Administration of a Soluble Recombinant Complement C3 Inhibitor Protects Against Renal Disease in MRL/lpr Mice

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Abstract. Complement receptor 1-related gene/protein y (Crry) in rodents is a potent membrane complement regulator that inhibits complement C3 activation by both classical and alternative pathways. To clarify the role of complement in lupus nephritis, MRL/lpr mice were given Crry as a recombinant protein (Crry-Ig) from 12 to 24 wk of age. Control groups were given saline or normal mouse IgG. Sera and urine were collected biweekly. Only 1 of 20 (5%) Crry-Ig-treated mice developed renal failure (BUN > 50 mg/dl) compared with 18 of 38 (47.4%) mice in control groups (P = 0.001). BUN levels at 24 wk were reduced from 68.8 ± 9.7 mg/dl in control groups to 38.5 ± 3.9 mg/dl in the Crry-Ig-treated group (P < 0.01). Urinary albumin excretion at 24 wk was also significantly reduced from 5.3 ± 1.4 mg/mg creatinine in the control groups to 0.5 ± 0.2 mg/mg creatinine in the Crry-Ig-treated group (P < 0.05). Of the histologic data at 24 wk, there was a significant reduction in scores for glomerulosclerosis and C3d, IgG, IgG3, and IgA staining intensity in glomeruli in complement-inhibited animals. Crry-Ig-treated animals were also protected from vasculitic lesions. Although there was no effect on relevant autoimmune manifestations such as anti-double stranded DNA titers or cryoglobulin IgG3 levels, circulating immune complex levels were markedly higher in complement-inhibited animals. Thus, inhibition of complement activation with Crry-Ig significantly reduces renal disease in MRL/lpr lupus mice. The data support the strategy of using recombinant complement C3 inhibitors to treat human lupus nephritis.

Systemic lupus erythematosus (SLE) is an autoimmune disease with widespread complement activation and deposition of complement fragments in involved tissues. Mice of the MRL/Mp background strain bearing the lpr gene in homozygosity (MRL/Mp-Ipr/Ipr or simply MRL/lpr), which leads to a deficiency of the apoptosis-promoting Fas protein (1,2), develop many features of human SLE, including autoantibodies, hypocomplementemia, and proliferative glomerulonephritis (GN) (3,4). Heavy proteinuria and impaired renal function can be observed after the onset of GN. The GN in MRL/lpr mice evolves from mesangial to diffuse proliferation, including a crescentic GN, and ultimately to glomerulosclerosis with renal failure and death in these mice (5). Decreased serum C3 levels and deposition of C3 activation fragments and other complement components in kidney suggest that complement is involved in the pathogenesis of murine as well as human lupus nephritis (3).

Complement activation can proceed via either the classical, alternative, or mannose-binding lectin pathways (6). Although each of these pathways have different activators, all three converge on C3 and then C5, after which the pathways share common proteins. Thus, activation through each of the three pathways leads to cleavage of C3 with generation of the pro-inflammatory and regulatory fragments C3a and C3b. C3b attaches covalently to immune complexes, followed by C5 binding and its cleavage to C5a and C5b. The former is a potent inflammatory molecule that can recruit and activate inflammatory cells, and apparently renal mesangial and proximal tubular cells as well (7–9), whereas the generation of C5b begins the nonenzymatic assembly of the C5b-9 membrane attack complex, which can result in cellular death or activation after membrane insertion (10).

Complement receptor (CR) 1-related gene/protein y (Crry) was identified by virtue of its protein and nucleotide similarity to human CR1 (11,12). Like human CR1, Crry is an intrinsic membrane complement inhibitor that inhibits C3 convertases of all pathways through decay-accelerating and factor I cofactor activities (13,14). Beginning with its use in a myocardial infarction model in rats (15), soluble recombinant CR1 has been used extensively in various rodent models of disease (16,17), including glomerular disease models (18). The limitation of the use of human CR1 in rodent models is that treatment can only be short-term given the inevitable generation of an immune response to the heterologous protein (19).

