C1q Deficiency and Autoimmunity: The Effects of Genetic Background on Disease Expression


*J Immunol* 2002; 168:2538-2543; doi: 10.4049/jimmunol.168.5.2538
http://www.jimmunol.org/content/168/5/2538

**References**

This article cites 25 articles, 11 of which you can access for free at:
http://www.jimmunol.org/content/168/5/2538.full#ref-list-1

**Subscription**

Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

**Permissions**

Submit copyright permission requests at:
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
C1q Deficiency and Autoimmunity: The Effects of Genetic Background on Disease Expression


Gene-targeted C1q-deficient mice have been shown to develop a syndrome reminiscent of human systemic lupus erythematosus with antinuclear Abs and proliferative glomerulonephritis. Initial phenotypic analysis conducted in (129 × C57BL/6) hybrid mice showed that background genes were a significant factor for the full expression of the autoimmune disease. To assess the contribution of background genes in the expression of the autoimmune phenotype, the disrupted C1qa gene was backcrossed for seven generations onto C57BL/6 and MRL/Mp+/+ strains. These were intercrossed with C57BL/6/lpr/lpr and MRL/Mp-lpr/lpr strains to generate C1q-deficient substrains. In C1q-deficient C57BL/6 mice, no evidence of an autoimmune phenotype was found, and C1q deficiency in both the C57BL/6/lpr/lpr and MRL/Mp-lpr/lpr strains did not modify the autoimmune phenotype observed in wild-type controls. However, in C1q-deficient MRL/Mp+/+ animals an acceleration of both the onset and the severity of antinuclear Abs and glomerulonephritis was seen. Disease was particularly pronounced in females, which developed severe crescentic glomerulonephritis accompanied by heavy proteinuria. In addition, the C1q-deficient MRL/Mp+/+ mice had an impairment in the phagocytic clearance of apoptotic cells in vivo. These data demonstrate that the expression of autoimmunity in C1q-deficient mice is strongly influenced by other background genes. The work also highlights the potential value of the C1q-deficient MRL/Mp+/+ strain as a tool with which to dissect further the underlying mechanisms of the autoimmune syndrome associated with C1q deficiency. The Journal of Immunology, 2002, 168: 2538–2543.

In humans, homozygous deficiencies of early classical complement pathway proteins are strongly associated with the development of systemic lupus erythematosus (SLE). In the case of C1q deficiency, SLE is found in >90% of reported cases (1). Gene-targeted C1q-deficient (C1qa−) mice were shown to develop spontaneously antinuclear Abs (ANA) and proliferative glomerulonephritis (GN) (2). This was demonstrated through the analysis of a large cohort of C1qa−/− mice on the hybrid (129 × C57BL/6) genetic background. Elevated numbers of apoptotic bodies were present in the glomeruli of nondiseased C1qa−/− animals compared with wild-type controls (2). This finding supported the hypothesis that C1q plays a role in the prevention of autoimmunity by facilitating the physiological clearance and processing of apoptotic debris (3–5). Further analysis of the initial C1qa−/− cohorts revealed that background genes were a significant factor in the expression of disease traits, as demonstrated by the presence of severe disease in a small proportion of (129 × C57BL/6) hybrid wild-type controls. In addition, it was observed that autoimmune traits were absent in a smaller cohort of C1q-deficient and wild-type pure inbred 129/Sv mice (2). These data suggested that C1q deficiency was acting as a disease-accelerating factor in a hybrid (129 × C57BL/6) genetic background predisposed to the development of spontaneous autoimmune disease.

