Expression of a Soluble Complement Inhibitor Protects Transgenic Mice from Antibody-Induced Acute Renal Failure

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Abstract. Crry is a potent complement regulator in rodents that inhibits C3 convertases. In rats, intrarenal arterial injection of anti-glomerular endothelial cell (GEN) antibodies leads to complement-dependent microvascular injury and acute renal failure. In this study, a mouse variant of this model and the effects of complement inhibition were examined. Transgenic mice that overexpressed soluble Crry systemically and in their kidneys were studied. Anti-GEN IgG was injected intravenously into eight Crry transgenic mice and seven transgene-negative littermates (which were used as control animals). Thirty h after injection, blood urea nitrogen (BUN) levels were 30.3 ± 4.4 and 114.8 ± 23.5 mg/dl for transgene-positive and -negative animals, respectively (P = 0.012). Four of five transgene-negative animals with BUN levels of >100 mg/dl were anuric; the remaining animal exhibited minimal albuminuria and no detectable urinary C3. In animals with renal failure, glomerular capillary collapse and tubular necrosis were observed. There was significant tubular staining for C3 in transgene-negative animals, with cellular and basal distributions, both of which were statistically greater than those in transgene-positive animals. Tubular cell C3 staining was strongly correlated with BUN values (r = 0.83, P < 0.001), as was C9 staining (r = 0.56, P = 0.037), suggesting that complement activation to the C5b–9 membrane attack complex had a causal role in renal failure. Thus, systemic injection of anti-GEN antibodies into mice leads to acute renal failure, with glomerular and tubular injury. Animals that overexpress soluble Crry in renal tubules and elsewhere are protected from the acute renal failure that occurs in this model, which ultimately seems to develop because of complement activation focused on tubules.

Acute renal failure can occur when there is severe renovascular, glomerular, or tubular injury. Most commonly, renal failure is associated with acute tubular necrosis, which can develop under numerous circumstances, such as sepsis, hypotension, or exposure to nephrotoxins. In contrast to having well described precipitants, the pathogenesis of acute tubular necrosis and the ultimate cause of renal failure remain poorly defined.

Complement activation can proceed via either the classical or alternative pathways. Central to both pathways is the cleavage of C3 and C5, with generation of proinflammatory fragments, such as C3a and C5a, as well as formation of the cell-damaging C5b–9 complex. The complement system is tightly regulated, being constrained in its activation as well as inhibited by a number of regulatory proteins when activation occurs. Beginning with early observations (reviewed in reference 1) that were later extended by Unanue and Dixon (2) and Salant et al. (3), a considerable amount of research has been performed with different experimental glomerular disease models, showing that complement activation is pathogenic (reviewed in reference 4).

In addition to glomerular effects, more recent information has demonstrated that complement activation may lead to tubulointerstitial injury. The circumstances under which this occurs include situations in which complement regulators located on the basal aspects of tubules are inhibited by function-neutralizing antibodies (5,6) and those in which the glomerular barrier to protein passage is impaired (7,8). In the latter setting, complement proteins become accessible to the apical surfaces of the proximal tubule; in such situations, the alternative pathway can be activated (9), because of the lack of C3 convertase regulators at this location (10,11).

A model of renal microvascular injury in which anti-glomerular endothelial cell (GEN) antibodies are injected directly into the renal artery has recently been described (12). Antibody binding to GEN is rapidly followed by complement activation, GEN injury, accumulation of platelets and fibrin in glomeruli, hemolytic anemia, and acute tubular necrosis, features reminiscent of the hemolytic uremic syndrome in human subjects. This renal injury is dependent on formation of the C5b–9 membrane attack complex, inasmuch as C6-deficient PVG rats were protected from disease (13) and inhibition of CD59 (a membrane regulator of C5b–9 formation) exacerbated disease (14).
The mouse protein originally termed p65 (15) was cloned as Crry (CR1-related gene y) (16), and this name has remained in use to signify the mouse and rat proteins (17–19). Mouse Crry has decay-accelerating and factor I cofactor activities toward alternative and classical pathway C3 convertases (17,20,21). We have produced transgenic mice in which Crry is expressed as a soluble protein (22). To accomplish this, the active region of mouse Crry was placed under the control of the broadly active and heavy metal-inducible metallothionein I promoter. These Crry transgenic mice exhibit complement-inhibiting levels of Crry in their sera and local production of Crry in various organs, including high levels of expression in renal tubules and glomeruli (22,23). As a result, these mice are protected from glomerular complement activation and injury in the nephrotoxic serum-induced nephritis (NSN) model. In this study, we examined whether these Crry transgenic mice are protected from injury in a model of acute renal failure induced by the intravenous injection of anti-GEN antibodies.