Because of the development of an inhibitory immune response to human CR1, the use of the homologous non-immunogenic rodent protein, Crry, is attractive to study long-term...
models such as murine lupus. In previous studies, we found that transgenic expression of Crry can protect MRL/lpr mice from developing renal failure and prolonged their survival (20). Unfortunately, transgenic expression is lifelong and thus not directly applicable to the human disease. We have previously developed a recombinant soluble form of Crry fused to the hinge CH2 and CH3 domains of mouse IgG1 (Crry-Ig), which was effective in ameliorating antibody-dependent short-term glomerular inflammation (21). In the present study, we used Crry-Ig to investigate the effects of chronic complement inhibition in MRL/lpr mice and provide evidence that the administration of a soluble complement inhibitor is of benefit in this accurate mouse model of human SLE.

Materials and Methods

Crry-Ig

Crry-Ig was produced and purified as described previously (21). The construct used to produce Crry-Ig utilizes the CMV promoter and encodes a signal peptide followed by the five short consensus repeats of Crry and the hinge, CH2 and CH3 domains of mouse IgG1, a non-complement fixing isotype. Crry-Ig exhibits a second order kinetic half-life of approximately 40 h (21). As a control for Crry-Ig, normal mouse IgG was used, which was purified from normal mouse serum by protein G affinity chromatography (Amersham-Pharmacia-Biotech, Uppsala, Sweden). Both Crry-Ig and mouse IgG were dia lyzed into normal saline. Endotoxin levels were < 5 ng/ml Crry-Ig or mouse IgG.

Experimental Protocol

All work with mice was approved by the University of Chicago Animal Care and Use Committee and were performed in accord with the NIH Guide for the Care and Use of Laboratory Animals. MRL/lpr mice were purchased from Jackson Laboratories (Bar Harbor, ME). Fifty-eight male mice were used in this study and were randomly divided into three groups to receive Crry-Ig (n = 20), mouse IgG (n = 20), or saline (n = 18). Starting at 12 wk of age, mice received intraperitoneal injections of a total volume of 0.3 ml every other day. Blood from the retroorbital plexus and urine collected in a metabolic cage were obtained every 2 wk. All surviving animals were sacrificed at 24 wk of age for tissue acquisition.

Measurements from Sera and Urine

Though Crry levels were determined in 16-wk-old animals receiving Crry-Ig by a previously described ELISA technique (21). As there is no circulating soluble Crry, these levels reflected injected Crry-Ig alone. Complement activity was measured in the same serum samples from Crry-Ig-injected animals as well as in saline and IgG-injected animals by a previously described assay in which C3 deposition on zymosan, a potent activator of the alternative pathway, was assessed by flow cytometry (21,22). Serum C3 levels were determined by ELISA. Plates were coated with goat anti-mouse C3 (Cappel Laboratories, Durham, NC), serum samples were loaded in serial dilutions starting at 1/1000, followed by HRP-goat anti-mouse C3 (Cappel). Results are expressed relative to sera from 24-wk-old MRL/+ mice.

Blood urea nitrogen (BUN) and urinary creatinine concentrations were detected with a Beckman Autoanalyzer (Beckman Coulter, Fullerton, CA). Urinary albumin concentration was measured by a mouse albumin ELISA kit (Bethyl Laboratories, Montgomery, TX), and normalized to urinary creatinine (23). Urinary albumin excretion in normal mice is < 0.025 mg/mg creatinine.

Circulating immune complex levels were measured by a modification of a previously described C1q binding ELISA (24). Briefly, 96-well plates were coated with human C1q (Quidel, San Diego, CA). After blocking, sera samples were loaded in serial dilutions starting at 1/1000, followed by HRP-goat anti-mouse IgG (Jackson Immunoresearch Laboratories, West Grove, PA). Sera samples from five 24-wk-old MRL/lpr mice were used as positive control, whereas sera from 24-wk-old MRL/+ and Balb/c mice were used as negative controls.

