Complement Activation Contributes to Both Glomerular and Tubulointerstitial Damage in Adriamycin Nephropathy in Mice

Daniel Turnberg, Margarita Lewis, Jill Moss, Yuanyuan Xu, Marina Botto and H. Terence Cook

*J Immunol* 2006; 177:4094-4102; doi: 10.4049/jimmunol.177.6.4094

http://www.jimmunol.org/content/177/6/4094
Complement Activation Contributes to Both Glomerular and Tubulointerstitial Damage in Adriamycin Nephropathy in Mice

Daniel Turnberg,* Margarita Lewis,* Jill Moss,† Yuanyuan Xu,‡ Marina Botto,* and H. Terence Cook†‡

Adriamycin nephropathy is a model of focal segmental glomerulosclerosis, characterized by proteinuria and progressive glomerulosclerosis and tubulointerstitial damage. In this study, we examined the role of complement in the etiology of adriamycin nephropathy in mice. We used mice deficient in C1q, factor D, C3, and CD59, and compared them with strain-matched controls. C3 deposition occurred in the glomeruli of wild-type mice as early as 48 h following a single i.v. injection of adriamycin. C3-deficient mice developed significantly less proteinuria and less podocyte injury at day 3 postadriamycin than controls, suggesting that complement is important in mediating the early podocyte injury. At later time points, C3-deficient mice were protected from glomerulosclerosis, tubulointerstitial injury, and renal dysfunction. Factor D-deficient mice were also protected from renal disease, confirming the importance of alternative pathway activation in this model. In contrast, C1q-deficient mice developed similar disease to controls, indicating that the complement cascade was not activated via the classical pathway. CD59-deficient mice, which lack adequate control of C5b-9 formation, developed significantly worse histological and functional markers of renal disease than controls. Interestingly, although more C9 deposited in glomeruli of CD59-deficient mice than controls, in neither group was tubulointerstitial C9 staining apparent. We have demonstrated for the first time that alternative pathway activation of complement plays an important role in mediating the initial glomerular damage in this in vivo model of focal segmental glomerulosclerosis. Lack of CD59, which regulates the membrane attack complex, led to greater glomerular and tubulointerstitial injury. The Journal of Immunology, 2006, 177: 4094–4102.

Adriamycin (also called doxorubicin) nephropathy is a rodent model of focal and segmental glomerulosclerosis (1, 2). It is characterized by rapid onset of glomerular podocyte damage and glomerular proteinuria, which progresses to segmental glomerular sclerosis. Accompanying this there is early tubulointerstitial damage with subsequent tubular atrophy, accumulation of myofibroblasts around damaged tubules, and interstitial fibrosis. It is of particular interest as it is thought to provide an animal model of the way in which glomerular proteinuria is associated with progressive tubulointerstitial scarring, which is thought to be a final common pathway in human glomerular disease.

There is increasing evidence that the complement system is important in mediating renal injury in proteinuric diseases. In adriamycin nephropathy, it has been shown recently that C6-deficient rats, which are unable to form the terminal membrane attack complex (MAC)3 of the complement system, are protected from peri-tubular myofibroblast accumulation and interstitial extracellular matrix deposition (3). These results have led to the suggestion that intraluminal activation of the complement cascade leading to the generation of the MAC is the principal mediator of progressive tubulointerstitial injury in proteinuric renal diseases whatever the cause of the glomerular injury. However, the role of other components of the complement cascade in mediating the glomerular and tubulointerstitial changes of adriamycin nephropathy has not been defined. We therefore studied adriamycin nephropathy in mice, which allowed us to take advantage of mouse strains with targeted deletions of other components of the complement system.

Adriamycin nephropathy has only been characterized using mice of a BALB/c genetic background, and indeed, other commonly used strains such as C57BL/6 are resistant to disease (1, 2). We therefore backcrossed mice lacking C3, C1q, factor D, or the complement regulatory protein CD59a onto the BALB/c genetic background and examined the course of the disease in these animals. CD59 is the only membrane-bound factor that prevents the formation of C5b-9 (4, 5). In contrast to humans, mice have two isoforms of C59: CD59a, which is widely expressed, and CD59b, which is predominantly limited to the testes (6). We have shown previously that mice lacking CD59a are more susceptible to glomerular immune complex injury (7) and to tubular injury and interstitial inflammation following ischemia-reperfusion injury (8). Although there is preliminary evidence that complement components, especially C3, are deposited in areas of tubulointerstitial injury in mice with adriamycin nephropathy, the functional role of the complement system in the pathogenesis of this injury has not been studied previously (9).

