Cutting Edge: C1q Protects Against the Development of Glomerulonephritis Independently of C3 Activation

Daniel A. Mitchell, Philip R. Taylor, H. Terence Cook, Jill Moss, Anne E. Bygrave, Mark J. Walport and Marina Botto

*J Immunol* 1999; 162:5676-5679; [http://www.jimmunol.org/content/162/10/5676](http://www.jimmunol.org/content/162/10/5676)

---

**References**
This article cites 23 articles, 12 of which you can access for free at:
[http://www.jimmunol.org/content/162/10/5676.full#ref-list-1](http://www.jimmunol.org/content/162/10/5676.full#ref-list-1)

**Subscription**
Information about subscribing to *The Journal of Immunology* is online at:
[http://jimmunol.org/subscription](http://jimmunol.org/subscription)

**Permissions**
Submit copyright permission requests at:
[http://www.aai.org/About/Publications/JI/copyright.html](http://www.aai.org/About/Publications/JI/copyright.html)

**Email Alerts**
Receive free email-alerts when new articles cite this article. Sign up at:
[http://jimmunol.org/alerts](http://jimmunol.org/alerts)

---

*The Journal of Immunology* is published twice each month by
The American Association of Immunologists, Inc.,
1451 Rockville Pike, Suite 650, Rockville, MD 20852
Copyright © 1999 by The American Association of Immunologists All rights reserved.
Print ISSN: 0022-1767 Online ISSN: 1550-6606.
Cutting Edge: C1q Protects Against the Development of Glomerulonephritis Independently of C3 Activation

Daniel A. Mitchell,2* Philip R. Taylor,2* H. Terence Cook,† Jill Moss,‡ Anne E. Bygrave,* Mark J. Walport,* and Marina Botto3*

C1q-deficient (C1qa−/−) mice develop antinuclear Abs and glomerulonephritis (GN) characterized by multiple apoptotic bodies. To explore the contribution of C3 activation to the induction of spontaneous GN, C1qa−/− mice were crossed with factor B− and C2-deficient (H2-Bf/C2−/−) mice. GN was present in 64% of the 45 C1qa/H2-Bf/C2−/− mice compared with 8% of the 65 H2-Bf/C2−/− mice and none of the 24 wild-type controls. IgG was detected in the glomeruli of diseased C1qa/H2-Bf/C2−/− kidneys. However, glomerular staining for C3 was absent. Increased numbers of glomerular apoptotic bodies were detected in undiseased C1qa/H2-Bf/C2−/− kidneys. These findings support the hypothesis that C1q may play a role in the clearance of apoptotic cells without the necessity for C3 activation and demonstrate that the activation of C3 is not essential for the development of GN in this spontaneous model of lupus-like disease. The Journal of Immunology, 1999, 162: 5676–5679.

The role of complement in the development and expression of autoimmunity in humans appears to be paradoxical. Complement components are present at the sites of tissue injury in inflammatory lesions, including glomerulonephritis (GN); however, inherited homozygous deficiencies of early classical complement pathway proteins, especially C1q and C4, are strongly associated with the development of systemic lupus erythematosus (SLE). The recent findings that C1q may bind specifically to the surface blebs of apoptotic keratinocytes and that C1q-deficient animals were the presence of increased numbers of apoptotic bodies in the glomeruli, a phenomenon also observed in the kidneys of C1qa−/− animals without histological evidence of GN. These observations support the hypothesis that C1q may protect against autoimmunity by serving as an opsonin in the efficient recognition and physiological clearance of apoptotic cells; however, these findings did not fully resolve the question surrounding the importance of complement activation in the development of the spontaneous GN. To address this question, we crossed the C1qa−/− strain with gene-targeted factor B/C2-deficient (H2-Bf/C2−/−) mice, generating mouse strains lacking both the classical and alternative pathways of complement activation in the presence or absence of C1q. These cohorts of mice were sacrificed after 8 mo and analyzed for the presence of autoantibodies and GN. Here, we demonstrate that C1qa−/− mice that also

Copyright © 1999 by The American Association of Immunologists
lack C2 and factor B develop GN without glomerular C3 deposition. Mice lacking C2 and factor B did not develop either GN or autoantibodies, showing a role for C1q alone or in conjunction with C4 in the protection against the development of autoimmunity.

Materials and Methods

Mice

C1qa−/− and H2-Bf/C2−/− mice were generated as described previously (4, 13). All mice were bred in a mixed genetic background (129Sv x C57BL/6) and kept in specific pathogen-free conditions but not in a germ-free environment. The C1qa−/− and H2-Bf/C2−/− mice were crossed to generate a C1qa/H2-Bf/C2−/− strain that was deficient in all three complement components. Animal care and procedures were conducted according to institutional guidelines.

