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Reconstitution of the Complement Function in C1q-Deficient (C1qa<sup>−/−</sup>) Mice with Wild-Type Bone Marrow Cells<sup>1</sup>

Franz Petry, Marina Botto, Rafaela Holtappels, Mark J. Walport, and Michael Loos<sup>6</sup>

Besides Ab-independent and Ab-dependent activation of the complement classical pathway in host defense, C1q plays a key role in the processing of immune complexes and in the clearance of apoptotic cells. In humans, C1q deficiency leads to systemic lupus erythematosus-like symptoms in over 90% of the cases, thus making this defect a strong disease susceptibility factor. Similarly, C1q-deficient mice (C1qa<sup>−/−</sup>) develop systemic lupus erythematosus-like symptoms, such as autoantibodies and glomerulonephritis. We have previously provided evidence that C1q is produced by cells of the monocyte-macrophage lineage. In this study, we have tested whether transplantation of bone marrow cells would be sufficient to reconstitute C1q levels in C1qa<sup>−/−</sup> mice. C1qa<sup>−/−</sup> mice received a single graft of 10<sup>7</sup> bone marrow cells from wild-type (wt) donors after irradiation doses of 6, 7, 8, or 9 Gy. Engraftment was monitored by a Y chromosome-specific PCR and a PCR that differentiated wt from C1qa<sup>−/−</sup> genotype. Serum levels of C1q Ag and C1 function increased rapidly in the recipient mice, and titers reached normal levels within 6 wk after bone marrow transplantation. In wt mice that received C1qa<sup>−/−</sup> bone marrow, serum levels of C1q decreased constantly over time and became C1q deficient within 55 wk. These data clearly demonstrate that bone marrow-derived cells are the source of serum C1q and are competent to reconstitute normal C1q serum levels in C1q-deficient mice. Therefore, stem cell transplantation could be a therapy for patients with hereditary C1q deficiency.

The complement component C1q plays an important role in host immune responses. C1q initiates the classical pathway activation of the complement system by binding to microbial ligands (innate immunity) or to Ag-Ab complexes (immune complexes, ICs)<sup>3</sup> via the Fc region of IgG and IgM molecules (adaptive immunity). Absence of C1q, as in hereditary C1q deficiency, leads to recurrent infections, demonstrating the role of C1q in host defense (1, 2). A second important function of C1q is linked with the clearance of ICs and the development of autoimmunity in cases of total or partial deficiency. C1q prevents the formation of large IC lattices. Pathological conditions such as systemic lupus erythematosus (SLE) lead to IC deposition, inflammation, and tissue damage. Patients with SLE develop secondary C1q deficiency due to C1q consumption in the acute phases of the disease, and they also generate autoantibodies against C1q (3). A third function of C1q has been recognized only recently. It has been demonstrated that the surface blebs of apoptotic cells bind C1q directly in the absence of Ig (4). Furthermore, C1q-deficient mice showed numerous apoptotic bodies in inflamed glomeruli (5). Both findings suggest a crucial role of C1q in the clearance of apoptotic cells, which has been demonstrated by Taylor et al. (6).

Hereditary C1q deficiency is strongly linked with SLE-like disease. Of 41 C1q-deficient patients described in the literature, 38 presented with SLE-like symptoms, including generation of autoantibodies, glomerulonephritis, and involvement of the CNS (3). Attempts to treat patients with hereditary C1q deficiency with fresh-frozen plasma as a source of C1q have been disappointing, as C1q levels dropped within hours after transfusion (7). Preformed ICs appeared to have fixed C1q, causing rapid depletion of infused C1q.

The generation of a C1q-deficient mouse model has opened new perspectives to study and understand the various biological roles of C1q. Whereas the majority of complement components are synthesized by hepatocytes, accumulating evidence has shown that the cells of the monocyte/macrophage lineage are responsible for the production of C1q (8–13). As these cells derive from precursors of hemopoietic stem cells, we tested the hypothesis that bone marrow cells (BMC) are capable and sufficient to reconstitute C1q serum levels in C1qa<sup>−/−</sup> mice.

Materials and Methods

Mouse strains and bone marrow transplantation (BMT)

C1qa<sup>−/−</sup> mice used were on a hybrid genetic background (129/Sv × C57BL/6) or backcrossed onto C57BL/6 for seven generations, as specified in each experiment, and were generated as previously described (5). Age- and sex-matched wild-type (wt) (129/Sv × C57BL/6 and C57BL/6) mice were used as controls. Animals were bred under specific pathogen-free conditions and kept in filtered cages during the experiments.