Materials and Methods

Crry Transgenic Mice

Transgenic mice expressing recombinant soluble Crry directed by the metallothionein I promoter were used in this study. These transgenic mice exhibit circulating levels of soluble Crry that are complement-inhibiting and display high intrinsic renal production of Crry, including in glomeruli and tubules (22). The Crry transgenic animals used in this study were derived from a single founder and have a CD-1 lineage. For all animals, the presence of the Crry transgene was documented by PCR. Soluble Crry in the serum, which is not present in normal mice (22), was confirmed by enzyme-linked immunosorbent assay for every animal. Littermates that lacked the Crry transgene were used as control animals.

Disease Protocol

Anti-GEN antiserum was raised in a single goat, as described previously (supplied by Dr. William Couser, University of Washington, Seattle, WA) (12). IgG was isolated by protein G affinity chromatography (Pharmacia, Piscataway, NJ) under endotoxin-free conditions. As assessed with the Limulus amebocyte lysate assay (Associates of Cape Cod, Falmouth, MA), anti-GEN IgG contained ≤0.3 EU/ml endotoxin. Anti-GEN IgG was injected intravenously into tail veins. Animals were allowed to recover from methoxyflurane anesthesia and disease induction and were then housed in metabolic cages for urine collection. Twenty h after injection, blood was obtained from the retro-orbital venous plexus. Thirty h after injection, blood was obtained by cardiac puncture and urine was collected from the urethral orifice using a Pasteur pipet, with gentle compression of the bladder. Urine samples collected in metabolic cages and those collected directly from the urethra were pooled. Animals were euthanized by cervical dislocation, and renal tissue was processed for the studies described below.

Two doses of anti-GEN were used in these studies. Eight Crry transgenic animals and seven Crry transgene-negative littermates received 2 mg intravenously. For examination of acute renal C3 deposition, seven transgene-positive and nine transgene-negative animals received injections of 2 mg of anti-GEN, followed by kidney collection 10 or 60 min later. In a separate group of studies designed to examine urinary protein excretion, five transgene-positive and five transgene-negative animals received 0.5 mg intravenously.

NSN Studies

The NSN model was used to determine the influence of proteinuria on acute renal failure. The advantages of this model for this study are that it is complement-independent at relatively high doses of NSN antibody and results in massive proteinuria (22,24,25). The basic protocol described above for the anti-GEN disease model was used to induce NSN. For these studies, transgene-positive or -negative mice received injections of either 1, 2, or 4 mg of NSN IgG (supplied by Dr. David Salant, Boston Medical Center).

Localization of Anti-GEN In Vivo

Two approaches were used to determine the localization of anti-GEN in normal mouse kidneys. The first involved standard indirect immunofluorescence (IF) microscopic examination of kidney sections from normal CD-1 mice. Four-micrometer cryostat sections were incubated with anti-GEN IgG, followed by FITC-conjugated antibody to goat IgG (Cappel, Durham, NC), and were viewed with a BX-60 IF microscope (Olympus Optical Co., Tokyo, Japan) (26). Control sections were similarly processed, except that nonimmune goat IgG was used instead of anti-GEN IgG.

The second approach involved injection of four normal CD-1 mice with 7.5 mg of anti-GEN IgG, followed by euthanasia 2 or 10 min later (two at each time point). Renal tissue was processed for IF microscopy for goat IgG and mouse C3 as described below.