In this study, we demonstrate for the first time that the absence of C3 protects from the initial glomerular injury in adriamycin
nephropathy. We have also shown that complement-mediated injury occurs via activation of the alternative, but not the classical pathway in this model. In addition, inhibition of the membrane attack pathway by its regulatory protein CD59 is important in controlling this type of complement-dependent glomerular injury and subsequent tubulointerstitial injury.

Materials and Methods

Animals

CD59-deficient (mCd59a−/−), C1q-deficient (Clqa−/−), and C3-deficient (C3−/−), and factor D (Cfd−/−) mice were generated, as described previously (10–13). All gene-targeted animals were then backcrossed for six generations onto a BALB/c genetic background and compared with strain- and age-matched controls. To ensure that C3−/− BALB/c (N6) mice were of the same H2 haplotype as wild-type BALB/c mice, H2 haplotype was checked for by PCR. Only female mice were used. Mice were kept in a specific pathogen-free environment, and experiments were performed according to institutional guidelines.

Experimental protocol

To induce adriamycin nephropathy, a single i.v. injection of doxorubicin (adriamycin; Pharmacia & Upjohn) diluted 1/2 with 0.9% saline was administered into the tail vein. For experiments with C3−/−, Cfd−/−, and Clqa−/− mice, a dose of 10 mg/kg adriamycin was used. A lower dose of 9 mg/kg was administered in experiments using mCd59a−/− mice, as preliminary experiments demonstrated that mCd59a−/− mice became rapidly unwell with large doses. Mice were killed at 8 wk postadriamycin injection unless they became ill at earlier time points. In addition, to ascertain the time course of renal complement deposition, some wild-type BALB/c mice were killed at earlier time points.

Histological analysis

Kidneys were fixed for 24 h in 4% buffered formalin saline, transferred to 70% ethanol, and embedded in paraffin. Sections were then stained with periodic acid-Schiff. All analyses were performed blinded to the sample identity. Tubulointerstitial damage was assessed by ranking of sections taking into account tubular necrosis, tubular dilatation, and cast formation in randomized sections. Glomerulosclerosis was assessed by grading the area of periodic acid-Schiff-positive sclerosis for each glomerulus, as follows: grade 0 = no sclerosis; grade 1 = 0–25%; grade 2 = 25–50%; grade 3 = 50–75%; and grade 4 = 75–100%. The mean glomerular sclerosis score for 50 glomeruli was then calculated. Paraffin-fixed sections were also stained with picrosirius red for assessment of collagen deposition. The mean area of positive staining was quantified using Image-Pro Plus software (Media Cybernetics), images were captured for analysis.

Immunofluorescence

Kidneys were snap frozen in isopentane and stored at −70°C. Frozen sections were cut at a thickness of 5 µm. An observer without knowledge of the sample identity performed all the quantitative immunofluorescence analyses. For C3 staining, a goat anti-mouse C3 FITC-conjugated Ab (Valeant Pharmaceuticals) was used. For C9 staining, a rabbit anti-rat C9 primary Ab (1:100) (cross-reactive with mouse C9 (14)) and a FITC-conjugated mouse anti-rabbit IgG secondary (1:50) (Sigma-Aldrich) were used. For the type IV collagen staining, a polyclonal rabbit anti-human type IV collagen Ab (1:100) (Research Diagnostics) and a FITC-labeled mouse anti-rabbit IgG secondary (1:50) (Sigma-Aldrich; F-4151) were used. All incubations were performed for 1 h at room temperature, and all Abs were diluted in PBS. A negative control (PBS) was used in each experiment to ensure there was no binding of the secondary Ab to the kidney section. In quantitative immunofluorescence studies, to exclude artifacts due to variable decay of the fluorochrome, all sections from one experiment were stained and analyzed at the same time. Sections were examined at ×400 magnification using an Olympus BX4 fluorescence microscope (Olympus Optical). A Photonic Science Color Coolview digital camera (Photonic Science) was attached to the microscope and, using Image-Pro Plus software (Media Cybernetics), images were captured for analysis. For glomerular analysis, 20 glomeruli were examined in each section, and for the tubulointerstitium 10 high-powered field (HPF) of the cortico-medullary area were assessed for each section. In each case, the mean fluorescence intensity was recorded, with results expressed in arbitrary fluorescence units.