Serum anti-double stranded (ds) DNA antibodies were measured by a previously described ELISA (20). Briefly, 96-well plates were coated with methylated BSA (Sigma-Aldrich, St. Louis, MO), followed by calf thymus dsDNA (Sigma-Aldrich). Serial dilutions of sera were plated and incubated at room temperature for 2 h, followed by HRP-conjugated goat anti-mouse IgG (Kierkegard & Perry Laboratories, Gaithersburg, MD) and OPD peroxidase substrate (Sigma). The OD450 was then measured. Sera from several 6 mo old MRL/lpr mice were pooled and served as a control. The amounts of anti-dsDNA were quantified by plotting against the standard curve and presented as relative units.

Because of the relevance of IgG3-containing cryoglobulins in complement-deficient MRL/lpr (25) mice, these were measured in these studies. Cryoglobulins were isolated from sera obtained from 24-wk-old mice. Blood samples were placed at 37°C for 2 h immediately after bleeding, followed by centrifugation at 300 × g at 37°C. Fifty microliters of serum from each sample was collected and incubated at 4°C for 5 d. The serum was centrifuged at 16,000 × g, and the precipitates were washed five times with cold PBS and dissolved in 50 μl of PBS at 37°C for 2 h before use. IgG3 levels in cryoglobulins were measured by ELISA conducted in a warm room maintained at 37°C. ELISA plates were coated with 1 μg/ml goat anti-mouse IgG kappa (Southern Biotechnology Associates, Birmingham, AL) overnight at 4°C. After the plates were warmed to 37°C, serial dilutions of unknowns or IgG3 standard (Sigma-Aldrich) were added and incubated for 1 h, followed by HRP-conjugated goat anti-mouse IgG3 (Caltag Laboratories, Burlingame, CA). After incubation for 1 h, HRP was developed with an OPD peroxidase substrate and the OD450 was determined. The concentration of IgG3 in cryoglobulins was calculated relative to the IgG3 control.

Measurements from Tissue

For immunofluorescence (IF) microscopy, tissues were snap frozen in 2-methylbutane in a container on dry ice. Four-micrometer cryostat sections were processed for direct IF. Sections were fixed in ethanol and stained with FITC-conjugated antibodies to mouse C3, IgG, IgG3, IgA (Cappel), IgM (Sigma), or human C3d (Dako, Carpenteria, CA), which is cross-reactive with mouse C3d (26). A semiquantitative score of staining intensity and distribution from 0 to 4 was provided in a blinded manner as described previously (23).

For light microscopy, tissues were fixed in 10% buffered formalin from which 4-μm sections were stained with periodic acid-Schiff. The extent of GN, glomerulosclerosis, and interstitial nephritis was graded from 0 to 4 according to the schema of Passwell et al. (27) as described previously (20). The extent of arteritis was graded by the following scale: 0, none; 1+, focal intimal, non-necrotizing arteritis only; 2+, diffuse intimal arteritis or focal transmural and/or necrotizing arteritis involving <20% of arteries present; 3+, transmural and/or necrotizing arteritis involving 20 to 50% of arteries; and, 4+, transmural and/or necrotizing arteritis involving >50% of arteries. Slides were identified only by number to the grader (MH).
Statistical Analyses

Data are expressed as mean ± SEM for parametric and mean ± Q1 for nonparametric data. In every variable measured, there was no difference between animals given saline or normal mouse IgG; therefore, these control groups were pooled. Where relevant, data from the individual groups are also provided in the Results. The statistical approach to compare albuminuria and BUN levels in groups over time was performed with the random effects model (‘proc mixed’ in SAS v. 8, SAS Institute Inc., Cary, NC) with the assumption that these change linearly over time. These results were confirmed using the area under the curve function (‘AUC’ in STATA for Windows v.7, STATA Corp., College Station, TX), comparing AUC between the two groups by two sample t and Wilcoxon rank sum tests. Comparing the risks of renal failure, skin lesions, ear necrosis, and survival in the groups was performed with Wilcoxon-Geham test in STATA, which were confirmed by the Cox proportional hazards model. \( \chi^2 \) analyses were used to compare fractions.