Recipient mice, generally 10–16 wk of age, received a total body γ-irradiation dose of 6–9 Gy delivered by a 137Cs γ-ray source. Donor femoral and tibial BMC were isolated by flushing medium through the bone shafts, washed three times, and filtered through nylon gauze to remove large particles. Recipients received 10<sup>7</sup> donor BMC of the opposite sex suspended in medium, which were infused i.v. into the tail veins within 6 h after irradiation. Starting at 2 wk after transplant, mice were bled by tail vein incision at various time points up to 55 wk.

PCR conditions

The successful reconstitution of the hemopoietic lineages after BMT was monitored by a Y chromosome-specific PCR. Blood leukocytes from recipient mice were used as a source of genomic DNA, which was prepared
by the Chelex-100 method from whole blood (14). A 402-bp DNA fragment of the male sex-determining gene tdy (15) was amplified by PCR, as described (16). Two PCR that discriminate wt from C1qa<sup>−/−</sup> genotypes were used to screen the recipient mice. For the amplification of an ~380-bp DNA fragment of the wt genotype, the primer pair mC1qA5’-GCGGGC CGTGTCAGCACGACAG; a sequence within exon 1 of the C1qa gene) and mC1qA/An1’-ACCAATGCTTCTACAGGACC; a sequence within intron 1 of the C1qa gene) were used. An ~180-bp fragment of the C1qa<sup>−/−</sup> genotype was received using primers mC1qA/An1’ and NEO (GGG GATCCGAGCATATAAAGAAGG; a sequence within the neomycin resistance gene of the gene-targeting construct). PCR amplification for both primer combinations was conducted for 3 min at 94°C, followed by 30 cycles of 60 s at 94°C, 30 s at 64°C, 30 s at 72°C, and finally 10 min at 72°C. Amplification products were visualized by ethidium bromide-stained agarose gel electrophoresis.

**Northern blot analysis and RT-PCR**

At various time points, individual mice were sacrificed and total RNA was extracted from various tissues using standard methods (17, 18). Electrophoresis, blotting, and hybridization of RNA and labeling of cloned mouse Northern blot analysis and RT-PCR cDNA were performed, as described (19). To amplify C1qa gene transcripts, a RT-PCR was performed using intron flanking primers (MA-5/INT, ACAGTTGGCTGAAGATGTCTG; MA-INT/3, CTGGTCC CTGATATGCGCTG), which can differentiate mRNA from genomic DNA PCR products. cRNA was synthesized from 4 to 8 μg RNA, as previously described (20). RT-PCR amplification was conducted for 3 min at 94°C, followed by 35 cycles of 45 s at 94°C, 45 s at 94°C, 45 s at 60°C, 60 s at 72°C, and finally 10 min at 72°C. Amplification of C1qa mRNA sequence results in an amplicon size of 279 bp.

**Complement assays**

Functional activity of serum C1q in C1qa<sup>−/−</sup>, wt, and mice after BMT was measured in a hemolytic C1 test, as described for human C1 (21), with purified guinea pig C4 and C2 preparations and EDTA-treated guinea pig serum as a source for C3 to C9 components. C1q Ag was estimated in an ELISA. Microtiter plates (Maxisorb; Nunc, Deisenhofen, Germany) were coated with 0.3 μg total mouse IgG1 (M-9269; Sigma, Deisenhofen, Germany) and blocked with 1% BSA in PBS. Serum samples were diluted 1:50 and incubated in coated plates for 1 h at room temperature. After two washes with PBS-0.05% Tween 20, bound C1q was incubated with a biotinylated goat anti-mouse C1q Ab (IgG preparation 

**Results**

**Control of graft establishment**

In preliminary experiments, the optimal irradiation dose that gave an efficient engraftment of donor BMC without causing mortality within the first 2 wk after irradiation was determined. Mice were irradiated with doses of 6–9 Gy and were given 10<sup>7</sup> BMC within 6 h after irradiation. In the group that received 9 Gy, over 50% (five of nine mice) died within the first week of the experiment; no mice of the other groups died in that period. A dose of 8 Gy was found to be optimal in terms of survival rates and the time frame necessary for a successful engraftment; therefore, it was applied in subsequent experiments. Recipient mice were screened for circulating donor-type leukocytes using a Y chromosome-specific PCR for the tdy gene and a second PCR for detection of wt or C1qa<sup>−/−</sup> genotypes. Two weeks after BMT, the tdy gene could be detected in DNA preparations from blood leukocytes in almost every female recipient of male BMC and in none of the male recipients of female bone marrow (Fig. 1A). In some wt males that received female BMC, the tdy gene could be amplified 6 wk posttransplant. Using the discriminating C1qa gene PCR, a complete conversion of the genotype was seen at this time point in all recipient mice (Fig. 1B). The same results were obtained from mice on hybrid 129/Sv × C57BL/6 genetic background as well as mice on C57BL/6 genetic background.