Measurement of C3 mRNA Levels by Reverse Transcription-PCR

To evaluate whether injection of anti-GEN IgG affected renal transcription of C3 mRNA (27–29), the following studies were performed. Twelve normal CD-1 mice received intravenous injections of 2 mg of anti-GEN IgG. At various times after injection, animals were euthanized and renal cortical tissue was isolated, from which RNA was extracted and reverse-transcribed to cDNA using oligo(dT) primers (19). PCR for mouse C3 was performed using the following primers designed on the basis of the mouse C3 sequence (GenBank accession number K02782), which span bases 735 to 1234 in mouse C3 and therefore yield a 500-bp product: forward primer, 5'-GAATACGGTGCTGCCAGTTT-3'; reverse primer, 5'-TGATGCACCACACGACATTT-3'. In preliminary studies, reverse transcription-PCR for C3 in normal mouse renal cortex resulted in linear accumulation of product from 20 to 35 cycles. For studies evaluating C3 mRNA accumulation after anti-GEN injection, 23 cycles of PCR were chosen, with the rationale that anti-GEN might lead to upregulated C3 transcription, which could be reliably determined using these limiting cycle numbers. As a control for RNA quantity and integrity, reverse transcription-PCR was also performed for glyceraldehyde-3-phosphate dehydrogenase (GenBank accession number M32599), using the following primers, which yield a 195-bp product: forward primer, 5'-CCATGGAGAAAGCCGGGG-3'; reverse primer, 5'-CAGAATGTGTCATGATGACC-3' (30). Twenty cycles of PCR were found to be in the linear range of product accumulation and were used in these studies. Photographs of ethidium bromide-stained gels were scanned into Tiff files and analyzed by Molecular Analyst software (Bio-Rad Laboratories, Hercules, CA).

Serum and Urine Measurements

Blood urea nitrogen (BUN) and urinary creatinine levels were measured using a Beckman autoanalyzer (Beckman Coulter, Inc., Fullerton, CA). Total bilirubin levels in serum were measured using a kit from Sigma Diagnostics (St. Louis, MO), according to the instructions provided. Urinary excretion of mouse C3 and albumin was measured using a Beckman autoanalyzer (Beckman Coulter, Inc., Fullerton, CA).
measured using previously described enzyme-linked immunosorbent assay protocols (31,32). Urinary values were normalized to creatinine excretion.

**Renal Tissue Processing**

Sagittal sections of renal tissue were fixed in buffered formalin. Five-micrometer sections were stained with periodic acid-Schiff stain and processed for light microscopic evaluation.

For IF microscopy, tissue was snap-frozen in isopentane on dry ice. Four-micrometer cryostat sections were processed for direct IF microscopy as described previously (26). FITC-conjugated antibodies to mouse C3 and fibrinogen and to goat IgG were obtained from Cappel. The anti-mouse C3 reacts with C3b, iC3b, and C3c but not C3d (31). Rabbit anti-rat C9 was generously provided by Dr. B. Paul Morgan (University of Wales) (33) and was conjugated with FITC (Sigma). This anti-rat C9 antibody cross-reacts with mouse C9, including that in the C5b–9 membrane attack complex, as demonstrated by strongly positive staining of glomeruli from MRL/lpr lupus mice (data not shown). Staining intensities were graded using a previously defined semiquantitative scoring system (32).

An immunohistochemical technique was used to evaluate the numbers of glomerular neutrophils. Cryostat sections were fixed with 4% paraformaldehyde and incubated with monoclonal antibody 7/4 (Sero-tec, Oxford, United Kingdom). The Vector ABC staining kit (Vector Laboratories, Burlingame, CA) was used according to the instructions provided by the manufacturer. The numbers of positively stained cells in \( \geq 50 \) glomeruli/animal were counted. In all instances when data were being compiled, the observer was blinded to the origin of the slides.

**Statistical Analyses**

All data are expressed as mean ± SEM. Comparisons between transgene-positive and -negative animals were made by \( t \) testing. For comparisons of multiple groups, one-way ANOVA, followed by Tukey’s pairwise comparisons, was used. Correlations among variables were examined by regression analysis. Minitab software (State College, PA) was used for these analyses.

**Results**

**Renal Localization of Anti-GEN**

As assessed using indirect IF, anti-GEN bound to glomeruli but not to tubules or renal blood vessels (data not shown). In animals given intravenous injections of high doses of anti-GEN, followed by euthanasia 2 or 10 min later, there was strong glomerular staining for IgG but no apparent vascular or tubular staining for goat IgG (Figure 1). C3 was activated in glomeruli as early as 2 min after injection (data not shown).

**Renal Function after Anti-GEN Injection**

BUN values were not different between the two groups of animals before injection of anti-GEN IgG (27.8 ± 1.6 and 32.1 ± 1.5 mg/dl in transgene-positive and -negative animals, respectively). Twenty h after anti-GEN injection, BUN levels were significantly higher in transgene-negative mice (111.0 ± 14.7 mg/dl), compared with transgene-positive mice (48.7 ± 11.7 mg/dl), and this persisted until 30 h after injection (\( P = 0.007 \) and 0.012 at 20 and 30 h, respectively). Figure 2 presents individual BUN values at the latter time point. As can be observed, five of seven transgene-negative animals exhibited BUN values of >100 mg/dl, whereas seven of eight transgene-positive animals exhibited normal BUN values. The remaining transgene-positive animal exhibited a BUN value of 58.1 mg/dl at the time of euthanasia.

