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Complement Component C3 Is Not Required for Full Expression of Immune Complex Glomerulonephritis in MRL/lpr Mice

Hideharu Sekine,* Christopher M. Reilly,* Ivan D. Molano,* Gérard Garnier,† Antonella Circolo,* Philip Ruiz,‡ V. Michael Holers,§ Susan A. Boackle,§ and Gary S. Gilkeson1*

Complement activation and tissue deposition of complement fragments occur during disease progression in lupus nephritis. Genetic deficiency of some complement components (e.g., Factor B) and infusion of complement inhibitors (e.g., Crry, anti-C5 Ab) protect against inflammatory renal disease. Paradoxically, genetic deficiencies of early components of the classical complement pathway (e.g., C1q, C4, and C2) are associated with an increased incidence of lupus in humans and lupus-like disease in murine knockout strains. Complement protein C3 is the converging point for activation of all three complement pathways and thus plays a critical role in biologic processes mediated by complement activation. To define the role of C3 in lupus nephritis, mice rendered C3 deficient by targeted deletion were backcrossed for eight generations to MRL/lpr mice, a mouse strain that spontaneously develops lupus-like disease. We derived homozygous knockout (C3−/−), heterozygous (C3+/−), and C3 wild-type (C3+/+) MRL/lpr mice. Serum levels of autoantibodies and circulating immune complexes were similar among the three groups. However, there was earlier and significantly greater albuminuria in the C3−/− mice compared with the other two groups. Glomerular IgG deposition was also significantly greater in the C3−/− mice than in the other two groups, although overall pathologic renal scores were similar. These results indicate that C3 and/or activation of C3 is not required for full expression of immune complex renal disease in MRL/lpr mice and may in fact play a beneficial role via clearance of immune complexes.


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Abbreviations used in this paper; IC, immune complex; GBM, glomerular basement membrane; TMB, 3,3′,5,5′-tetramethylbenzidene; SLE, systemic lupus erythematosus; RT, room temperature; DAF, decay accelerating factor; MCP, membrane cofactor protein.

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which the MRL/lpr (129) F1 C3-deficient mice. Heterozygous F1 offspring were backcrossed (22). MRL/lpr genomic tail DNA into a 25-bp flanking region and the first 105 bp of exon 1 of the C3 gene with the neomycin resistance gene as previously described (22). MRL/lpr mice deficient in C3 were derived by genetic backcrosses in which the MRL/lpr parental strain was initially mated to heterozygous (B6 × 129)/F1, C3-deficient mice. Heterozygous F2 offspring were backcrossed for seven generations with MRL/lpr mice, resulting in eight genetic backcrosses with the MRL/lpr genetic background. The eighth backcross generation was interbred to yield Fas-/-C3-/-, Fas-/- C3+/-, and Fas+/- C3-/- mice. The mice were housed and bred under the pathogen-free conditions initially in the Division of Comparative Medicine at the Washington University School of Medicine (St. Louis, MO) and subsequently at the animal facility of the Ralph H. Johnson Veterans Affairs Medical Center (Charleston, SC).

Materials and Methods

Generation of complement C3-deficient MRL/lpr mice

MRL/lpr (MRL/lpr2) mice were purchased from The Jackson Laboratory (Bar Harbor, ME). The C3-deficient construct was generated by replacing 2.3 kb of the 5’ flanking region and the first 105 bp of exon 1 of the C3 gene by the neomycin resistance gene as previously described (22). MRL/lpr mice deficient in C3 were derived by genetic backcrosses in which the MRL/lpr parental strain was initially mated to heterozygous (B6 × 129)/F1, C3-deficient mice. Heterozygous F2 offspring were backcrossed for seven generations with MRL/lpr mice, resulting in eight genetic backcrosses with the MRL/lpr genetic background. The eighth backcross generation was interbred to yield Fas-/-C3-/-, Fas-/- C3+/-, and Fas+/- C3-/- mice. The mice were housed and bred under the pathogen-free conditions initially in the Division of Comparative Medicine at the Washington University School of Medicine (St. Louis, MO) and subsequently at the animal facility of the Ralph H. Johnson Veterans Affairs Medical Center (Charleston, SC).