The influence of background genes on disease expression in other murine autoimmune models has been described, especially with respect to the lpr and Yaa disease susceptibility genes. In MRL/Mp mice, the presence of the lpr gene accelerates the development of high level and broad spectrum autoantibody production and lethal GN in addition to marked lymphoproliferative disease. In contrast, homozygosity of the lpr gene in other strains such as C57BL/6, AKR, LG/J, and C3H leads only to autoantibody production and lymphoproliferation; GN is largely absent (6). The Y-chromosome-linkled Yaa gene in BXSB and MRL/Mp+/+ males enhances the rapid development of autoantibodies and GN (7, 8). However, in the C57BL/6 background, the Yaa gene does not lead to an autoimmune phenotype (7). Extensive mapping analyses of (New Zealand Black × New Zealand White)-derived cohorts of mice have provided insights into the genetic aspects of disease inheritance in murine models of SLE. Significant conclusions drawn from these studies indicate that murine SLE exists as a complex trait disorder in which specific combinations of susceptibility alleles are required for expression of the full phenotype (9, 10).

To investigate further the role of C1q deficiency in the spontaneous development of autoimmune disease and to provide a clearer perspective on the effects of background genes, the disrupted C1qa gene was backcrossed for seven generations onto C57BL/6 and MRL/Mp+/+ strains. These were intercrossed with C57BL/6/lpr/lpr and MRL/Mp-lpr/lpr strains to generate C1q-deficient substrains. The C57BL/6/lpr/lpr and MRL/Mp-lpr/lpr strains spontaneously develop autoantibodies and lymphoproliferative disease associated with the lpr mutation that gives rise to Fas deficiency, although severe GN and early mortality are only seen in MRL/

---

*Rheumatology Section and †Department of Histopathology, Imperial College School of Medicine, Hammersmith Hospital, London, United Kingdom

Received for publication April 18, 2001. Accepted for publication December 19, 2001.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 This work was supported by Wellcome Trust Grant 054838 and the Arthritis Research Campaign. D.A.M. was the recipient of an Arthritis Research Campaign Ph.D. studentship, and J.C.-H. was the recipient of a fellowship from the National Institute of Health, Spain (BEFI 99/9212).

2 Address correspondence and reprint requests to Dr. Mark J. Walport, Rheumatology Section, Imperial College School of Medicine, Division of Medicine, Hammersmith Hospital, Du Cane Road, London, U.K. W12 ONN. E-mail address: m.walport@ic.ac.uk.

3 Abbreviations used in this paper: SLE, systemic lupus erythematosus; ANA, antinuclear Ab; GN, glomerulonephritis; AEU, arbitrary ELISA unit.
Mp-lpr/lpr mouse (6). The MRL/Mp+/+ strain shares ~99% of its background genome with the MRL/Mp-lpr/lpr strain (11), except that Fas expression is normal. MRL/Mp+/+ mice do not develop the massive lymphoproliferative disorder seen in their lpr/lpr counterparts, although late-onset ANA production and mild GN are found, typically after 12 mo.

We report in this work the results of studies of the effects of homozgyous C1q deficiency on the autoimmune phenotype in C57BL/6 and MRL/Mp+/+ mice in the presence and the absence of the lpr gene.

Materials and Methods

Mice

The disrupted murine C1qa gene was backcrossed for seven generations onto the C57BL/6 strain (Harlan Olac, Bicester, U.K.). The C57BL/6.C1qa+/− (N7) animals were intercrossed with commercially obtained C57BL/6/Lpr/lpr mice (The Jackson Laboratory, Bar Harbor, ME) to generate a novel C57BL/6/Lpr/lpr.C1qa−/− strain. Cohorts of MRL/Mp+/+ Lpr/lpr.C1qa+/− (N7) and MRL/Mp+/+ C1qa−/− (N7) mice were generated in a similar fashion. Throughout the backcrossing procedure, the targeted C1qa and C1qa+ genes were assayed via PCR analyses. All the mice involved in this experiment were maintained in the same specific pathogen-free, but not germ-free, environment. All animal care and procedures were conducted according to institutional guidelines.