Autoantibody assays and serum biochemistry

Mice were bled at 3, 5, and 8 mo of age; at 8 mo, all of the animals were sacrificed. The serum was stored at −70°C before analysis. Levels of IgG ANA were sought by indirect immunofluorescence using Hep-2 cells (14). Anti-dsDNA Abs were detected by indirect immunofluorescence on Crithidia luciliae (15). Serum samples were screened at a 1/80 (ANA) or 1/20 (anti-dsDNA) dilution, and the positive samples were titrated to endpoint. Abs to ssDNA (calf thymus) were measured by ELISA as described previously (16). Samples were screened at a 1/50 dilution, and the results were expressed in arbitrary ELISA units (AEU) relative to a standard positive sample (derived from an MRL/lpr/lpr mouse) that was assigned a value of 100; samples were scored as positive at ≥7.0 U (3 SD above the lower limit of detection).

Serum creatinine and serum albumin were measured by an autoanalyzer using standard methods.

Histology

Kidney portions were fixed in Bouin’s solution for 4 h, transferred into 70% ethanol, and processed into paraffin. The sections were stained with hematoxylin and eosin and scored for GN as described previously (17). Glomerular hypercellularity was graded on a scale of 0–IV; grade 0 represents no involvement, and grade IV represents severe proliferative GN. For electron microscopy, kidneys were fixed in 4% glutaraldehyde, postfixed in 1% osmium tetroxide, and embedded in Spurr’s resin. Fluorescence microscopy was conducted on snap-frozen sections incubated with FITC-conjugated polyclonal Abs to mouse IgG (Sigma, Poole, U.K.) and mouse C3 (Cappel/ICN Biomedicals, Aurora, OH). For C4 staining, a monoclonal rat anti-mouse C4 (Cedarlane, Ontario, Canada) and a monoclonal FITC-labeled mouse anti-rat IgG secondary Ab (Cappel/ICN Biomedicals, Aurora, OH) were used. Apoptotic bodies were quantified by light microscopy on coded sections. A cell was considered apoptotic when it showed loss of cell volume, chromatin condensation along the nuclear membrane with intensely basophilic staining, and/or nuclear fragmentation into spherical structures containing condensed chromatin.

Statistics

Statistics were calculated using GraphPad Prism version 2.0 (GraphPad Software, San Diego, CA). Nonparametric statistical tests were applied as appropriate.

Results

Autoantibody analysis

Three cohorts of mice, consisting of 45 C1qa/H2-Bf/C2−/−, 65 H2-Bf/C2−/−, and 24 wild-type (wt) animals, were analyzed for the presence of autoantibodies at 3, 5, and 8 mo of age. IgG ANA were detected in 20% of the C1qa/H2-Bf/C2−/− mice at 5 mo, increasing to 40% at 8 mo (range 1:80–1:1280). In comparison, low levels of ANA were detected in only 4% of the wt mice (titer 1:80) and in 1% of the H2-Bf/C2−/− mice (titer 1:80) at 8 mo of age (Mann-Whitney U test, p < 0.0001) (Fig. 1). At 8 mo of age, Abs to ssDNA were detected in 17% of the C1qa/H2-Bf/C2−/− mice (range 14.6–150 AEU) compared with only one of the wt mice (12.9 AEU) and none of the H2-Bf/C2−/− mice (Kruskal-Wallis test, p = 0.0026). Abs to dsDNA were detected in only two C1qa/H2-Bf/C2−/− animals.

Renal histology

Histological examination showed GN in 29 of 45 (64%) of the C1qa/H2-Bf/C2−/− mice at 8 mo compared with only 5 of 65 (8%) of the H2-Bf/C2−/− mice and none of the 24 wt mice (χ² = 55.17, p < 0.0001) (Table I). In the C1qa/H2-Bf/C2−/− group, GN was observed predominantly in females (87%) compared with 41% of the males). The severity of GN and levels of ANA did show a significant correlation, although the correlation was weak (Spearman correlation: p = 0.0443, r = 0.3013). Morphologically, the GN consisted of glomerular hypercellularity with increased numbers of cells in mesangial areas and capillary lumens (Fig. 2A). Renal functional analysis showed no differences in serum creatinine (nephritic kidneys: 37.30 ± 3.20 μmol/l (mean ± SEM); non-nephritic kidneys: 40.00 ± 2.89) and in serum albumin (nephritic kidneys: 24.67 ± 2.60 g/l; non-nephritic kidneys: 23.67 ± 1.30).