Results

Complement Inhibition with Crry-Ig Reduces Albuminuria and Prevents Renal Failure in MRL/lpr Mice

To follow the effect of complement inhibition on the development of renal disease over time, BUN levels and albuminuria were determined every other wk during the treatment with Crry-Ig from 12 to 24 wk of age. In the control groups, BUN levels increased as the mice aged (Figure 1). In contrast, complement inhibition with Crry-Ig led to significantly decreased BUN levels (\( P < 0.01 \)). For instance, BUN levels at 24 wk were 38.5 ± 3.9 mg/dl in the Crry-Ig-treated group compared to 68.8 ± 9.7 mg/dl in control groups (70.4 ± 13.5 and 67.4 ± 14.3 mg/dl in animals given saline and IgG, respectively).

Examined a different way is the cumulative development of renal failure, as defined by BUN values over 50 mg/dl. As shown Figure 2, complement inhibition with Crry-Ig prevented the development of renal failure compared with the control groups. Only 1 of the 20 Crry-Ig-treated animals developed renal failure by 24 wk of age compared with 18 of 38 control animals (10 in saline-treated and 8 in mouse IgG-treated groups). In the single Crry-Ig-treated animal with renal failure, BUN levels were 40.2 mg/dl at 22 wk of age and 101.7 mg/dl at 24 wk of age, at which time it was sacrificed. Thus, complement inhibition with Crry-Ig prevented the development of renal failure in MRL/lpr mice.

Albuminuria was used as another important index of renal disease distinct from BUN levels. Control MRL/lpr mice treated with saline or mouse IgG had a progressive rise in albuminuria over time (Figure 3). In contrast, animals treated with Crry-Ig had minimally elevated albuminuria, which did not increase from 18 to 24 wk of age (\( P < 0.001 \) versus control groups). At 24 wk of age, urinary albumin excretion was 0.5 ± 0.2 mg/mg creatinine in Crry-Ig-treated animals compared with 5.3 ± 1.4 mg albumin/mg creatinine in control groups.

Despite the clear reduction in renal disease, complement inhibition with Crry-Ig did not have a statistically significant
effect on 24 wk survival, which was 14 (36.8%) of 38 and 10 (50%) of 20 in control and Crry-Ig-treated groups, respectively. Of the 24 animals that died spontaneously but that were not treated with a complement inhibitor, six had evidence for renal failure before death, whereas none of the Crry-Ig–treated animals had evidence for renal failure before spontaneous death \((P = 0.081)\) by \(\chi^2\). Examined another way is a comparison of premorbid BUN values (obtained within 2 wk of death), which were 61.9 ± 13.1 mg/dl in controls and 27.8 ± 1.6 mg/dl in Crry-Ig–treated mice \((P < 0.02)\). Thus, while complement inhibition with Crry-Ig prevents renal disease, animals in this group appeared to die from a condition(s) other than renal failure.

Pathologic Findings

All surviving mice were sacrificed at 24 wk of age. Histologic data are shown in Table 1 and representative photomicrographs in Figure 4. Although the scores for GN, glomerulosclerosis, and interstitial nephritis were all the lowest in Crry-Ig–treated groups, the only statistically significant difference was in glomerulosclerosis. Reconciling these results with the renal functional data may be complex but could include the relatively small numbers of surviving animals, the semiquantitative nature of the scoring system, and that control animals with worse histologic disease were removed from analysis because of death (so-called informative censoring).

As expected, complement inhibition with Crry-Ig reduced glomerular deposition of C3 and C3d (Table 2), although only the latter was statistically different. Surprisingly, Crry-Ig–treated animals had significantly less glomerular deposition of IgG, IgG3, and IgA than controls (Table 2). The montage in Figure 5 shows representative IF micrographs for these different immunoreactants from animals treated with Crry-Ig and saline.