Four of the five transgene-negative animals with BUN values of >100 mg/dl were anuric. For the remaining animal, urinary albumin excretion was 42.0 µg/mg creatinine (normal values, <25 µg/mg creatinine). For all other animals, urinary albumin excretion was only minimally elevated, with average values of 60.2 µg albumin/mg creatinine for the two transgene-negative animals with normal renal function and 73.0 ± 58.2 µg albumin/mg creatinine for the transgene-positive animals. Urinary C3 was not detectable for any animal. Therefore,
injection of anti-GEN antibodies produces acute renal failure but only minor apparent alterations in the glomerular permeability barrier to protein passage. Animals bearing the Crry transgene are protected from acute renal failure.

To further investigate the possibility that anti-GEN might induce glomerular proteinuria, five transgene-positive and five transgene-negative animals were given injections of 0.5 mg of anti-GEN, with the rationale that the lower dose would not lead to anuria in the most severely affected animals. Thirty h after injection, BUN values were 32.4 ± 1.4 and 30.4 ± 2.6 mg/dl for the transgene-positive and -negative animals, respectively. Urinary albumin excretion values were 67.9 ± 12.8 and 160.2 ± 82.5 μg albumin/mg creatinine for transgene-positive and -negative animals, respectively. Although they suggested a difference, these values were not statistically different, because of the wide variability. Again, urinary C3 was not detectable for any animal.

Renal Histologic Features

Irrespective of transgene status, animals with BUN values of <100 mg/dl exhibited normal glomerular (Figure 3a) and tubular (Figure 4a) histologic features. In the five transgene-negative animals with renal failure, as defined by BUN values of >100 mg/dl, there were varying degrees of focal glomerular capillary collapse (Figure 3b) and acute tubular necrosis (Figure 4b). Occasional polymorphonuclear leukocytes were observed in the glomeruli of most animals, but marked exudative changes were not apparent (further quantified below). Interstitial infiltrates were not present.

IF Findings

Thirty h after injection, goat IgG persisted in the glomeruli of all animals (data not shown). Although there was some variability in staining intensity among animals, there was no statistical difference between transgene-negative and -positive animals (Table 1). Coincident with the presence of goat IgG in glomeruli, modest amounts of mouse C3 were apparent in glomeruli. Interestingly, there was no difference between transgene-positive and -negative animals (Table 1 and Figure 5). To examine complement activation occurring acutely after anti-GEN injection, animals were euthanized 10 or 60 min after antibody injection. In transgene-negative animals, there was segmental, finely granular, glomerular capillary wall staining for C3 in two of four and five of five animals at 10 and 60 min, respectively, which was not present in any of the seven transgene-positive animals at these two time points.

In contrast to glomerular C3 staining, in transgene-negative animals there was prominent tubular staining for C3 both in a basal distribution (Figure 6b) and in tubular cells (including those that may have been sloughed into the tubular lumina) (Figure 6c), which was significantly less than that observed for transgene-positive animals (Table 1 and Figure 6a). Interestingly, the tubular cell staining for C3 was strongly correlated with BUN values (r = 0.83, P < 0.001), suggesting a causal role in renal failure.

IF staining for C9 protein was also examined. In contrast to C3, C9 was not identified to an appreciable extent in glomeruli, nor was it present in the basal aspects of tubules (data not shown). However, tubular cells stained for C9 to an extent equivalent to that for C3 (Table 1), and the two values were thus strongly correlated (r = 0.79, P < 0.001). As with C3, C9 staining was correlated with BUN levels (r = 0.56, P = 0.037). These data further suggest that complement activation, leading to the generation of C5b–9 on tubules, is pathogenic in this model.

