection in a severe IRI model by extending the duration of ischemia to 30 min. After 30 min IRI, more severe kidney injuries were observed, as evidenced by the levels of BUN (Figure 4C) and creatinine (Figure 4D), which are much higher than that in 25 min IRI, and caused mortalities (Figure 5). The treatment of C3-siRNA prevented the abrupt increase of BUN and creatinine caused by severe IRI (Figure 4C and 4D). Hence, treatment with C3-siRNA significantly attenuated IRI-induced renal injury.

C3-siRNA protects against lethal kidney ischemia

The above results showed that C3-siRNA treatment prevented renal dysfunction. We therefore evaluated the survival of treated versus vector-treated or PBS-treated mice following 30 min of ischemia. The severe ischemia was selected to clearly delineate survival differences in the siRNA treatment group. Since most mice with renal failure die by the fourth day, long-term survival was defined as surviving for the past 8 days. As can be seen in Figure 5, siRNA injection improved survival after IRI. Of 10 siRNA-treated mice, 7 survived till the eighth day, compared to 2 of 10 in both PBS-treated control mice or vector-treated control mice (p < 0.001 vs. PBS and vector control).
Preventing I/R Injury by Silencing C3

Figure 3: Silencing C3 in vivo. (A) Tracking siRNA in kidney. Fifty micrograms of pRNAT U6.1 vector that contains C3-siRNA and a GFP report gene were injected i.v. into CD1 mice, as described in Materials and Methods. Forty-eight hours after injection of PBS (a & c) or siRNA vector (b & d), kidneys were recovered and sectioned. The distribution of siRNA vectors was tracked by green fluorescence using fluorescence microscopy. (a & b): Bright-field phase images. (c & d): GFP fluorescence images. (B) Gene silencing in kidney was detected by real-time PCR. Forty-eight hours after gene silencing, kidneys were clamped for IRI. Twenty-four hours after reperfusion, kidney tissues were recovered and total RNAs were extracted. Transcripts of C3 and GAPDH were determined by quantitative real-time PCR. C3 expression was compared among healthy (unclamped) mice, clamped but not silenced mice, vector-treated and clamped mice and silenced and clamped mice (n = 6, per group). Relative quantity of C3 mRNA was expressed as mean ± SEM. Statistical significance when compared with controls (*) was denoted at p < 0.05. (**) at p < 0.001. (C) C3 gene silencing in kidney was detected by immunohistochemistry. Unclamped (a), Non-silenced and clamped (b), vector-treated and clamped (c) and C3-silenced and clamped (d) mice were subjected to kidney IRI. Twenty-four hours after reperfusion, kidneys were recovered and sectioned. The expression of C3 was determined by immunohistochemical staining with antibody against C3. (D) C9 deposit was detected by immunohistochemistry. (a). A small amount of C9 can be seen in unclamped mice. (b) C9 deposit was upregulated in ischemia mice. (c). Vector treatment could not inhibit C9 deposit. (d). C3-siRNA treatment before ischemia can prevent C9 deposit.

Inhibition of C3 prevents IRI-induced inflammatory response

We then examined the kidneys for evidence of histopathology changes of IRI, comparing kidneys in (1) healthy, unclamped mice; (2) mice exposed to IRI but untreated with siRNA; (3) mice exposed to IRI and treated with siRNA and (4) mice exposed to IRI and treated with vector. By the blinded observer, kidneys were graded for percentage of total area showing evidence of tubular infarction, vacuolization, cast formation and neutrophil invasion. Renal influx of neutrophils, an important feature of IRI-induced inflammation, was also assessed by counting the number of infiltrating cells present in the tissue sections. As Table 1 presents, compared to healthy kidneys, renal IR induced significant pathological changes, particularly infarction and cast formation, tubular vacuolization, edema and neutrophil infiltration. In contrast, mice pre-treated with C3-siRNA demonstrated significant attenuation of all pathological
changes except for tubular vacuolization, which remained unchanged. Remarkably, neutrophil infiltration was almost entirely eliminated as well as edema and cast formation. Infarction was significantly reduced but still involved approximately 25% of the renal tissue (Table 1).

TNF is an important cytokine associated with IRI, which can magnify injury directly and by neutrophil attraction. Complement may or may not upregulate TNF expression in different models. To further understand whether complement activation is responsible for the generation of
Figure 4: siRNA reduces the effect of renal ischemia and reperfusion on renal function. Renal pedicles were clamped for 25 min (A & B) or 30 min (C & D). Blood was collected from unclamped healthy mice or mice with IRI, treated with PBS, vectors or C3-siRNA. BUN (A & C) and serum creatinine (B & D) were determined as described in Materials and Methods. Data shown are mean ± SEM (*p < 0.05, C3-siRNA-treated vs. untreated and clamped mice, **p < 0.001, C3-siRNA-treated vs. vector-treated and clamped mice (n = 8, per group).

Figure 5: siRNA protects against lethal kidney ischemia. CD1 mice were i.v. treated with 50 µg C3-siRNA vectors following clamping for 30 min. Survival of C3-siRNA-treated (n = 10), non-treated control (n = 10) and control-vector-treated (n = 10) mice was observed over 8 days. (p < 0.05, C3-siRNA-treated vs. PBS-treated and clamped mice, vector-treated and clamped mice).

Discussion

It has been documented that complement is associated with IRI (14); however, effective therapy of IRI by blocking a specific component of the complement activation cascade has not been reported. In this study, we have demonstrated that silencing the C3 gene using siRNA can inhibit the renal expression of C3 and that successful delivery of C3-siRNA to the kidneys resulted in the prevention of renal IRI.