Crry-Ig Levels and Complement Inhibition

Average Crry-Ig levels were 5.9 \(\mu g/ml\) in Crry-Ig–treated animals at a time immediately preceding the next dose of Crry-Ig \((i.e.,\) trough levels). Even with these relatively low serum levels of Crry-Ig \((21)\), animals had significant complement inhibition. C3b deposition on zymosan was 8.4 ± 1.2 U/ml in the control group \((8.9 ± 1.6\) in saline and \(8.4 ± 1.8\) in normal IgG groups), whereas it was reduced nearly 60% to 3.9 ± 0.8 U/ml in Crry-Ig–treated animals \((P = 0.002)\).

As expected, levels of C3 in serum were depressed in control MRL/lpr animals at both 16 and 24 wk relative to MRL/+ animals (Table 3). At both time points, MRL/lpr animals treated with Crry-Ig had higher serum C3 levels than control MRL/lpr mice \((but lower than MRL/+ animals)\), although the difference between control animals did not reach statistical significance \((P = 0.084\) at 16 wk).

Autoimmune Features in MRL/lpr Mice Treated with Crry-Ig

The excoriating dermatitis in the scapular region and ear necrosis are felt to be manifestations of small vessel vasculitis in MRL/lpr mice \((25,28)\). As shown in Figure 6, Crry-Ig–treated animals demonstrated a decreased incidence of dermatitis (panel A) and ear necrosis (panel B) compared with control groups. At 18 wk of age and beyond, among the control group mice, those treated with normal mouse IgG had greater incidences of dermatitis and ear necrosis than animals treated with saline (not shown).

Complement inhibition with Crry-Ig had no effect on anti-

Table 1. Histologic data in 24-wk-old MRL/lpr mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Glomerulonephritis</th>
<th>Glomerulosclerosis</th>
<th>Arteritis</th>
<th>Interstitial Nephritis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crry-Ig ((n = 9))</td>
<td>1.7 ± 0.3</td>
<td>0.8 ± 0.3</td>
<td>1.4 ± 0.4</td>
<td>1.3 ± 0.1</td>
</tr>
<tr>
<td>Control ((n = 14))</td>
<td>1.9 ± 0.3</td>
<td>1.6 ± 0.2</td>
<td>1.4 ± 0.3</td>
<td>1.7 ± 0.2</td>
</tr>
</tbody>
</table>

a Semiquantitative scores \((0\) to \(4)\) for the extent and severity of histologic indices of renal disease in animals treated from 12 to 24 wk of age with Crry-Ig or mouse IgG or saline as controls, are presented as mean ± SEM.

b \(p < 0.05\) versus control group.

Figure 4. Effects of complement inhibition with Crry-Ig on renal histology in MRL/lpr mice. Shown are representative micrographs from 24-wk-old MRL/lpr mice treated with Crry-Ig (A), mouse IgG (B), or saline (C). In panels A and B, there is diffuse mesangial proliferation. In panel B, there is segmental hyalinosis (arrowhead). In panel C, there is diffuse proliferative GN with one extensively sclerotic glomerulus (asterisk) and one with segmental hyalinosis (arrowhead). The latter and one additional show cellular crescents (arrows). Magnification, \(×200\).
dsDNA levels at any time relative to the other two control groups (Figure 7). Spleen weights were also no different among the groups (0.66 ± 0.05 and 0.59 ± 0.06 g in control and Crry-Ig-treated animals, respectively). Thus, while complement inhibition affects disease in kidney and small vessels, it does not appear to affect these relevant features of autoimmunity in MRL/lpr mice.

Given the protection from small vessel vasculitis, as well as the significant reduction in IgG3 deposition in glomeruli, it was important to consider whether complement inhibition with Crry-Ig had any effect on IgG3 in cryoglobulins, which are felt to be relevant to these manifestations (25,28). However, there was no difference in the levels of cryoglobulin IgG3 between groups (1.3 ± 0.4 and 1.6 ± 0.5 mg/ml in control and Crry-Ig-treated animals, respectively).