**Gene expression**

RT-PCR experiments showed that within 2 wk after BMT, organs of C1qa<sup>−/−</sup> recipients were colonized by wt donor cells expressing normal C1q. C1qa mRNA-specific PCR products could be demonstrated in cDNA preparations of peritoneal cells, heart, liver, spleen, and kidney (Fig. 2A). Northern blot experiments confirmed these results. An increase of C1qa mRNA over time could be shown in C1qa<sup>−/−</sup> mice reconstituted with wt BMC, whereas no specific hybridization was detected in mRNA from untreated C1qa<sup>−/−</sup> control mouse spleen (Fig. 2B). C1qa<sup>−/−</sup> mice that received C1qa<sup>−/−</sup> BMC had no detectable C1qa mRNA levels, whereas wt mice reconstituted with wt BMC showed hybridization signals comparable with untreated wt controls. Similar results were seen in RNA preparations from liver, heart, and kidney (results not shown). At wk 4 after BMT, the Northern blot hybridization signal from thioglycollate-stimulated peritoneal cells in C1qa<sup>−/−</sup> recipients reconstituted with wt BMC was indistinguishable from wt recipients reconstituted with wt BMC and nontreated wt mice, whereas in C1qa<sup>−/−</sup> recipients receiving C1qa<sup>−/−</sup> BMC, no C1qa mRNA was detectable even after prolonged exposure of the Northern blot (Fig. 2C).

**Detection of C1q Ag and functional activity in sera of reconstituted mice**

Functional activity of C1q was tested in a hemolytic C1 assay. In C1qa<sup>−/−</sup> mice on the hybrid 129/Sv × C57BL/6 genetic background reconstituted with wt BMC, C1 functional activity was in the range of C1 titers of wt mice within 4–6 wk after transplant.

**FIGURE 1.** Control of engraftment of donor BMC in irradiated recipient mice. Agarose gel electrophoresis of PCR products for the detection of Y chromosome tdy gene (A) and for differentiation of C1q genotypes (B) amplified from blood leukocyte DNA. A. Recipient mice were transplanted with 10<sup>5</sup> donor BMC of the opposite sex after receiving an irradiation dose of 8 Gy. Recipient and donor mice were of mixed 129/ Sv × C57BL/6 genetic background. At wk 2 and 6 posttransplant in almost every female recipient, male donor-derived circulating leukocytes were detectable. In male recipients, the tdy gene was absent at wk 2, but was detectable again at wk 6 posttransplant in some mice. B, C1qa<sup>−/−</sup> recipients showed a complete conversion of the C1q genotype posttransplant.
C1qa−/− mice and wt mice were of mixed 129/Sv background, except where noted differently. A, Agarose gel electrophoresis of RT-PCR products for the detection of C1qa mRNA. Lane 1, cDNA preparation from wt spleen; lanes 2–6, cDNA preparations from C1qa−/− mice at wk 2 posttransplant, as specified; lane 7, cDNA preparation from wt DBA1 mice; marker (100-bp ladder). B, Agarose gel electrophoresis (top) and Northern blot hybridization (bottom) of spleen RNA preparations from C1qa−/− recipients + wt BMC at wk 2, 3, and 4 (lanes 2–4). Lane 1, Untreated C1qa−/−; lane 5, C1qa−/− recipients + C1qa−/− BMC; lane 6, wt recipient + wt BMC; lane 7, untreated wt; lane 8, untreated wt C57BL/6. C, Agarose gel (top) and Northern blot (bottom) of peritoneal cell RNA from recipients 4 wk posttransplant. Mice were stimulated with thioglycolate medium i.p. 4 days before cell harvest. Blots in B and C were hybridized with a C1qa cDNA-specific probe. Exposure times, 7 days (B) and 16 h (C). The size of the C1qa mRNA is indicated.