Renal Cortical C3 mRNA Levels

After injection of anti-GEN IgG, C3 mRNA levels in renal cortex were evaluated, with the rational that de novo synthesis of C3 may occur locally after injury in this model. However, there was no significant difference with time in C3 mRNA levels (Figure 7). These results indicate that C3 transcription is not increased in the renal cortex in this model. In fact, there was a slight decrease in C3 mRNA levels at the later time points (≥20 h), which possibly could be ascribed to tubular cell damage.
Neutrophil Accumulation

Thirty h after injection of anti-GEN IgG, there was a modest accumulation of neutrophils in glomeruli. However, there was no difference between transgene-positive and -negative animals (0.35 ± 0.17 and 0.33 ± 0.09 neutrophils/glomerulus, respectively).

Thrombotic Microangiopathy

In rats given anti-GEN directly in the renal artery, a model of the hemolytic uremic syndrome develops, characterized by hemolysis and glomerular fibrin accumulation (12). In this study, however, there was no specific glomerular accumulation of fibrin, as assessed using IF microscopy (data not shown). Furthermore, total bilirubin levels in sera 30 h after disease onset were not elevated and were not different between the groups (0.67 ± 0.39 and 0.58 ± 0.17 mg/dl in transgene-positive and -negative animals, respectively). Therefore, this model in mice seems to be distinct from that induced in rats.

NSN Studies

In mice given relatively high doses of NSN IgG, the resultant glomerular injury is complement-independent and leads to massive proteinuria (22,24,25). Comparable amounts of NSN antibody were used in these studies. As demonstrated in Figure 8, acute renal failure developed in all animals and was related to the dose of NSN antibody used. The resultant albuminuria was marked, with values of >10,000 µg albumin/mg creatinine for every animal, and values were positively correlated with BUN values (r = 0.69, P < 0.001). In contrast to studies in animals with acute renal failure induced by anti-GEN, no animal in these studies was anuric. Furthermore, instead of diffuse tubular cell staining for mouse C3, there was punctate staining for mouse C3 in apical portions of tubular cells, consistent with these being endocytic vacuoles (Figure 9) (34).

Discussion

In this study, injection of anti-GEN antibodies into mice led to acute renal failure in five of seven genetically normal mice. As with the rat model, injected antibodies rapidly bound to glomeruli and activated complement, ultimately leading to renal failure with glomerular and tubular morphologic abnormalities (12). Also as with the rat model, proteinuria and neutrophil accumulation were present but were relatively mild. However, in distinct contrast to the rat model, microangiopathic hemolytic anemia was not present. Transgenic animals that overexpressed the complement inhibitor Crry systemically as a soluble protein and locally in both glomeruli and renal tubules were protected from acute renal failure.

The finding that Crry transgenic animals were protected from acute renal failure implicates the complement system as being pathogenic in this model, as is the case in the rat model (13). The possible sites of complement activation within the kidney in this model include the glomerular, tubular, and vascular compartments. On the basis of our previous results with the NSN model, in which glomerular complement activation was eliminated in 17 of 20 Crry transgenic mice (22), it was logical to assume that a similar degree of complement inhibition would occur in this model. This was true early in the course of this disease model, but by 30 h the difference in C3 immunostaining was no longer present. Crry acts as a factor I cofactor to cleave C3b into the inactive fragment, iC3b (21).

Because this is also recognized by the anti-C3 antiserum used for IF (31), a plausible explanation for these findings is that anti-GEN did activate complement but in Crry transgenic animals the C3b generated was inactivated by Crry but remained covalently bound in glomeruli. The next logical site of anti-

Table 1. Semiquantitative immunofluorescence staining scores for mice given intravenous injections of anti-glomerular endothelial cell IgG

<table>
<thead>
<tr>
<th>Staining Score</th>
<th>Transgene-Positive</th>
<th>Transgene-Negative</th>
</tr>
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<tbody>
<tr>
<td>Glomerular IgG</td>
<td>1.4 ± 0.2</td>
<td>1.1 ± 0.2</td>
</tr>
<tr>
<td>Glomerular C3</td>
<td>0.9 ± 0.3</td>
<td>0.8 ± 0.2</td>
</tr>
<tr>
<td>Basal tubular C3</td>
<td>0.7 ± 0.3</td>
<td>2.1 ± 0.3</td>
</tr>
<tr>
<td>Tubular cell C3</td>
<td>0.1 ± 0.1</td>
<td>1.5 ± 0.4</td>
</tr>
<tr>
<td>Tubular cell C9</td>
<td>0.4 ± 0.3</td>
<td>1.9 ± 0.3</td>
</tr>
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*P < 0.005 versus transgene positive.

bP < 0.05 versus transgene-positive.
body binding and complement activation is within the renal
vasculature, as observed with anti-GEN injection in rats (12),
as well as with injection of neutralizing anti-Crry antibodies
(5). However, we could not identify specific antibody binding
or C3 deposition in the renal microvasculature, either acutely
or 30 h after injection of anti-GEN antibodies.