Because the complement system is well known to be important to clear circulating immune complexes (29) along with our finding that there was less immune complex deposition in glomeruli of Crry-Ig-treated MRL/lpr mice, at least as judged by IF microscopy, levels of circulating immune complexes were measured over time in all animals. Surprisingly, beginning at 18 wk of age and extending to the termination of the study when the animals were 24 wk of age, Crry-Ig-treated animals had markedly higher levels of circulating immune complexes compared with control MRL/lpr animals treated with either saline or normal mouse IgG (Figure 8). In these latter two groups, there were minimal differences (hence, the standard error bars are contained within the symbols at all ages in this group). Sera from MRL/MRL+ and Balb/c animals had undetectable circulating immune complexes by this technique.

### Table 3. Serum C3 levels in 16- and 24-wk-old MRL/lpr mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>16 wk</th>
<th>24 wk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crry-Ig</td>
<td>87.4 ± 5.4 (n = 16)</td>
<td>83.5 ± 4.9 (n = 10)</td>
</tr>
<tr>
<td>Control</td>
<td>76.0 ± 3.3 (n = 35)</td>
<td>73.1 ± 6.8 (n = 14)</td>
</tr>
</tbody>
</table>

* Serum levels of C3 were measured by ELISA in 16- and 24-wk-old MRL/lpr mice treated from 12 wk of age with Crry-Ig or mouse IgG or saline as controls. Data are presented as percent of C3 levels present in control (non-diseased) 24-wk-old MRL+/+ mice (mean ± SEM).
To investigate the role of chronic complement inhibition at the point of C3 activation in lupus using a strategy that mimics human disease treatment, we treated MRL/lpr mice with a recombinant soluble form of the mouse membrane complement inhibitor, Crry. To provide this soluble Crry with optimal pharmacokinetic properties, two Crry molecules were fused to the hinge, CH2 and CH3 domains of mouse IgG1. This was chosen because it is a non-complement-activating isotype and the resulting Crry-Ig protein would not be recognized as foreign when used chronically in mice. Crry-Ig has a two-phase serum elimination profile with a rapid initial loss followed by a second prolonged decline with a $t_{1/2}$ of 40 h (21). Although our previous studies have shown that Crry-Ig was effective in the nephrotoxic serum nephritis model occurring over 18 h, the true advantage of Crry-Ig could be exploited in chronic studies in mice as we have done here. At an alternate day dosing schedule, sufficient Crry-Ig was present even at trough levels to result in complement inhibition roughly 60% that of control animals.

We found that complement inhibition with Crry-Ig nearly completely inhibited the development of renal disease in the MRL/lpr model. This was shown by several measures of renal disease, including cumulative or preterminal BUN values and the cumulative incidence of renal failure. In addition, Crry-Ig-treated mice had only mild albuminuria over the time period in which glomerular permselectivity defects progressively worsen and as shown in this study in control animals not treated with a complement inhibitor. Thus, these robust results allow us to state definitively that complement inhibition with Crry-Ig clearly influences renal disease in MRL/lpr lupus mice.

Of the histologic measures of renal disease, the only one that was significantly different between surviving Crry-Ig-treated animals and controls was glomerulosclerosis. Although scores for the severity of both GN and interstitial nephritis were lower in complement-inhibited animals, neither was statistically significant. Potential reasons why pathologic scores were not different while renal function was included were the relatively small numbers of surviving animals, limitations in the scoring system itself, and informative censoring through loss of the most severely affected animals. Glomerulosclerosis is related to renal functional demise is supported by the strong correlation between the extent of sclerosis and BUN ($r = 0.80$, $P < 0.001$) and albuminuria ($r = 0.85$, $P < 0.001$). These data also support that glomerulosclerosis is affected by complement inhibition.