When C1qa−/− mice on the C57BL/6 background became available, experiments were repeated with 10 mice per group. Mice were monitored over a period of 55 wk after BMT. Analysis of the C1q genotype of these mice gave similar results as those obtained in the previous three BMT experiments. Fig. 4 represents the results of the C1q ELISA of each recipient at all time points. An increase of C1q Ag can be seen in C1qa−/− mice as a result of wt BMC engraftment. By wk 6 post-BMT, each recipient had C1q antigenic concentrations in the normal range. Seven of ten mice remained within that range until the end of the experiment at wk 55 post-BMT. Of the three mice that dropped below that range, one mouse did not receive the full 10^7 BMC due to a leakage of the vein during injection. Another mouse in which C1q levels dropped dramatically after wk 32 died at wk 53.

Wt mice that received C1qa−/− BMC developed a linear decrease of C1q Ag over the observation period. At wk 55 post-BMT, all but one mouse had C1q levels in the range of the ELISA values of C1qa−/− sera.

Discussion

BMT experiments were conducted to determine whether monocyte/macrophage precursors in BMC were competent and sufficient to restore the wt C1q phenotype in C1q-deficient mice. To ensure effective engraftment of hemopoietic stem cells, recipients were preconditioned with high dose total body γ-irradiation. The BMT itself did not modify the C1q phenotype of the recipient, as irradiated wt mice receiving wt BMC had full C1q function, and C1qa−/− mice receiving C1qa−/− BMC tested negative (data not shown). Furthermore, C1q mRNA hybridization signals were identical in mice given homologous transplants as those seen in untreated mice (Fig. 2C). In mice preconditioned with 8 or 9 Gy, the heterologous donor C1q phenotype established faster than in mice given lower irradiation doses. Therefore, a total body irradiation dose of 8 Gy was used in subsequent experiments.
Accumulating data have demonstrated that the major source of C1q biosynthesis are cells of the monocyte/macrophage lineage. Functional assays, biosynthetic labeling experiments, and mRNA studies have shown that peritoneal macrophages are capable of producing C1q (8–11). C1q biosynthesis could also be demonstrated in cultured peripheral blood monocytes (12, 13), follicular dendritic cells and interdigitating cells of the spleen (22), Kupffer cells of the liver (23), and microglial cells of the brain (24–26). A recent report has demonstrated C1q gene activity in nonmonocyte/macrophage neuronal cell lines by RT-PCR (27). These findings are in contrast to the majority of the other complement components for which hepatocytes are the major sites of biosynthesis.

Therefore, we tested the hypothesis that BMT, containing the precursors of monocytes/macrophages, is capable and sufficient to restore C1q levels in C1q-deficient mice. The results showed that a single graft of 10^7 BMC was sufficient to restore normal serum levels of C1q in genetically deficient mice. Furthermore, wt mice were made C1q deficient by a single transplant of BMC from C1qa/−/− donor mice. However, there was a marked difference in the time course of the increase and decrease of C1q levels between C1qa/−/− and wt mice, respectively. In C1q-deficient mice, reconstitution of C1q to normal levels was detected within 6 wk after transplantation, whereas a much slower decrease of C1q was identified in wt mice that received C1qa/−/− BMC. C1q mRNA studies in C1qa/−/− mice preconditioned with 6 Gy showed that within 2 wk, various organs were colonized by C1q-synthesizing donor cells (Fig. 2A).

Although the actual ratio of donor and recipient macrophages in the various organs was not determined with the methods applied, it is most likely that resident tissue macrophages that have been demonstrated to produce C1q in situ (22) are only slowly replaced by donor cells. The γ-irradiation doses that destroy hemopoietic stem cells and other rapidly proliferating cells are ineffective for differentiated cells such as tissue macrophages. Therefore, recipient macrophages continue to produce C1q over a long period of time (i.e., months) and, as a consequence, C1q serum levels decrease only slowly in wt mice that were reconstituted with C1qa/−/− BMC.

From the results of the experiments performed, we cannot determine the location of the C1q-producing cells that are responsible for the serum levels that have been measured. Apparently, only a proportion of the total C1q-producing cells is required to reach normal C1q levels in serum. This implies that even sublethal total body irradiation or other myeloablative procedures for preconditioning individuals undergoing BMT could be sufficient for correction of a hereditary C1q defect. The data presented in this work suggest that transplantation of hemopoietic stem cells, which has become more and more a routine in hematological practice, might be a potential treatment for patients with hereditary C1q deficiency.

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References