Autoantibody assays

Assays were conducted using serum obtained from the various cohorts of mice at designated time points determined by the anticipated and observed stages of disease. Serum was stored at −70 °C before analysis. Levels of IgG ANA were sought by indirect immunofluorescence using Hep-2 cells. Anti-dsDNA Abs were detected by indirect immunofluorescence on Crithidia luciliae (12). Serum samples were screened at a 1/200 (ANA) or a 1/20 (anti-dsDNA) dilution, and the positive samples were titrated to end point. Abs to ssDNA were measured by ELISA as described previously (13). Samples were screened at a 1/50 dilution, and the results were expressed with arbitrary ELISA units (AEU) relative to a standard positive sample (derived from an MRL/lpr mouse) that was assigned a value of 100. Serum samples were considered positive if they were above the mean ± SD of the negative serum. The intra-assay coefficients of variation were between 3.5 and 9.5%, and the interassay coefficients were between 3.6 and 18.9% for the samples measured.

A flow cytometric test was used to detect anti-mouse RBC Abs in the sera of 5-wk-old mice. About 1% of RBC were suspension-prepared from BALB/c mice, in 1% BSA/PBS for 30 min at 4 °C. After washing三次 with 1% BSA/PBS, the RBC were incubated with biotinylated rat anti-mouse κ-chain mAb (H139.52.1; Southern Biotechnology Associates, Birmingham, AL), followed by PE-conjugated streptavidin (BD Pharmingen, Oxford, U.K.) and analyzed with a FACScan (BD Biosciences, San Jose, CA). Results were expressed as the fluorescence intensity. New Zealand Black female serum was used as a positive control, and BALB/c serum was used as a negative control. Serum samples were considered positive if they were above the mean ± SD of the negative serum.

Rheumatoid factor activity was measured by ELISA as previously described (14). Briefly, microtiter plates were coated with 5 μg/ml (4-hydroxy-3-isoo-5-nitropheny)acetetyl conjugated BSA, followed by a mouse IgG2α anti-(4-hydroxy-3-isoo-5-nitropheny)acetetyl Ab (NIP-23) (15). The plates were then incubated with appropriately diluted sera, and the assay was developed with alkaline phosphatase-labeled rat anti-mouse κ-chain mAb (H139.52.1; Southern Biotechnology Associates). The results were expressed in AEU, referring to a standard curve derived from a serum pool of MRL/Mp+/+ mice.

Histology

Kidney portions were fixed in Bouin’s solution for 2 h, transferred into 70% ethanol, and processed into paraffin. The sections were stained with period acid-Schiff reagent and scored for GN as previously described (16). Glomerular hypercellularity was graded on a scale of 0–IV, in which grade 0 represents no involvement and grade IV represents severe proliferative GN in >90% of glomeruli. Additional morphological characteristics such as crescent formation and periglomerular fibrosis were also noted. Proteinuria was assessed in selected MRL/Mp+/+ mice using Harman’s protein-bistix (Bayer Diagnostics, Newbury, U.K.). Apoptotic bodies present in 50 glomeruli/section 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.

Immunofluorescence staining for glomerular IgG and C3 deposits was conducted on selected samples of tissue that were snap-frozen in liquid nitrogen-cooled isopentane. Frozen sections (5 μm) were cut and fixed in acetone and air-dried before incubation with FITC-conjugated anti-mouse IgG (1/40 dilution in PBS; Sigma-Aldrich, Poole, U.K.) or anti-mouse C3 (1/50 dilution; ICN Pharmaceuticals, Costa Mesa, CA). Sections were subsequently washed, and the slides were mounted in Permafluor reagent (Immunon, Pittsburgh, PA) and viewed under a fluorescence microscope.