Genotyping of the mice by PCR

Primers for PCR for C3 were as follows: V789 (5’- C3-’AGGGACACAG CCCAGGTTACG-3’), V788 (5’- Neo5 5’-TCTGCTGCAGCTTCAT- TCAG-3’), and Fas C 5’-GATCCCATGAGCCTAA-GT-3’. DNA was isolated from tail snips (3- to 4-week-old mice) using a QiAamp Tissue kit (Qiagen, Santa Clarita, CA). PCR was performed by adding 500 ng of genomic tail DNA into a 25-μl reaction mixture containing 1.5 mM MgCl2, 0.5 μM concentrations each of oligonucleotide mix, 0.2 mM concentrations each of dNTP mix, and 1.5 U of AmpliTaq Gold (Perkin-Elmer, Norwalk, CT). PCR was performed with a 10-min denaturation step of 94°C followed by 40 cycles of 94°C for 1 min, 60°C for 2 min, and 72°C for 3 min with a final extension of 10 min at 72°C in a Perkin-Elmer GeneAmp 9600. After PCR amplification, samples were electrophoresed in 1% agarose gels containing 3,3’-diaminobenzidine (DAB; Sigma) and TMB substrate. The same method was used for measurement of serum Ig isotype levels and total Ig in sera and in cryoglobulins, except HRP-conjugated goat anti-mouse IgG (γ-chain specific; Sigma) and TMB for color development. Amax was measured as above. Results are shown as the Amax at a 1/100 dilution. dsDNA was derived from S1 nuclease (Sigma) treatment of phenol-purified calf thymus DNA.

Measurement of anti-DNA Ab

Anti-DNA Ab levels were measured by ELISA as previously described (24). Briefly, 96-well ELISA plates were coated with 5 μg/ml double-stranded calf thymus DNA (Sigma) in sodium salt citrate buffer at 37°C overnight. After washing with PBS-T, sera were added in serial dilutions starting at 1/100 and incubated for 45 min at RT. After washing with PBS-T, HRP-conjugated goat anti-mouse IgG Ab (γ-chain specific; Sigma) was added, followed by TMB for color development. A380 was measured as above. Results are shown as the A380 at a 1/100 dilution.

Isolation of cryoglobulins from sera

Cryoglobulins were isolated from sera as previously described (26). Briefly, the mice were bled and blood samples were immediately placed at 37°C for 2 h. After centrifugation at 300 × g at 37°C, supernatants were collected and incubated at 4°C for 72 h. After incubation, the samples were centrifuged at 2000 × g, supernatants were removed, and precipitates were washed five times with cold PBS. After being washed, they were resuspended in PBS at the same volume as the original sera. IgG content was then measured. The isolated cryoglobulins were placed at 37°C for 5 h before use. All assays of cryoglobulins and their activity were performed using warmed plates and reagents. Assays were conducted in a hot room maintained at 37°C.

Measurement of Igs

Total IgG Ab levels in sera or in cryoglobulins were determined by ELISA using a standard curve of known concentrations of mouse IgG. ELISA plates were coated with 1 μg/ml goat anti-mouse Ig (κ-chain specific; Southern Biotechnology Associates, Birmingham, AL) overnight at 4°C and then warmed to 37°C for cryoglobulin assays. After washing with PBS-T, sera or cryoglobulins were added in serial dilutions starting at 1/100 dilution and incubated for 45 min at RT. Color development was measured as described above by using a HRP-conjugated goat anti-mouse IgG (γ-chain specific; Sigma) and TMB substrate. The same method was used for measurement of serum isotype levels and total Ig in sera and in cryoglobulins, except HRP-conjugated goat anti-mouse IgG1, IgG2a, IgG2b, or IgG3 or HRP-conjugated goat anti-mouse H and L chains were used (Southern Biotechnology Associates).
Measurement of circulating ICs, rheumatoid factor, and C3 levels in serum

Circulating ICs levels were determined by the C1q ELISA methods previously described with some modifications (27). ELISA plates were coated with 10 μg/ml human C1q (Sigma) in 0.1 M carbonate buffer (pH 9.6), incubated for 48 h at 4°C, blocked for 2 h at RT with 1% BSA in PBS, and washed with PBS. EDTA-treated sera samples were added in serial dilutions starting at a 1/150 dilution and incubated for 1 h at RT and overnight at 4°C. The assay was then performed as described above with HRP-conjugated goat anti-mouse IgG (γ-chain specific; Sigma). Aggregated human γ-globulin was used as a positive control. BALB/c mice sera (The Jackson Laboratory) were used as a negative control.