Electron microscopy

Specimens (1 mm³) of renal cortex were fixed in 3% glutaraldehyde in 0.1 M cacodilate buffer for 4 h at 4°C. The samples were then postfixed in 2% aqueous osmium tetroxide, block stained in 2% aqueous uranyl acetate, dehydrated through ascending grades of ethanol, and embedded in Spur’s resin at 70°C overnight. Ultrathin sections were collected on 400 mesh copper grids and stained with 1% aqueous uranyl acetate and Reynold’s lead citrate.

Renal function assessment

Serum urea was measured using an Olympus AU600 analyzer (Olympus Diagnostics). Proteinuria was measured by dipstick of urine (Hema-Com-bistix; Bayer) daily for the first week and then weekly. In addition, mice were placed in metabolic cages for 24-h collection of urine to allow more accurate assessment of urine albumin concentration. The albumin concentration was measured by radial immunodiffusion. Samples and standards (mouse albumin) (Sigma-Aldrich) were placed in wells (4 µl/well) in 1.2% agarose in PBS containing rabbit anti-mouse albumin (Biogenesis). Gels were dried and stained with Coomassie blue. Albumin concentration was calculated with reference to a standard curve.
Statistical analysis

All values described in the text and figures are expressed as median and range for \( n \) observations. Statistical analysis was conducted using GraphPad Prism 3.02 (GraphPad). Data were analyzed using a Mann-Whitney \( U \) test for all comparisons, and a \( p \) value of \(<0.05\) was considered to be significant. For survival analysis, a Kaplan-Meier statistic was used and a \( p \) value of \(<0.05\) was also considered significant.

Results

Initial dose-ranging experiments using between 7 and 11 mg/kg adriamycin in wild-type BALB/c female mice suggested that a dose of 9–10 mg/kg adriamycin produced significant irreversible injury characterized by glomerulosclerosis and tubular injury at 2 wk postinjection, followed by interstitial inflammation and fibrosis at later time points (4–6 wk; data not shown). Higher doses produced much more severe injury that developed more rapidly, while lower doses produced only mild reversible histological abnormalities. However, in all experiments, independently of the dose administered, significant proteinuria was observed (+ + + + on dipstick) from weeks 1 to 6.

Glomerular C3 deposition occurs by 48 h after adriamycin administration

Guided by these findings, we decided to investigate the role of the complement system in mediating the initial glomerular injury following adriamycin injection, we killed BALB/c mice at various time points to study glomerular complement deposition. Immunofluorescence microscopy revealed that in unmanipulated BALB/c mice kidneys, C3 was present around tubules and along Bowman’s capsule, but no C3 was detected in glomeruli (Fig. 1A). Electron micrographs at day 3 following adriamycin administration show extensive foot process effacement in wild-type animals (B), while podocytes were relatively well preserved in C3−/− mice (C).
C3−/− animals compared with BALB/c controls. Small amounts of albumin could be detected in the urine of wild-type animals as early as day 2 postadriamycin injection and reached a maximum level by day 6. In contrast, the onset of albuminuria was markedly delayed in C3−/− mice, but achieved a similar upper limit to the wild-type mice by day 10 after the administration of adriamycin (Fig. 4A). Consistent with these findings, electron microscopy of glomeruli at day 3 after adriamycin showed that there was better preservation of podocyte foot processes in C3−/− mice than wild-type animals (Fig. 2, B and C).

**Absence of C3 reduced progressive renal injury**

Four weeks postadriamycin injection, C3−/− mice were significantly protected from chronic glomerular damage (median sclerosis score 0.8; range 0.3–1.1; n = 8 for C3−/− vs 2.0; 0.8–3.6; n = 8 for wild type, p = 0.007; Fig. 3A) and tubulointerstitial injury (median rank of tubular injury 6; range 3–9; n = 8 for wild type vs 2; 1–5; n = 8 for C3−/−, p = 0.004; Fig. 3B). Consistent with these structural differences, C3−/− animals were protected from renal impairment when compared with wild-type controls (median urea 7.2 mmol/L 5.7–9.1; n = 8 for C3−/− vs 12.8; 10.3–60.0; n = 8 for controls, p = 0.0002; Fig. 3C). Histological changes showed severe, often global sclerosis in wild-type animals (Fig. 4A) with only relatively minor, segmental abnormalities in C3−/− animals (Fig. 4B). Comconitantly, wild-type animals displayed severe tubular damage typified by loss of the epithelial brush border, flattening of tubular cells, and tubular cast formation (Fig. 4C). In contrast, tubular injury was mild in C3−/− mice (Fig. 4D). In wild-type animals, C3 was deposited especially in areas of most severe renal damage, but also in a peritubular distribution (Fig. 4E). No C3 staining was seen in C3-deficient mice (Fig. 4F).