In vivo apoptotic cell clearance by peritoneal macrophages

Phagocytosis of apoptotic cells by peritoneal macrophages was investigated in vivo as previously described (5). Briefly, inflammatory macrophages were recruited into the peritoneum by injecting 1 ml of sterile 4% thioglycollate. Four days later the mice were injected with 1 × 107 apoptotic Jurkat T cells or 3 × 107 apoptotic murine thymocytes. Jurkat T cells were induced to undergo apoptosis by exposure to UV radiation, followed by 2-h culture in RPMI 1640/0.4% BSA. This resulted in a population of cells that was ~40% apoptotic and ~95% trypan blue negative. Mouse thymocytes were obtained by mechanical dissociation of thymi from 3- to 5-wk-old mice and were induced to undergo apoptosis by 3-h culture in RPMI 1640/0.4% BSA in the presence of 1 μM dexamethasone (Sigma-Aldrich). This resulted in a population of cells that was ~30% apoptotic and ~95% viable. Apoptosis was confirmed by annexin V binding, propidium iodide staining (assayed by flow cytometry), and morphological changes, including nuclear fragmentation and condensation, loss of cell volume, and membrane blebbing (assayed on cytospin preparations). Cells were considered viable when they excluded propidium iodide and trypan blue. After 30 min the mice were sacrificed, the peritoneal cells were recovered by lavage, and phagocytosis was scored on coded cytospins stained with DiffQuick (Dade Behring, Marburg, Germany). Phagocytosis was expressed as the percentage of macrophages ingesting apoptotic cells.

Statistics

Statistics were calculated using GraphPad Prism version 2.0 (GraphPad Software, San Diego, CA). Nonparametric statistical tests were applied throughout. Differences were considered significant for p < 0.05.

Results

Analysis of C57BL/6.C1qa−/− mice

Three cohorts consisting of 54 C1qa−/− (25 males and 29 females), 48 C1qa+/− (23 males and 25 females), and 45 wild-type (22 males and 23 females) C57BL/6 mice were analyzed. ANA titers were measured at 6, 9, and 12 mo of age, and all samples tested were negative. In view of this result, no further autoantibody analyses were conducted. Renal tissue obtained at 12 mo was assessed for the presence of GN. The great majority of mice in all three cohorts showed normal morphology, and no significant differences among the three groups were observed (data not shown). Similarly, the quantification of glomerular apoptotic bodies in the kidneys of these mice did not show a significant difference among any of the three experimental cohorts (data not shown).

Analysis of C57BL/6/lpr.C1qa−/− mice

A total of 33 C57BL/6/lpr.C1qa−/− animals (16 males and 17 females) and 44 C57BL/6/lpr controls (21 males and 23 females) were studied. At 3 mo of age low ANA titers (range, 1/80 to 1/160) were detected, but no significant difference between the two groups was found. A similar picture was seen in the analysis of anti-ssDNA Abs (range, 7–576 AEU, in 15% of the C1qa−/− and 20% of the wild-type mice). A similar analysis was conducted at 4 and 6 mo of age. As expected, the overall prevalence and titer of autoantibodies were increased at these later time points, although no significant difference between the two groups were found. GN was assessed in renal tissue collected at 8–10 mo of age. Severe GN was detected in a small proportion of animals from both groups, and overall no significant difference in the prevalence
or severity of GN existed between the two groups. Apoptotic bodies (expressed as the number found in 50 glomeruli) were counted in 10 C57BL/6.lpr/lpr.C1qa−/− and 10 wild-type C57BL/6.lpr/lpr mice with no histological evidence of GN at 8 mo of age, and significantly greater numbers were found in C1q-deficient kidneys (C57BL/6.lpr/lpr.C1qa−/−, 1.1 ± 0.27 (mean ± SEM); wild-type, 0; by Mann-Whitney U test, p < 0.012). However, at 10 mo of age in 12 C57BL/6.lpr/lpr.C1qa−/− and 10 wild-type C57BL/6.lpr/lpr mice, no significant difference was found (C57BL/6.lpr/lpr.C1qa−/−, 0.539 ± 0.22; wild-type, 0.818 ± 0.38).