Rheumatoid factor levels in serum were measured by ELISA as previously described (21). ELISA plates were coated with rabbit IgG (1 μg/ml) overnight at 4°C. After washing, sera were added in serial dilutions, starting at 1/150 dilution, and incubated for 45 min at RT. The assay was then performed as described above with HRP-conjugated rabbit anti-mouse IgG (Fc specific; Pierce, Rockford, IL) or rabbit anti-mouse IgM (μ-chain specific; Pierce).

C3 levels in serum were measured by ELISA. ELISA plates were coated with goat anti-mouse C3 (Cappel) and incubated overnight at 4°C. After washing and blocking with 5% BSA in PBS for 1 h, sera were added in serial dilutions, starting at 1/200 dilution, and incubated for 1 h at RT. The assay was then performed as described above with HRP-conjugated goat anti-mouse C3 (Cappel). BALB/c mice sera (The Jackson Laboratory) were used as a positive control.

Pathology

At the time of sacrifice (24 wk), the kidneys were removed. One kidney was fixed with 10% buffered Formalin, embedded in paraffin, and then sectioned before staining with hematoxylin and eosin. The other kidney was snap frozen in liquid nitrogen and placed in OCT medium. Frozen sections (4-μm thick) were stained with fluorescein-conjugated anti-mouse IgG or C3. The hematoxylin and eosin kidney slides were interpreted in a blinded fashion and graded for glomerular inflammation, proliferation, crescent formation, and necrosis. Interstitial and tubular changes were also noted. Scores from 0 to 4+ (0, none; 1+, mild; 2+, moderate; 3+, moderate-severe; 4+, severe; scores of crescent formation and necrosis were doubled) were assigned for each of these features and then added together to yield a final renal score. Changes were also assessed as to whether they were focal or diffuse. Vasculitis was judged as either being present or absent. Immunofluorescence slides were read blinded and graded 0–4+ (0, none; 1+, mild staining; 2+, moderate staining; 3+, moderate-high staining; 4+, high staining) for fluorescence intensity.

Statistics

The unpaired Student’s t test was used to test for significant differences between groups. The Mann-Whitney two-tailed U test or Kruskal-Wallis test was used to determine the significance of changes in renal score (Table I), glomerular C3 deposition (Table II), and glomerular IgG deposition (Fig. 6). A p < 0.05 was considered to be statistically significant.

Results

Genotype and phenotype of the mice

The genotype of the mice was determined by PCR for C3 and Fas. After the eighth backcross to MRL/lpr mice, C3+/+ mice were bred to generate double homozygous animals: C3+/−/C3+/−, Fas+/−/Fas+/− (MRL/lpr C3+/−/C3+/−), C3+/−/Fas+/− (MRL/lpr C3+/−/C3−/−), and C3−/−/Fas+/− (MRL/lpr C3−/−/C3−/−) mice. In the pups from these breedings, the C3-null gene was inherited in Mendelian fashion with no significant observable fetal loss. The MHC class II genotype of the mice was determined by PCR RFLP analysis. All mice selected for study in each group were H-2k.

To verify that the genotype of the mice was expressed phenotype, serum C3 levels were measured in the study mice over time. As shown in Fig. 1, C3 was undetectable in the serum of the MRL/lpr C3+/− mice and was at intermediate and highest levels in the C3+/− and C3+/+ mice, respectively (p < 0.05), indicating a gene dose effect on serum C3 levels. There was a trend toward decreasing serum C3 levels in the C3+/− and C3+/+ mice with aging and disease expression.