**Complement activation by the alternative pathway caused renal injury**

C3 activation can occur by the classical, alternative, or lectin pathways. To dissect out the role of alternative pathway activation, we used mice deficient in the alternative pathway component factor D. Cfd−/− mice were protected from early proteinuria in a similar fashion to C3−/− mice (Fig. 5A), suggesting that alternative pathway activation mediated the early complement-induced podocyte damage. Three weeks after adriamycin administration, Cfd−/− mice were significantly protected from both glomerular injury (median injury score 3.2; range 0.8–3.5; n = 6 for controls vs 0.0–0.8; n = 5 for Cfd−/− mice, p = 0.02; Fig. 5B) and tubulointerstitial damage (median injury rank 9; range 6–11; for controls vs 3.5; 1–7; for Cfd−/− mice, p = 0.009; Fig. 5C). In addition, renal function was preserved in Cfd−/− mice as compared with matched controls (median urea 9.4 mmol/L; 8.6–12.9; for controls

**FIGURE 4.** Periodic acid-Schiff staining and C3 immunofluorescence of renal sections following adriamycin injection. Glomerular damage 4 wk postadriamycin injection was characterized by segmental or global sclerosis in wild-type animals (A) compared with either normal appearances or mild segmental sclerosis (B) in C3−/− mice. Tubulointerstitial damage in the wild-type animals (C) was exemplified by tubular cell thinning, loss of brush border epithelium, and tubular cast formation, while C3−/− mice (D) were protected to a significant extent from these changes. In wild-type animals, C3 was deposited especially in areas of most severe renal damage, but also in a peritubular distribution (E). C3-deficient animals were used as negative controls (F).
vs 4.8; 4.2–5.6; for Cfd−/− mice, p = 0.036; Fig. 5D). To confirm that the absence of factor D protected Cfd−/− mice from renal injury by preventing C3 activation, we stained renal sections for C3. No C3 immunofluorescence staining was apparent in either glomerular or tubulointerstitial compartments in the Cfd−/− mice (Fig. 5F).

Classical pathway activation of complement was not involved in the pathogenesis of adriamycin nephropathy

To explore possible classical pathway involvement, we examined adriamycin-induced nephropathy in C1q-deficient mice. The levels of proteinuria were similar in C1qa−/− and wild-type animals during the entire period of observation (Fig. 6A). Structural injury 4 wk postadriamycin administration was not significantly different between C1qa−/− mice and BALB/c controls in terms of either glomerulosclerosis (median injury score 2.8; range 0.4–3.2; n = 6 for C1qa−/− vs 1.9; 1.1–2.7; n = 7 for controls, p = 0.48; Fig. 6B) or tubulointerstitial injury (median rank of tubular injury 7; range 2–12; n = 6 for C1qa−/− vs 6; 1–11; n = 7 for controls, p = 0.76; Fig. 6C). In addition, there were no functional differences in terms of serum urea between the two groups of mice (median urea 12.5 mmol/L; 6.6–32.2; n = 6 for C1qa−/− vs 8.4; 7.4–23.1; n = 7 for controls, p = 0.6; Fig. 6D).

The terminal complement pathway played an important role in mediating renal damage

To determine the role of the membrane attack pathway in the pathogenesis of adriamycin-induced renal damage, we used mCd59a−/− mice that lack the major regulator of MAC formation. Despite a similar degree of proteinuria (Fig. 7A), mCd59a−/− mice