### Discussion

To investigate the role of chronic complement inhibition at the point of C3 activation in lupus using a strategy that mimics human disease treatment, we treated MRL/lpr mice with a recombinant soluble form of the mouse membrane complement inhibitor, Crry. To provide this soluble Crry with optimal pharmacokinetic properties, two Crry molecules were fused to the hinge, CH2 and CH3 domains of mouse IgG1. This was chosen because it is a non-complement-activating isotype and the resulting Crry-Ig protein would not be recognized as foreign when used chronically in mice. Crry-Ig has a two-phase serum elimination profile with a rapid initial loss followed by a second prolonged decline with a $t_{1/2}$ of 40 h (21). Although our previous studies have shown that Crry-Ig was effective in the nephrotoxic serum nephritis model occurring over 18 h, the true advantage of Crry-Ig could be exploited in chronic studies in mice as we have done here. At an alternate day dosing schedule, sufficient Crry-Ig was present even at trough levels to result in complement inhibition roughly 60% that of control animals.

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Complement inhibition with Crry-Ig did lead to a modest increase in survival, as half of the MRL/lpr mice (10 of 20) treated with Crry-Ig survived to 24 wk compared with 36.8% of control animals (14 of 38), although this improvement was not statistically significant. Our careful analyses of renal function (BUN and albuminuria) allow us to state with assuredness that none of the ten complement-inhibited animals that died spontaneously did so from renal disease. Clearly there are other organ systems involved in lupus mice (20,30), as is true in the human disease.

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Potential cellular sources of excessive matrix production include intrinsic glomerular cells, fibroblasts, and macrophages under stimulation from transforming growth factor-β, basic fibroblast growth factor, platelet derived growth factor, interleukin-1, and tumor necrosis factor (31). These cytokines can be released in vitro and in vivo by complement activation products (32–35), and each has been shown to be relevant to murine lupus nephritis (36–40). Coincident with the generation of this inflammatory milieu is the upregulation of the mRNA for types I, III, and IV collagen, laminin, and heparan sulfate proteoglycan (41), with the end result being renal scarring. Thus one can envision potential mechanisms by which inhibiting the complement system and decreasing release of C3a, C5a, and C5b-9 membrane attack complex could lessen the ultimate development of glomerulosclerosis.

As expected from the chronic use of Crry-Ig, there was less C3d immunostaining in glomeruli. This glomerular C3d is likely to be the result of deposited C3b being acted on by factor I using CR1 as cofactor (42–45) and gives an indication of past complement activation (46). Although C3 was also reduced in animals receiving Crry-Ig, this was not statistically different, a reason for which is not fully apparent.

A striking finding in this study was the clear differences in immune complex handling in blood and glomeruli comparing complement-inhibited animals with control groups. Consistent with the role for complement activation to the level of C3b to facilitate the clearance of immune complexes in mice (23,47) (as well as primates [48]) is that complement inhibited animals had much higher circulating immune complex levels. What was surprising given the finding of high circulating immune complex levels in Crry-Ig-treated mice was the significant reduction in glomerular staining for IgG, IgG3, and IgA in these animals, which occurred without apparent effect on other relevant features of autoimmunity. This discordance between circulating and glomerular deposited immune complexes is undoubtedly very complex but may reflect the sizes of the complexes (23), their clearance through the immune adherence receptors on platelets (in mice) (47,49), and effects of complement activation directly in the glomerulus (50). Furthermore, in preliminary studies using microarrays, we have seen significant MHC class II and Ig gene expression in glomeruli and cortex of control animals, which was markedly reduced by complement inhibition. These data raise the possibility that complement activation products influence the local appearance of antigen-presenting cells and immune effector cells such as lymphocytes, which are likely to be relevant to immune renal injury (51–53). Separating which of these potential mechanisms are responsible for that which we have observed here is the subject of ongoing studies in our lab.