The gross appearance of the mice changed with aging. At 20 wk of age, >50% of the mice in each group began to develop an excoriated skin rash on the shoulder area as well as ear necrosis (>50%). These skin changes are characteristic of MRL/lpr disease and are attributed to IgG3 cryoglobulinemia (28, 29). There were no differences in the incidence or severity of skin rash or ear necrosis among the three groups.

At the time of sacrifice of the mice at 24 wk of age, there were no differences in body weights or spleen weights among the three groups (data not shown). Splenocytes were assayed by flow cytometry and no significant differences were noted in cell number or percentage of CD19+, CD21+, CD44+, CD8+, IgM+, or CD3+ cells (data not shown).

Ig levels in sera or in cryoglobulins

Deficiency of C3 is known to have effects on overall IgG levels, whereas complement receptor deficiency affects IgG isotype levels (2, 30, 31). It is also known that MRL/lpr renal disease and vasculitis are linked with IgG3 cryoglobulin production (28, 29, 32, 33). We measured serum levels of total IgG and IgG3 by ELISA and found no statistically significant differences among the three groups (Fig. 2, A and B). Measurements of the other IgG isotypes (IgG1, IgG2a, and IgG2b) and total IgG also demonstrated no statistically significant differences among the three groups, although the C3−/− mice trended toward lower IgG2a levels (data not shown).

Serum levels of IgG3 cryoglobulins were also measured by ELISA. There were no statistically significant differences among the three groups, although at the age of 22 wk, the C3−/− mice trended toward lower IgG3 cryoglobulin levels compared with the C3+/− and C3+/+ mice (Fig. 2C).

Measurement of serum autoantibody levels

Production of anti-dsDNA Abs, a T cell-dependent autoimmune response, is linked with renal disease in MRL/lpr mice, and it is known that deficiency of C3 inhibits T cell-dependent B cell responses (2, 21, 34, 35). To determine whether C3 deficiency had any effect on anti-DNA and/or anti-GBM Ab production, serum levels of these autoantibodies were measured by ELISA. As shown...
in Fig. 3, serum levels of anti-dsDNA and anti-GBM Ab in the three groups increased as the mice aged. However, there were no significant differences among the three groups at any time point. Similar to the anti-DNA and anti-GBM Ab responses, the lack of C3 did not affect IgG or IgM rheumatoid factor production (data not shown).

**Circulating ICs**

To determine the role of C3 on clearance of circulating ICs, we measured circulating ICs by ELISA using the C1q binding assay. As shown in Fig. 4, the mice in all three groups developed increasing serum levels of ICs with aging, although there were no significant differences among the three groups at any time point. Sera from control BALB/c mice had no detectable circulating ICs by this assay (data not shown).

**Urinary albumin excretion**

To determine the effect of C3 on autoimmune nephritis, we measured 24-h urinary albumin excretion by ELISA beginning at 12 wk of age. As shown in Fig. 5, the MRL/lpr C3+/+ mice developed increasing albuminuria at 16 wk of age, while albuminuria remained <0.1 mg/mouse/day in the other two groups. At 20 wk of age, the C3+/+ mice also started to develop albuminuria, while the C3+/− mice had a further delayed onset and a lower level of albuminuria. At 24 wk of age, there was a significant difference in albuminuria between the C3+/− group and the other two groups. Due to the death of the most severely affected C3+/− mice between 24 and 30 wk, there was a decrease in albuminuria at 30 wk in this group.

**Renal pathology**

A group of mice was sacrificed at the age of 24 wk (nine C3+/+, four C3+/−, and nine C3−/− mice). Despite the increased albuminuria in the C3+/− mice, there were no differences among the
three groups of mice in the overall renal pathology scores (Table I). In the glomerular region, each of the three groups had marked glomerular hypercellularity, mesangial expansion, inflammation, and epithelial reactivity. In the interstitial region, all of the mice had chronic interstitial inflammation with infiltration of inflammatory cells. None of the kidneys from the three groups had significant tubular changes.

Medium vessel vasculitis in the kidney is a pathologic feature of renal disease in MRL/lpr mice (29). At 24 wk of age, there was no significant difference in the incidence or severity of vasculitis between the three groups (Table I).