---

FIGURE 5. Markers of renal injury after adriamycin injection in Cfd−/− and control mice. Cfd−/− mice developed less proteinuria measured by dipstick than wild-type animals after adriamycin administration (A). Renal sections from Cfd−/− animals displayed significantly less glomerulosclerosis (B) and tubulointerstitial injury (C) compared with matched controls. In addition, Cfd−/− mice were protected from renal dysfunction (measured by serum urea) compared with controls (D). Wild-type mouse kidneys showed significant glomerular C3 staining (E). In contrast, C3 staining was notably absent from Cfd−/− mouse kidneys (F). The error bars denote SEM.
developed significantly more glomerulosclerosis (median injury score 2.8; range 1.1–2.7; n = 8 for mCd59a-/- vs 0.8, 0.1–2.0; n = 9 for controls, p = 0.0078; Fig. 7, B, E, and F) and tubulointerstitial injury than wild-type animals (median rank of tubular injury 8; range 4–10; n = 8 for mCd59a-/- vs 3; 1–6; n = 9 for controls, p = 0.03; Fig. 7C). As a functional correlate of these structural differences, serum urea was significantly greater in the mCd59a-/- mice than strain-matched controls (median urea 23.6 mmol/L; 19.2–28.0; n = 8 for mCd59a-/- vs 10.3; 8.0–14.3; n = 9 for controls, p = 0.0006; Fig. 7D). Interestingly, although glomeruli from mCd59a-/- mice showed strikingly more C9 deposition (as a marker of MAC) than wild-type animals (median C9 staining 47.0 arbitrary fluorescence units; 31.2–77.4; n = 10 for controls, p = 0.001), there was very little tubulointerstitial C9 staining in either group. Importantly, mortality in mCd59a-/- mice was significantly greater than wild-type animals following adriamycin administration (4 of 8 mCd59a-/- mice died compared with 0 of 9 controls; p = 0.01), and therefore, in this series of experiments mice were killed 3 wk following adriamycin dosing.

To characterize further the development of interstitial fibrosis, we stained sections for smooth muscle actin (as a marker of myofibroblast transformation) and with picrosirius red (to detect types I and III collagen deposition). There was greater picrosirius red (median area of staining 0.02; range 0.005–0.08; n = 8 for mCd59a-/- vs 0.008, 0.001–0.03; n = 9 for controls, p = 0.04; Fig. 8A) and smooth muscle actin staining (median score 5; range 4–8; n = 8 for mCd59a-/- vs 4; 1–7; n = 9 for controls, p = 0.02; Fig. 8B) in mCd59a-/- animals compared with wild-type animals. In addition, renal sections of mCd59a-/- mice displayed a significantly greater number of TUNEL-positive apoptotic cells than controls (median number of apoptotic cells per HPF 8.3; range 4.7–11; n = 8 for mCd59a-/- vs 3.3; 1.7–7.6; n = 9 for controls, p = 0.0003; Fig. 9).

Discussion

The model of adriamycin nephropathy in rodents has been studied as a model of proteinuric renal disease in which there are progressive glomerulosclerosis and tubulointerstitial injury. In particular, it has been used to elucidate the role of proteinuria in leading to progressive tubulointerstitial scarring. In this study, we examined the role of the complement system in this model in mice. We confirmed previous descriptions on the time course of the renal disease in this model in BALB/c mice (15) and found that after a single i.v. dose of adriamycin there was rapid onset of albuminuria, which was first detectable by day 2. Renal sections demonstrated focal and segmental glomerulosclerosis and subsequent, progressive tubulointerstitial scarring. These changes were accompanied by increased deposition of C3 in glomeruli. We therefore studied the course of the model in C3-deficient mice.

Mice lacking C3 showed a reduction in severity of the disease. The earliest manifestation of this was a delay in the onset of detectable albuminuria accompanied by a reduction in podocyte damage as assessed by effacement of foot processes. Subsequently, mice without C3 showed less glomerulosclerosis and less tubulointerstitial scarring and, functionally, this was reflected by better renal function with a lower serum urea. The classical pathway of complement activation was not involved in initiating this complement-dependent injury, as the absence of C1q did not alter disease severity. To investigate whether complement-mediated damage ensued from alternative pathway activation, we studied mice deficient in the alternative pathway component factor D. We demonstrated that Cfd-/- mice were protected from early proteinuria, and from later glomerular and tubulointerstitial injury. This was also manifested functionally as an improvement in renal function as measured by serum urea. It would have been interesting to study mice deficient in factor B. However, the factor B gene lies on chromosome 17 in the same region as the MHC, and therefore, backcrossing factor B-deficient mice (that are H2b) onto a BALB/c background (H2d) would have meant comparing mice with different H2 haplotypes, which could have confounded the results thereby obtained.

We hypothesize that adriamycin causes initial toxic injury to the podocyte, and then the damaged cells are able to activate the alternative pathway possibly because of reduced surface expression of complement-inhibitory molecules. The deposition of C3 then
exacerbates podocyte injury, leading to earlier onset of proteinuria and subsequently more severe glomerular injury and sclerosis. Once activated, the complement system may mediate renal damage through either the leukocyte chemoattractant effect of C5a or via MAC. To investigate further the role of the membrane attack pathway, we used mCd59a−/− mice and demonstrated that the lack of CD59, and therefore unregulated MAC deposition, significantly exacerbated renal disease in terms of glomerulosclerosis, tubulointerstitial injury, and serum urea. We have shown previously exacerbation of glomerular injury in nephrotoxic nephritis in mice lacking CD59 (16), and the present results emphasize further the important role that CD59 plays in controlling glomerular injury due to complement activation.