Tubular C3 staining was significantly greater in wild-type
mice, compared with Crry transgenic mice; this finding was
true for C3 staining in both basal (presumably the tubular
basement membrane) and cellular distributions. This basal
distribution of C3 is a normal finding in mice, as is focal and
segmental staining of Bowman’s capsule, albeit in lesser
amounts than present in this study in experimental transgene-negative mice. This staining is specific for the C3 protein, because it is not present in C3-deficient mice (32). The basal staining for C3 was significantly increased in transgene-negative controls. However, the fact that this staining bore no relationship to BUN levels \( (r = 0.32) \) makes it less likely that basal complement activation is responsible for the acute renal failure in this model.

In contrast to the findings presented above, the high degree of correlation between tubular cell staining for C3 and BUN levels \( (r = 0.83) \) suggests that C3 deposition is at least a marker for renal failure and may be causally related. This finding is despite the fact that the defect in glomerular permselectivity was not marked, as indicated by mild albuminuria and the absence of detectable urinary C3. To further examine the relationship between proteinuria and acute renal failure, we used the NSN model. Animals developed acute renal failure, the magnitude of which was proportional to the amount of NSN antibody injected and the degree of albuminuria. Rather than the diffuse tubular cell staining for C3 observed in anti-GEN-treated animals, mice with NSN exhibited evidence of endocytic vacuoles (34), consistent with active reabsorption of C3 leaking across the glomerular capillary wall. Whether tubular complement activation contributes to pathogenesis in the NSN model but overwhelms protection by the soluble Cry protein is not clear.

Taken together, the anti-GEN model developed here must include the following initial glomerular events: binding of injected antibodies to glomerular endothelium, complement activation, modest impairment of permselectivity, and accumulation of neutrophils. Complement activation on tubules then ensues, leading to acute renal failure. Potential mechanisms for this tubular complement activation include ischemia-reperfusion injury (35–37) related to the glomerular damage; complement activation in peritubular capillaries by goat anti-GEN or natural murine anti-endothelium antibodies (37); and the appearance of complement proteins in the urinary space, either derived from plasma by glomerular filtration or directly produced by tubules (27,29). In this study, we have provided evidence that antibody binding and complement activation do not occur in peritubular capillaries, glomerular filtration of C3 is not apparent, and renal expression of C3 mRNA is not increased. Therefore, of these mechanisms, primary glomerular damage leading to ischemic and possibly reperfusion-related injury of downstream tubular segments is the most plausible explanation that incorporates the available data. A similar degree of widespread tubular complement activation has been noted in human kidney specimens after renal infarction (38). The means by which complement is activated in ischemia-reperfusion injury remain to be fully defined. Animals that express soluble Cry in tubules are resistant to tubular complement activation and the resultant acute renal failure.

Acute renal failure in the setting of well defined complement activation was previously observed in two situations. The first is the anti-GEN model in rats. In this model, prior complement depletion with cobra venom factor reduced BUN levels from 186.1 to 28.3 mg/dl (13). The second is acute renal failure in rats in which massive glomerular proteinuria was induced with
puromycin aminonucleoside (7). Complement inhibition with soluble recombinant human CR1 reduced the pathologic indices of tubular damage but did not affect the markedly reduced GFR. Here we show complete prevention of acute renal failure in Crry transgenic mice.

There are distinct advantages to the use of Crry transgenic mice, as in these studies. The first is that complement inhibition is continuous and relatively constant. The second is related to the local production of the complement inhibitor Crry. In the design of these Crry transgenic mice, we focused on the kidney. In the absence of a suitable promoter to yield kidney-specific production of Crry, we chose the metallothionein I promoter; although this promoter is widely active, it exhibits relative high renal expression (39). We think that high tubular expression of the Crry transgene, which leads to significant release of biologically active Crry in tubular lumina (22), constitutes a significant benefit, particularly given the normal absence of C3 convertase regulators at this site (7–10). Success in these studies supports the design of similar genetic strategies for human subjects, such as the use of kidney-specific promoters to direct the production of complement inhibitors (40).

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