Immunofluorescence analysis

To determine the role of C3 genotypes on glomerular IC deposition, immunofluorescence analysis was performed. Frozen sections of the kidneys from the three groups were stained with fluorescein-conjugated anti-mouse C3 or IgG. As expected, there was no C3 deposition in the glomeruli of the C3+/− mice, while it was readily evident in the glomeruli of the other two groups (Table II).

As shown in Fig. 6, there was significantly greater IgG deposition overall in the C3−/− mice compared with the wild-type C3+/+ mice, although there was overlap in graded values between the two groups. The small numbers of C3−/− mice precluded statistical comparison of IgG deposition to the other two groups, although the glomerular IgG deposition in the C3−/− mice was overall similar to the C3+/− mice.

Survival

MRL/lpr C3−/− and C3+/− mice had increased early mortality compared with the C3+/+ group with 57% mortality at 30 wk compared with 22% of the mice in the C3+/+ mice (Fig. 7). The difference in the survival between the C3+/− and C3+/+ groups and the C3−/− group, however, did not reach statistical significance.

Discussion

To determine the role of C3 in lupus nephritis, we backcrossed C3-deficient mice to MRL/lpr mice for eight generations and then intercrossed C3+/− mice. The results presented in this report indicate that C3 deficiency in MRL/lpr mice had minimal to no effect on skin disease, spleen size, B cell or T cell number, B cell activation, pathologic renal scores, or production of autoantibodies. C3-deficient MRL/lpr mice did, however, manifest significantly greater albuminuria and glomerular IgG deposition compared with wild-type C3-producing littermates.

Our initial hypothesis was that the genetic deficiency of C3 would likely be protective against the development of renal disease in MRL/lpr mice by preventing the inflammatory response and cellular damage mediated by complement activation. We were also aware, however, that C3 deficiency might worsen disease by altering IC clearance in a deleterious fashion. As described, genetic C3 deficiency in MRL/lpr mice had minimal effect on disease expression with the only significant differences being greater IC deposition in the kidneys and earlier onset of albuminuria in the C3-deficient mice.

Why C3 deficiency in MRL/lpr mice was not protective against renal disease is not clear and is in contrast with previously published reports of the effects of specific complement deficiencies on chronic inflammatory renal disease. Our laboratory recently described the beneficial effect of genetic deficiency of the alternative complement pathway component Factor B in MRL/lpr renal disease (36). In the Factor B-deficient mice, there was decreased C3 activation likely due to the lack of an amplification loop for C3 activation via Factor B. We postulated that this lack of C3 activation may partially explain the protection afforded by Factor B deficiency. It is also possible that MHC differences between the Factor B-deficient mice and the wild-type mice played a role in disease expression. The Factor B-deficient mice were H-2b/k whereas the wild-type mice were H-2a/k, perhaps affecting Ag presentation leading to differences in pathogenic Ab production. There is no published data to date suggesting a role of H-2 in disease in MRL/lpr mice; therefore, the mechanisms underlying renal protection in the Factor B-deficient mice warrants further investigation.

Further evidence for a critical role of complement activation in chronic inflammatory glomerulonephritis was demonstrated by blocking the activation of C5. Anti-C5 Ab treatment effectively decreased proteinuria and proliferative renal disease in NZB/NZW
F1 mice, another murine model of SLE (13). Although we did not measure levels of C5a in our mice, we believe that levels of C5a would be low in mice deficient in C3 given that C3 activation is required for activation of C5. Thus, although the C3-deficient mice were also likely deficient in activation of C5, they still developed renal disease. The lack of effect of C3 deficiency on disease in MRL/lpr and C1q-deficient mice, we believe, reflects the multiple biologic effects of C3. C3 represents a double-edged sword for IC glomerulonephritis. Depending on the model of renal injury or whether disease is acute or chronic, C3 may be harmful due to its activation of inflammatory pathways or beneficial based on its effects in enhancing IC clearance.