Analysis of MRL/Mp-lpr/lpr.C1qa−/− mice

The MRL/Mp-lpr/lpr model develops a highly accelerated and aggressive autoimmune disease. Therefore, ANA titers and the presence of GN were assessed in a small sentinel group of 1.5-mo-old mice (11 MRL/Mp-lpr/lpr.C1qa−/− and 10 MRL/Mp-lpr/lpr wild-type mice). All the samples analyzed were ANA negative, and no statistically significant difference in the prevalence or severity of GN was observed between the two groups. More extensive analysis was conducted in a group consisting of 19 MRL/Mp-lpr/lpr.C1qa−/− and 24 MRL/Mp-lpr/lpr wild-type animals at the later time point of 2 mo of age. Although at this time high ANA titers (range, 1/80 to 1/1280) were found in 84% of the MRL/Mp-lpr/lpr.C1qa−/− and in 64% of the MRL/Mp-lpr/lpr wild-type mice, there was no significant difference between the two groups. Similarly, no differences were seen with regard to measurements of anti-ssDNA Ab levels or anti-dsDNA titers. At 2 mo of age severe GN (grades I–IV) was detected in both groups, but no significant increase in mortality, although not marked, was observed within the C1q-deficient group (by log-rank test, p = 0.022; Fig. 1).

Analysis of MRL/Mp+/+.C1qa−/− mice

In contrast to the results obtained from the study of the above cohorts, analysis of the MRL/Mp+/+ strain revealed marked differences between C1q-deficient and wild-type animals. Studies were conducted in 47 MRL/Mp+/+.C1qa−/− (29 males and 18 females) and 34 MRL/Mp+/+ controls (22 males and 12 females).

The initial analysis was conducted on serum obtained at 3 mo of age, at which point no ANA titers were detected in either of the two groups. However, by 6 mo of age a discernable phenotype began to emerge, in which the presence and titer of ANA in the MRL/Mp+/+.C1qa−/− group were significantly greater than those found in controls (by Mann-Whitney U test, p = 0.0001; Fig. 2a). In addition, significantly higher levels of anti-ssDNA Abs were detected in MRL/Mp+/+.C1qa−/− mice (by Mann-Whitney U test, p = 0.0057; Fig. 2b). Analysis of the distribution of autoantibody levels within the individual male and female populations measured at 6 mo showed that in both experimental groups the females had a higher incidence of autoantibody production compared with the males. At 6 mo of age MRL/Mp+/+.C1qa−/− mice developed significantly higher, although not marked, levels of rheumatoid factor (by Mann-Whitney U test, p = 0.0422; Fig. 2c). However, no significant levels of anti-dsDNA and anti-erythrocyte Abs were detected in either of the two groups of mice at 6 mo (data not shown). In the course of the study after 5 mo of age

**FIGURE 2.** Autoantibody profiles in MRL/Mp+/+.C1qa−/− and MRL/Mp+/+ wild-type mice at 6 mo of age. a, ANA titers. Each circle represents one mouse. Serum samples were titrated to the end point. b, Anti-ssDNA Ab levels expressed in AEU relative to a standard positive sample, which was assigned a value of 100 AEU. The symbols are the same as in a. c, Rheumatoid factor levels expressed in AEU referring to a standard curve derived from a serum pool of MRL/Mp-lpr/lpr mice, which was assigned a value of 1000 U. The symbols are the same as in a.
an accelerated mortality and morbidity within the female MRL/Mp+/+.C1qa−/− group was noticed. The clinical presentation of disease in these mice was characterized by kidney failure, resulting in heavy proteinuria and anasarca. Mortality data were collected up to 12 mo of age, at which time point the mice were sacrificed. From this analysis it emerged that significantly greater mortality was recorded within the MRL/Mp+/+.C1qa−/− female group, in which there was 41% mortality by 12 mo compared with no mortality in the wild-type group (by log-rank test, p = 0.014; Fig. 3).