Immunostaining revealed the presence of prominent deposits of IgG in the glomeruli of C1qa/H2-Bf/C2−/− mice assessed as positive for GN. The staining was mostly mesangial, with some on the capillary wall. The wt mice with no histological evidence of GN had only weak focal staining in some glomeruli, a pattern also seen in the H2-Bf/C2−/− mice. Kidneys from C1qa−/− mice showed staining that was similar to that observed in the C1qa/H2-Bf/C2−/− mice (Fig. 2B).

Table I. Histological assessment of kidney sections

<table>
<thead>
<tr>
<th>Strain</th>
<th>Sex (n)</th>
<th>0</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1qa/H2-Bf/C2−/−</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>wt (+/+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wt (−/−)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H2-Bf/C2−/−</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C1qa/H2-Bf/C2−/−</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* All of the experimental animals were sacrificed at 8 mo of age to obtain age-matched autopsy specimens. Bouin’s fixed kidney sections were scored for GN. Glomerular hypercellularity was graded on a 0–IV scale, where 0 = no involvement and IV represents severe proliferative GN in >90% of glomeruli.
In the kidneys of wt mice, there was strong peritubular staining for C3, with very weak staining in mesangial areas. Similar weak mesangial C3 staining was seen in the undiseased kidneys of H2-Bf/C2−/− mice and in the nephritic kidneys of C1qa/H2-Bf/C2−/− animals. No peritubular staining was seen in either of these groups. Kidneys from nephritic C1qa−/− mice used as positive controls showed extensive mesangial C3 deposition (Fig. 2C). Immunostaining showed C4 in the mesangium of all of the experimental groups of mice in similar quantity and distribution (data not shown). There was no enhancement of the mesangial staining of C4 by light microscopy, the H2-Bf/C2−/− mouse shows normal glomerular morphology; there is faint glomerular localization of IgG and C3. The glomerular morphology of macrophages were present in capillary lumens.

Electron microscopy in selected cases showed expansion of mesangial areas with multiple electron-dense deposits. Some capillary loops showed subendothelial deposits with formation of a new layer of basement membrane on the luminal side of the deposits and mesangial cell interposition (Fig. 3). Cells with the morphology of macrophages were present in capillary lumens.

As we have reported previously, the most striking histological feature of the GN in the C1qa−/− mice was the presence of multiple apoptotic bodies in glomeruli (4). Assessment of the kidneys of C1qa/H2-Bf/C2−/−, H2-Bf/C2−/−, and wt mice with no histological evidence of GN revealed increased numbers of apoptotic bodies in the glomeruli of the C1qa/H2-Bf/C2−/− animals compared with the other two groups (wt (n = 23): 0.174 ± 0.388 (mean of apoptotic bodies in 50 glomeruli ± SEM); H2-Bf/C2−/− (n = 56): 0.089 ± 0.288; C1qa/H2-Bf/C2−/− (n = 16): 0.875 ± 0.272; Kruskal-Wallis test, p < 0.0013).

Discussion

We have reported recently that C1qa−/− mice develop autoimmunity characterized by the production of ANA and immune complex-mediated renal damage. Deficiency of the FcR complex γ-chain backcrossed onto the autoimmune-prone (NZB × NZW)F1.
background resulted in protection from the development of GN (11) without affecting the production of autoantibodies. An alternative approach to the same question involved the use of the nephrotoxic serum GN model in FcR γ-chain deficient mice and also showed a dramatic amelioration of lupus-like autoimmune disease (4). Detection of glomerular C3 deposition in the diseased kidneys of C1qa−/− animals indicated that complement was activated, but its relevance to the disease (20). Our initial studies in C1qa/H2-Bf/C2−/− mice confirmed a protective role for C1q in the development of GN (4). Detection of glomerular C3 deposition in the diseased kidneys of C1qa−/− animals indicated that complement was activated, but its relevance to the pathogenesis of the GN was unknown. The findings reported here, which showed GN with no C3 deposition in the C1qa/H2-Bf/C2−/− mice compared with the other two groups, would indicate that the development of glomerular damage associated with C1q deficiency occurs predominantly via a complement-independent, and perhaps FcR-mediated, mechanism. In addition, the presence of peritubular C3 staining in the kidneys of the wt mice, but not in kidneys of the H2-Bf/C2−/− mice, suggests that this staining may reflect deposited C3. In contrast, the low level of mesangial staining for C3 and C4 in all groups of mice is most likely the product of local synthesis, supporting a role for glomerular cells in the local production of complement components (21–23).

In conclusion, the data described in this study of complement-deficient mice, when considered in association with recent studies on the role of FcRs in GN, support the hypothesis that the early components of the classical pathway protect from the development of nephritis. If this mechanism is impaired, as in C1q deficiency, renal inflammation may proceed, with FcRs as the dominant transducers of IgG-mediated injury.

Acknowledgments

We thank the staff of our animal facility for the care of the animals used in this work.

References