It is well known that the activated complement components C4b and C3b bind covalently to ICs, which are then cleared from the circulation by the binding of C4b and C3b to complement receptors on the surface of erythrocytes in humans and platelets in rodents (3, 37, 40). One possible reason for the increased IC deposition in the C3-deficient mice glomeruli is from a lack of C3b. If this is the mechanism for the increased glomerular IC deposition in the C3-deficient mice, then serum IC levels should also be higher in C3-deficient mice, which they were not. The presence of C4b in the C3-deficient mice may have compensated for the lack of C3b in the clearance of circulating IC. It is also possible, although unlikely, that the murine strains differ in the role of C5 in renal disease or that acute glomerulonephritis may be more complement dependent than chronic glomerulonephritis. More likely, however, the differential effect on renal disease reflects the other biologic effects of C3 beyond activation of C5 and its resultant mediation of cellular effector function. In addition, compensatory inflammatory pathways were likely activated in the MRL/lpr mice congenitally deficient in C3. In mice with an intact complement cascade, C5a is a key factor in recruitment of inflammatory cells to the kidney. In mice deficient in the ability to generate C5a from birth, other chemotactic factors, such as IL-8, likely compensate for the lack of C5a. Such compensation may not occur or may be delayed when C5 activation is blocked pharmacologically in an otherwise intact animal.

Our results, however, are in agreement with a recent study by Mitchell et al. (39). Using C1q-deficient mice that develop a lupus-like disease, these investigators determined the role of C3 in renal disease in this alternative murine model of SLE. Mice that are genetically deficient in Factor B and C2 were bred onto the C1q-deficient background. Lacking C2 and Factor B, these mice were unable to activate C3 by either the classical or alternative pathways and thus were functionally C3 deficient. Similar to our results, there was minimal to no effect of C3 deficiency on renal disease in the C1q-deficient mice, indicating that significant lupus-like renal disease can develop without the activation of C3.

Table I. Renal pathology of kidneys from MRL/lpr mice

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Renal Score</th>
<th>Focal</th>
<th>Diffuse</th>
<th>Vasculitis</th>
</tr>
</thead>
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<tr>
<td>C3+/+</td>
<td>9</td>
<td>8.7 ± 6.5</td>
<td>1.6 ± 1.1</td>
<td>1.3 ± 1.1</td>
<td>4/9</td>
</tr>
<tr>
<td>C3−/−</td>
<td>4</td>
<td>12.4 ± 3.4</td>
<td>1.1 ± 0.6</td>
<td>1.5 ± 0.4</td>
<td>1/4</td>
</tr>
<tr>
<td>C3−/−</td>
<td>9</td>
<td>10.3 ± 4.5</td>
<td>1.7 ± 0.8</td>
<td>1.6 ± 0.8</td>
<td>4/9</td>
</tr>
</tbody>
</table>

*At the time of sacrifice (24 wk), the kidneys were removed, then sectioned before staining with hematoxylin and eosin. The kidney slides were graded for glomerular inflammation, proliferation, crescent formation, and necrosis. Scores from 0 to 4 were assigned for each of these features and then added together to yield a final renal score. Data are the mean renal score ± SD. Interstitial changes were also noted. Vasculitis was judged as either being present or absent.

Table II. Glomerular C3 deposition in MRL/lpr mice

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Glomerular C3 Deposition</th>
</tr>
</thead>
<tbody>
<tr>
<td>C3+/+</td>
<td>9</td>
<td>2.10 ± 1.24</td>
</tr>
<tr>
<td>C3−/−</td>
<td>4</td>
<td>2.50 ± 0.58</td>
</tr>
<tr>
<td>C3−/−</td>
<td>9</td>
<td>0.00 ± 0.00</td>
</tr>
</tbody>
</table>

*Glomerular C3 deposition was graded based on the amount of immunofluorescence intensity on a scale from 0 to 4+ by a blinded observer. Data are the mean ± SD of the graded immunofluorescence.

FIGURE 6. Glomerular IgG deposition in MRL/lpr mice. Glomerular IgG deposition was graded based on the immunofluorescence intensity on a scale from 0 to 4+ by a blinded observer. Data are the mean ± SD of the graded immunofluorescence. Significantly higher IgG deposition was observed in the glomeruli of the C3−/− mice than in the wild-type mice. *, p = 0.048 C3+/+ mice vs C3−/− mice.
unlikely, that there were differences in serum levels of IC that we did not detect using the Clq assay.