In parallel with the effects on glomerular injury, we found that tubulointerstitial injury was reduced in mice lacking C3 and exacerbated in mice without CD59, implying an important role for the MAC. Mice lacking CD59 showed increased tubulointerstitial apoptosis, as demonstrated by TUNEL staining, increased interstitial collagen deposition, and increased interstitial myofibroblasts identified by α smooth muscle actin staining. The pathogenic mechanisms that link proteinuric renal disease and progressive tubulointerstitial fibrosis are of considerable interest and have been reviewed recently (17). There is a clear relationship between the degree of proteinuria and the severity of interstitial disease in rodent models, such as protein-overload proteinuria and puromycin aminonucleoside nephritis. Studies using these models have suggested that the proteinuria itself could induce the progressive interstitial injury, and several hypotheses have been proposed as potential mechanisms of proteinuria-induced tubulointerstitial injury. These include direct tubulotoxicity of high protein concentrations (18) or that specific proteins such as growth factors, lipoproteins (19, 20), transferrin (21), or activated complement components (22, 23) may be damaging. Recent studies have emphasized the role of the MAC. Rangan et al. (3) examined adriamycin nephropathy in C6-deficient rats that were unable to form MAC. They found that MAC deposition occurred predominantly on the luminal surface of proximal tubules in wild-type C6-sufficient rats. Despite a similar level of proteinuria and glomerular injury, C6-deficient rats had fewer peritubular myofibroblasts than controls. These data suggest that proteinuria-induced intraluminal formation of C5b-9 is responsible for myofibroblast formation, and thereby contributes to progressive interstitial damage and renal failure in proteinuric glomerular disease. An alternative hypothesis for the tubulointerstitial injury in focal and segmental glomerulosclerosis is that podocyte injury causes adhesion of the glomerular tuft to Bowman’s capsule, followed by accumulation of misdirected filtrate around the origin of the proximal tubules with subsequent occlusion and tubular atrophy (24). These two hypotheses of tubular...
damage due to proteinuria and misdirected filtration with subsequent nephron loss are not mutually exclusive.

Our study does not allow us to determine which mechanism leads to tubulointerstitial scarring in Adriamycin nephropathy in mice. However, it is notable that, although we found markedly increased staining for C9 in the glomeruli of mice lacking CD59, we found very little C9 staining associated with tubules in wild-type or CD59-deficient mice, which argues against a major role of tubular MAC deposition in causing direct tubular injury, but rather suggests that in the absence of CD59 there is increased glomerular MAC leading to increased glomerular scarring, and this then leads to tubulointerstitial scarring by a process not dependent on tubular complement activation.

Clearly, there are significant differences between our model and the model of Adriamycin nephropathy in the rat described by Rangan et al. (3), in which tubular MAC was detected. In their model, no C3 was found in glomeruli, whereas we observed clear C3 deposition that increased with time. The reason for these differences is not clear and may reflect either a species difference or differences in the dose of Adriamycin and severity of the model. They found only a mild glomerulosclerosis, but no difference in the progression of glomerulosclerosis in rats lacking C6, suggesting that MAC did not play a role in the glomerular lesions, whereas our results in CD59-deficient mice clearly show that if MAC activity is uncontrolled, then this leads to increased glomerulosclerosis.

In conclusion, we have demonstrated an important functional role of the complement system in mediating injury in Adriamycin nephropathy. We have shown that lack of C3 reduces early glomerular injury and proteinuria and ameliorates subsequent glomerular and tubulointerstitial scarring with preservation of renal function. We have demonstrated for the first time that lack of a functional alternative pathway markedly reduces both histological and functional markers of renal injury in this model. We have also shown that CD59, by limiting deposition of the MAC, protects the glomerulus from scarring. CD59 also limits tubulointerstitial scarring, although whether this is indirect via the effect on glomerular scarring or a specific role for tubular CD59 is unclear.

Acknowledgments
We thank all of the staff in the animal facility for their technical assistance. We thank Ian Shore (Charing Cross Hospital, London, U.K.) for his help with the electron microscopy. We express our appreciation to Youhong Zhang, Gregory A. Skibinski, and Mark A. McCrory for their technical assistance.
The authors have no financial conflict of interest.

Disclosures

The authors have no financial conflict of interest.

References