Of interest was our finding that the skin lesions affecting both the back and ears of MRL/lpr mice were clearly affected by complement inhibition. The evidence to date indicate that this is likely the result of IgG3-containing cryoglobulins depositing in dermal vasculature (28,54). One potential explanation for our observed effect is that complement inhibition directly affected the levels of these in sera (25), although this was not the case in our studies. Alternatively, it is possible that despite their deposition in dermal capillaries, complement inhibition prevented the subsequent inflammation. Although these complexes have the capacity to activate complement, a direct role for complement activation in resulting inflammation has not been shown in previous studies (54). It is thus possible that other immune complexes were responsible for the dermal vasculitis we observed, and the ensuing inflammation was favorably affected by treatment with Crry-Ig. In contrast, the vasculitis occurring around larger vessels, such as in the kidney, was not affected by complement inhibition with Crry-Ig. This arteritis is composed of accumulations of so-called double-negative (CD4+CD8+ B220+TCRαβ+) T lymphocytes and is therefore unlikely to have a complement mediation.

The use of animals in which specific gene products have been targeted through homologous recombination (knockouts) has permitted a great deal of insight into the roles of specific gene products. Such studies have been extended to the lupus mouse models through the backcrossing of specific deficiencies into lupus strains. Deficiency of C1q accelerated disease in MRL/Mp+/+ mice (55), whereas deficiencies of C4 or CR1/CR2 resulted in enhanced autoimmune disease, including lupus nephritis, in C57BL/6.lpr/lpr mice (56–58). Although almost certainly an oversimplification, these data have been interpreted to indicate that classical pathway activation on apoptotic debris, rich in nuclear components, is necessary for their appropriate clearance and maintenance of tolerance, as signaled through the B lymphocyte CR1/CR2 (59,60).

What is hard to reconcile with the various hypotheses is that C3 deficiency does not affect the development of lupus in MRL/Mp-lpr/lpr (24) or C57BL/6.lpr/lpr mice (with or without C4 deficiency) (56). Detailed serologic studies illustrated the C3 deficiency did not affect the autoimmunity per se. C3 occupies the central portion of all three complement pathways; therefore, these studies argued that complement activation plays no role in glomerular pathology. However, C3 deficiency also led to an increase in glomerular IgG deposition, reflecting immune complex processing abnormalities that clearly occur in these C3-deficient mice (23,50,50). Glomerular-deposited immune complexes interact with Fc receptors on inflammatory cells, creating a complement-independent but cell-dependent disease (61,62). On balance, it seems likely that potential complement dependence in C3-deficient mice can be eliminated with increasing amounts of immune complex deposition. In contrast, using Crry-Ig to partially and intermittently block C3 activation allows for effective immune complex processing (even in a decrease in glomerular IgG, as we have shown here) along with diminished inflammation in the kidney. Thus, a predominantly “protective” phenotype is present in Crry-Ig-treated MRL/lpr mice that is absent in mice completely lacking C3.

Consistent with the findings here supporting a complement dependence of lupus nephritis is the finding that MRL/Mp-lpr/lpr mice deficient in factor B are protected from lupus nephritis (25). These findings are also somewhat surprising, as traditional thinking has lupus nephritis occurring through immune complex-directed classical pathway activation. However, in
that setting again partial C3 activation allows effective immune complex processing by the classical pathway, but the decrease in total C3 activation in the absence of factor B provides a protective effect to the kidney. Thus, our results are more comparable to factor B deficiency than complete C3 deficiency.

In summary, we have shown that chronic complement inhibition with Crry-Ig, a potent inhibitor of complement C3 convertases, beginning at the onset of autoimmune disease in MRL/lpr lupus mice and extending through the evolution of the disease, dramatically protects against the development of renal and dermal disease manifestations. Protection from lupus nephritis occurred in several aspects of the renal disease, those being albuminuria, indicating abnormalities in glomerular permselectivity to protein passage, elevated BUN levels, reflective of impaired glomerular filtration, and the development of glomerulosclerosis, the end result of glomerular inflammation. Our findings have clear relevance to human lupus nephritis, as these animal models are felt to have parallels to the human disease. Furthermore, compounds with similar activity profiles to Crry-Ig (in particular soluble recombinant human CR1) are currently available for use in human diseases (16,17,19,63). These data support the use of complement inhibitors in human SLE.

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See related editorial, “Complement Inhibitors and Glomerulonephritis: Are We There Yet?” on pages 815–818.

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