Another possible reason for increased IC deposition in the glomerulus in the setting of C3 deficiency is altered IC transportation through the GBM. The complement system promotes transfer of IC across the GBM (41, 42). Recently, Sheerin et al. (43), by using an induced model of membranous glomerulonephritis in C3-deficient C57BL/6 mice, demonstrated that C3 deficiency retarded the passage of ICs across the GBM and led to an accumulation of ICs in the glomeruli. C3 and/or subsequent activation of the complement system in the glomeruli presumably alters the physiochemical characteristics of ICs in the subendothelial site that stimulates transmembrane passage. Our data in lupus-prone mice support this hypothesis regarding altered IC clearance in C3-deficient mice. The glomerulus is structurally adapted to filtering, causing it to be continually exposed to IC trapping; therefore, some mechanism is essential to remove ICs. The current data suggest that C3 is a critical mediator for removal of bound ICs in the glomerulus.

In the kidney, complement activation is regulated by a number of complement regulatory proteins such as decay accelerating factor (DAF), CD59, membrane cofactor protein (MCP), and CR1 (44). DAF, MCP, and CD59 are ubiquitously expressed on glomerular endothelial, epithelial, and mesangial cells, while CR1 is localized exclusively on podocytes. In rodents, a single membrane protein “Cry” possesses both DAF- and MCP-like functions and is expressed on the same resident glomerular cells as DAF and MCP (45, 46). Expression of these complement regulatory proteins is altered by complement attack itself and other factors such as cytokines (47–50). In human lupus nephritis, expression of DAF and CD59 is increased in the glomerular capillary wall in epithelial and mesangial cells (47, 51, 52). Similarly, Cry expression is up-regulated in the kidneys of MRL/lpr mice as renal disease progresses (53). This up-regulation of complement regulatory proteins might be a protective response against the complement-mediated injury in this disease. Indeed, injection of soluble Cry and Cry-overproducing transgenic mice protects against injected Ab-induced renal disease, further supporting a deleterious role for complement in these models of glomerulonephritis (53, 54).

The complement system does not act in isolation in modulating inflammation in glomerulonephritis. Other pro-and anti-inflammatory pathways are activated by ICs. Mesangial cells are located in the glomeruli and border directly on endothelial cells (capillaries), which possess numerous microholes at the juxta-capillary region (55). This histological structure allows ICs access to FeRs expressed on mesangial cells (56–58). Mesangial cells play an important role in inflammation in the kidney (59, 60). Interaction of ICs with mesangial cells via Fe receptors triggers a cascade of renal injury characterized by cellular proliferation, matrix protein accumulation, chemokine release, and mononuclear cell recruitment (56, 61–65). A recent study of lupus-prone mice (NZB/NZW F2 mice) deficient for Fe receptor γ-chain I and III expression (FcγRI and FcγRIII) indicated that the presence of FcγRs was required for development of proliferative glomerulonephritis (66). Despite the glomerular deposition of ICs, including C3 and the activation of the complement cascade, FcγR-deficient mice developed significantly less renal disease and had significantly increased survival compared with control mice. These results suggest that C3 activation alone is insufficient to induce renal disease without mesangial cell/macrophage activation through Fe receptors. Complement activation, however, clearly enhances immune responses mediated through Fe receptors and as demonstrated by the Cry and anti-C5 experiments remains a viable therapeutic target in treating glomerulonephritis.

In conclusion, our results demonstrate that 1) C3 and/or activation of C3 is not required for full expression of glomerulonephritis in MRL/lpr mice, and 2) C3 may in fact be beneficial in MRL/lpr mice through direct or indirect effects on the clearance of IC deposition in the glomeruli. These results are in contrast to a number of studies demonstrating beneficial effects of inhibiting complement factors in inflammatory glomerulonephritis. Additional studies are needed to define the factors that mediate the role of complement activation in glomerulonephritis, including the role of local vs systemic production and activation of complement. MRL/lpr C3-deficient mice provide a novel and useful model for dissecting the role of C3-dependent and C3-independent effects in autoimmune disease.

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