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

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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

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 autoimmune in humans appears to be paradoxical. Complement components are present at the sites of tissue injury in inflammatory lesions, including glomerulonephritis (GN); however, inherited homoyzogous deficiencies of early classical complement pathway proteins, especially C1q and C4, are strongly associated with the development of systemic lupus erythematosus (SLE) (1). The recent findings that C1q can bind specifically to the surface blebs of apoptotic keratinocytes (2) and that the common autoantigens targeted in SLE can be found in high concentrations on the surface of cells which have undergone apoptosis (3) have led to the new hypothesis that C1q deficiency may cause SLE as a result of an impaired clearance of apoptotic cells (2, 4). In addition, immunization with apoptotic cells has been shown to stimulate autoantibody production (5), suggesting that defects in the pathways by which apoptotic cells are processed could play an important role in driving an autoimmune response.

Recently, there has been considerable debate surrounding the role of complement in the induction and maintenance of inflammation, with growing evidence stressing the important role of Fc receptors (FcRs) in the mediation of the inflammatory responses triggered by immune complexes. Experiments involving the reverse passive Arthus reaction in the skin suggest a dominant role for FcRs, as opposed to complement activation, in initiating inflammation (6–9), although, in a model of reverse passive Arthus reaction in the lungs, there was evidence that complement played a key role in the initiation of the inflammatory injury (10). FcRγ-chain deficiency has been shown to confer marked protection against renal damage both in the spontaneous (NZB × NZW)F1 model of autoimmunity (11) and in a nephrotoxic serum GN model in C57BL/6 mice (12). In these experiments, the mice were protected from severe GN, whereas the glomerular deposition of IgG and C3 remained unaffected, providing strong evidence for a predominant role for FcRs in driving inflammation in autoimmune nephritis.

Gene-targeted homozygous C1q-deficient (C1qa−/−) mice have been shown to develop a spontaneous autoimmune disorder with high titers of antinuclear Abs (ANA) and GN that was associated with renal IgG and C3 deposition (4). A striking feature of the GN in C1qa−/− animals was 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 supported 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 (13), 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
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 (129/Sv × 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 lucilae (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 (AU) relative to a standard positive sample (derived from an MRL/Mp-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 4 represents severe proliferative GN in ≥90% of glomeruli. For electron microscopy, kidneys were fixed in 4% glutaraldehyde, postfixed in 1% osmium tetroxide, and embedded in Spur’s resin. Fluorescence microscopy was conducted on snap-frozen sections incubated with FITC-conjugated polyclonal Abs to mouse IgG (Sigma–Aldrich, St. Louis, MO), mouse IgM (Bioss, Woburn, MA), and mouse IgA (Bioss) (18). For C4 staining, a monoclonal rat anti-mouse C4 (Cedarlane, Ontario, Canada) and a monoclonal FITC-labeled mouse anti-rat IgG secondary Ab (Sigma) 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 throughout.

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 (Kruskal-Wallis 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 AU) compared with only one of the wt mice (12.9 AU) 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>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>wt (+/+)</td>
<td>(n=11)</td>
<td>11</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>H2-Bf/C2/−/−</td>
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<td>28</td>
<td>1</td>
<td>0</td>
<td>0</td>
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<tr>
<td>C1qa/H2-Bf/C2/−/−</td>
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<td>32</td>
<td>3</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>C1qa/H2-Bf/C2/−/−</td>
<td>(n=23)</td>
<td>13</td>
<td>4</td>
<td>3</td>
<td>2</td>
</tr>
</tbody>
</table>

* All of the experimental animals were sacrificed at 8 mo of age to obtain age-matched autopsy specimens. Bowin’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 in the H2-Bf/C2−/− mouse. The C1qa−/− mouse shows hypercellular GN with prominent mesangial staining for both IgG and C3 (all at ×340 magnification).

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−/− mouse 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 histo logical 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 GN associated with the presence of increased numbers of apoptotic bodies (4). IgG and C3 were present in the glomeruli of the diseased kidneys, which suggested that complement was being activated, most likely by the alternative pathway. This possibility led to the question of whether the development of glomerular injury in this model was dependent upon the activation of C3 in glomeruli by the alternative pathway. To test this hypothesis, we crossed mice deficient in C1q with mice deficient in factor B and C2.

Mice deficient in complement activation by disruption of the C2 and factor B genes did not develop spontaneous autoimmunity. When deficiency of C1q was added, renal damage and autoantibody production developed, suggesting a discrete role for the first component of the classical pathway, and possibly C4, in protection from autoimmunity. A striking feature of the glomeruli of aged, undiseased C1qa−/− mice was the presence of elevated numbers of apoptotic bodies (4). This observation coupled with the knowledge that 1) C1q can directly bind to apoptotic cells (2), 2) the surface blebs of apoptotic cells express the common autoantigens of SLE (3), and 3) immunization with apoptotic cells can stimulate autoantibody production (5), has led to the hypothesis that C1q may be involved in the clearance of apoptotic cells, and that this activity may protect from the development of autoimmunity. An elevated number of apoptotic bodies were also present in the undiseased kidneys of the C1qa/H2-Bf/C2−/− mice but not in the H2-Bf/C2−/− animals, suggesting that the proposed defect in the clearance of apoptotic cells in C1qa−/− animals did not require C3 activation.

Recent studies using both spontaneous and induced models of GN have suggested a dominant role for FcRs in the generation of 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 in NZB/W F1 mice after treatment with a blocking monoclonal antibody specific for complement component C5. Prog. Natl. Acad. Sci. USA 93:8563.