Serological analysis of the sera collected at 12 mo confirmed the results obtained at 6 mo of age, except that a number of female MRL/Mp+/+.C1qa−/− mice showed reduced autoantibody titers compared with those measured at 6 mo, probably because of renal dysfunction with proteinuria and fluid accumulation. Assessment of GN was conducted on renal tissue obtained at 12 mo of age, with the exception of a number of female MRL/Mp+/+.C1qa−/− mice that died or had to be sacrificed prematurely due to the development of severe anasarca. The prevalence and severity of GN were significantly greater in the MRL/Mp+/+.C1qa−/− group compared with the controls (by Mann-Whitney U test, p < 0.0001) regardless of gender (Table I). However, within each experimental group females suffered a higher incidence of severe GN compared with males. In a number of cases of diseased females light microscopy revealed the presence of grossly abnormal glomeruli showing crescent formation and periglomerular fibrosis (Fig. 4). Simple dipstick tests before sacrifice showed marked proteinuria (>20 mg/ml) in all female MRL/Mp+/+.C1qa−/− mice assessed positive for severe GN. Immunofluorescence staining in selected GN-positive kidneys revealed the presence of extensive IgG and C3 deposits distributed both within the mesangium and along capillary walls (data not shown).

Assessment of the kidneys of MRL/Mp+/+.C1qa−/− and MRL/Mp+/+ wild-type mice with no histological evidence of GN revealed a nonsignificant trend toward an increase in the number of glomerular apoptotic bodies in the C1q-deficient mice compared with wild-type animals (MRL/Mp+/+.C1qa−/− animals (n = 10), 1.3 ± 0.538 (mean of apoptotic bodies in 50 glomeruli ± SEM); MRL/Mp+/+ wild-type mice (n = 27), 0.48 ± 0.18; by Mann-Whitney U test, p = 0.22). Only 10 C1q-deficient kidneys were available for the analysis due to the very high prevalence of GN in this cohort. Phagocytosis in vivo of heterologous (Jurkat T cells) and autologous (murine thymocytes) apoptotic cells by elicited peritoneal macrophages was significantly impaired in the MRL/Mp+/+.C1qa−/− mice compared with the MRL/Mp+/+ wild-type animals (p = 0.0012 and p = 0.0043, respectively, by Mann-Whitney U test; Fig. 5). Comparison of the phagocytic uptake of an equivalent number of viable thymocytes confirmed that the peritoneal macrophages preferentially engulfed apoptotic cells (percentage of macrophages ingesting apoptotic thymocytes, 30.1 ± 1.4% (n = 5) compared with 3.4 ± 1.86% (n = 4) with viable cells (mean ± SEM); p = 0.0159, by Mann-Whitney U test).

**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>MRL/Mp+/+</td>
<td>M (22)</td>
<td>21</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>F (12)</td>
<td>7</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>MRL/Mp+/+</td>
<td>M (29)</td>
<td>9</td>
<td>3</td>
<td>11</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>C1qa−/−</td>
<td>F (18)</td>
<td>3</td>
<td>4</td>
<td>0</td>
<td>1</td>
<td>10</td>
</tr>
</tbody>
</table>

*All the experimental animals were sacrificed at 12 mo of age with the exception of a number of MRL/Mp+/+.C1qa−/− that died or had to be sacrificed prematurely. Bourn’s fixed kidney sections were scored for GN as described in Materials and Methods. M, Male; F, Female.

**Discussion**

The work described in this paper highlights the importance of genetic background on the expression of autoimmunity in C1q-deficient mice. In C57BL/6.C1qa−/− mice no evidence of autoimmunity was found, demonstrating that in this nonpermissive genetic background the absence of C1q was not sufficient to drive a detectable disease phenotype. By analogy, the Yaa gene that is a potent accelerator of disease in the BXSB strain did not stimulate the expression of disease traits in C57BL/6 mice (7). In C57BL/6.lpr/lpr mice, C1q deficiency did not appear to affect any of the phenotypic markers of disease, as the serological and histological profiles obtained did not differ significantly from wild-type controls. This would suggest that despite the presence of a predisposing autoimmune factor (i.e., the lpr gene), the effects of C1q deficiency on modifying the pattern of disease, especially the

**FIGURE 3.** Survival analysis of 22 MRL/Mp+/+.C1qa−/− and 12 MRL/Mp+/+ wild-type female mice. By 12 mo the mortality rate in the MRL/Mp+/+.C1qa−/− was 41% compared with no mortality observed in the MRL/Mp+/+ wild-type group (by log-rank test, p = 0.014).