Complement is a complex cascade of more than 30 proteins that are activated in a sequential manner. The cascade has three initiating arms, including classical, lectin and alternative pathways, to produce enzymatic complexes, namely C3 and C5 convertases. All three pathways result in C3 activation and merge into a common pathway that results in the formation of the MAC or C5b-9 (29). C3 was selected as a target for gene silencing in this study, since C3 is the central and critical plasma protein of the complement pathway and its inhibition should effectively terminate activation of the downstream complement cascade. Indeed, levels of the C9 component were also significantly decreased in C3-siRNA-treated kidneys, supporting this complement blocking strategy.
IRI was graded using the following criteria: 0, <10% area of injury; 1, 10–25%; 2, 25–50%; 3, 50–75% and 4, 75–100%.

1 Nontreated and nonclamped mice.
2 Clamped with PBS treatment.
3 Clamped with vector treatment.
4 Clamped with C3-siRNA treatment.

*p < 0.05, **p < 0.01, ***p < 0.001.

Table 1: Pathological change of kidney

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<th>Cast formation</th>
<th>Edema</th>
<th>Neutrophil infiltration</th>
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<tr>
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Figure 6: C3-siRNA downregulates TNF-α expression. Forty-eight hours after gene silencing, kidneys (n = 4, per group) were clamped for IRI. Twenty-four hours after reperfusion, kidney tissues were recovered and sectioned. Expression of TNF-α was determined by real-time PCR (A) and immunohistochemistry staining with an antibody against TNF-α (B). (p < 0.05, C3-siRNA-treated vs. untreated and clamped mice, **p < 0.001, C3-siRNA-treated vs. vector-treated and clamped mice). TNF-α expression detected by immunohistochemistry was graded using the following criteria: 0, <10%; 1, 10–25%; 2, 25–50%; 3, 50–75% and 4, 75–100%.

IRI is also associated with the expression of pro-inflammatory cytokines, which can promote further injury. TNF-α is a potent inflammatory cytokine, expressed by renal parenchymal cells as well as mononuclear cells residing within the kidney, and contributes to neutrophil infiltration and kidney injury. Various models of IRI have linked complement and TNF-α expression. Wada et al. (30) showed that inhibition of C5 significantly inhibited local TNF-α expression after intestinal IRI. The role of TNF-α in renal IRI has not been established, as de Vries et al. (17) reported that inhibition of complement activation did not affect the IR-induced upregulation of TNF-α in their renal ischemia model. Our results demonstrating the reduced expression of TNF-α following C3-siRNA silencing suggest that TNF-α is linked to IRI, and while our data do not identify the renal source of TNF-α, the benefit in renal injury that we have demonstrated may have been due in part to attenuation of TNF-α as well as complement. Both renal epithelial and endothelial cells are capable of complement synthesis (31–33), and therefore, kidneys are well positioned to initiate complement activation by means of locally secreted components. Kidneys can express massive amounts of complement, as in the case of rejecting allografts, and can contribute up to 16% of the circulating C3 pool (34). Thus, the consequences of renal C3 expression can be both local and systemic. Using a mouse IRI model, we have demonstrated that the renal expression or deposition of C3 occurs early after reperfusion and is localized to cellular debris and injured tubular epithelial cells consistently, with the view that the complement pathway is crucially involved in renal IRI. This is supported by previous studies showing C3 deposition in rodent kidney after IRI and also in infarcted human kidneys (7,13,35,36). In our present study, we extend the link between renal IRI and complement by demonstrating that specific blocking of C3 expression by siRNA can largely prevent renal IRI and attenuate IRI-induced renal dysfunction. While the role of complement as an important component of renal IRI has been supported by previously published reports (31–33), attempts to block renal IRI have been variably successful. Complement receptor 1-related protein y (Cry) is normally expressed on the proximal tubular epithelium and plays an important role in preventing alternative pathway complement activation. The loss of Cry polarity results in loss of protection from
IRI (12). Heterozygous gene-targeted mice that expressed lower amounts of Cry were more sensitive to ischemic injury (12). However, exogenous Cry-Ig was unable to significantly reduce renal injury in vivo, even in high doses. This may be because Cry-Ig requires precise targeting to specific tubular cell compartments to be effective (41). Antibodies, directed at C5 mAb BB5.1 and C5aR, can inhibit complement activation and ameliorate IRI. However, these antibody treatments could not prevent early stage complement upregulation. Furthermore, these treatments require multiple administrations, which may limit therapeutic attractiveness (17,42). Lysophosphatidic acid (LPA) has been shown to inhibit complement activation and IRI-mediated renal dysfunction to levels similar to what we have shown with siRNA (43). This suggests that LPA may be an alternative strategy, although it is less specific and may interact with other targets that could be important for renal homeostasis if long-term administration is used. siRNA provides an alternative therapy for preventing IRI, which can not only be used in the late stage of IRI, but may also prevent early IRI through an siRNA-containing organ-storage solutions (22).

Presently, there are no clinically accepted therapies that block renal IRI using a specific inhibitor of C3 delivered locally to renal tubular epithelium, the major target during renal ischemia. In this study, C3-siRNA, incorporated into a pRNAT U6.1 vector with a GFP reporter gene to allow tracking, demonstrated efficient silencing of C3 in renal tubular epithelial cells and with preservation of renal function following a severe ischemic insult. This work strongly supports the notion that siRNA may be a potential clinically applicable therapy for renal IRI.

In summary, the data presented here indicate that the systemic administration of C3-specific siRNA is capable of renal cell entry and reducing renal C3 synthesis. These results further clearly demonstrate that the systemic application of C3-siRNA can prevent renal IRI. Consequently, the use of systemically delivered C3-siRNA may represent a novel approach to preventing complement-mediated renal damage and may become therapeutically useful, both in the setting of renal transplantation and in other conditions associated with renal IRI.

Acknowledgments

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References

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