**FIGURE 4.** Representative renal histology of MRL/Mp+/+.C1qa−/− (a) and MRL/Mp+/+ wild-type mice (b). a, Enlarged glomerulus from a MRL/Mp+/+.C1qa−/− mouse showing proliferation of cells in Bowman’s space forming a crescent (arrows) and periglomerular fibrosis (periodic acid-Schiff staining; magnification, ×420). b, Glomerulus with normal morphology from a MRL/Mp+/+ wild-type mouse.
development of severe GN, were not detectable. Similarly, the study of C1q-deficient MRL/Mp-lpr/lpr mice did not show evidence of enhancement or acceleration of disease markers. The MRL/Mp-lpr/lpr strain exhibits a very aggressive and early onset disease that is heavily influenced by the lpr mutation, a defect with profound immunological consequences (17). Therefore, in such an accelerated phenotype the presence of any possible disease-modifying roles of C1q deficiency would be difficult to resolve clearly, although mortality appeared to be subtly increased in the MRL/Mp-lpr/lpr.C1qa−/− group, as indicated by the survival study. In contrast, analysis of the MRL/Mp+/+ C1qa−/− strain showed a marked acceleration of autoimmune disease compared with wild-type MRL/Mp+/+ controls. Both autoantibody production and the prevalence and severity of GN were significantly increased in MRL/Mp+/+ C1qa−/− animals. Although ANA and anti-ssDNA Ab titers and the prevalence of GN was higher in MRL/Mp+/+ C1qa−/− mice, the development of anti-dsDNA Abs within the entire experimental group, including controls, was not detected. Previous analysis of the MRL/Mp+/+ strain has failed to show significant anti-dsDNA Ab production (18), whereas in MRL/Mp-lpr/lpr mice this species of autoantibody is common. With regard to the analysis of C4- and CD21/CD35-deficient C57BL/6 mice, the genetic background was a hybrid of 129 and C57BL/6. It is known that the hybrid (129 × C57BL/6) genetic background is predisposed to the development of SLE (2, 24). This raises the possibility that the modification of disease in this case could have been influenced primarily by combinations of uncharacterized background genes interacting with the lpr mutation and not necessarily due to the action of the targeted deletions. In addition, recent data have suggested that the capacity of C4 to protect from the development of autoimmunity in the permissive (129 × C57BL/6) host strain operates independently of CD21/
CD35 function (25). This supports the hypothesis that early classical pathway complement proteins, upstream from C3 activation, are the principal components of the complement system involved in the prevention of autoimmune responses, a mechanism for which may involve the handling of apoptotic cells (5). In this context it is of note that the MRL/MpJ-C1qa−/− mice exhibited an in vivo impairment in the phagocytic uptake of heterologous and autologous apoptotic cells by elicited peritoneal macrophages compared with the wild-type animals (Fig. 5).

In conclusion, the effect of genetic background on the expression of disease in gene-targeted C1q-deficient animals has been found to be critical. Analysis of C1q deficiency in C57BL/6, C57BL/6.lpr/lpr, MRL/Mp-lpr/lpr, and MRL/Mp+/+ mice only showed evidence of disease acceleration in MRL/Mp+/+ animals. The MRL/Mp+/+ C1qa−/− strain may prove to be a very useful tool with which to dissect the contributions of C1q deficiency to autoimmune pathogenesis in light of the genetic uniformity of this model. In addition to the relevance of this work to the relationship between C1q deficiency and autoimmunity, this study also illustrates the importance of the genetic background in the analysis of gene-targeted mice in general. The majority of the original reports describing SLE-like disease in gene-targeted mice have presented data obtained from hybrid mouse strains.

Acknowledgments

We thank all the staff of the animal facility for their technical assistance. We are grateful to Margarita Lewis for her technical help with the preparation of the histological specimens.

References