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Local Synthesis of C3 Within the Splenic Lymphoid Compartment Can Reconstitute the Impaired Immune Response in C3-Deficient Mice

Michael B. Fischer, Minghe Ma, Nelson C. Hsu, and Michael C. Carroll

Mice bearing a disrupted C3 locus (C3−/−) have an impaired Ab response to T-dependent Ags (bacteriophage φX174 and nuclear protein-keyhole limpet hemocyanin) characterized by a reduction in number and size of germinal centers and impaired retention of Ag by follicular dendritic cells. To test the importance of C3 synthesized locally within the lymphoid compartment during an immune response to T-dependent Ag, we reconstituted C3−/− mice with wild-type bone marrow of MHC-identical littermates. Engraftment not only restored local C3 synthesis in the spleen, but also rescued the impaired humoral response. The major source of C3 mRNA was MOMA-2+ macrophages localized within the white pulp areas of the spleen. Interestingly, C3 expression is apparently regulated as C3 mRNA was not detected in splenic sections of nonimmune mice. Furthermore, local C3 synthesis by donor macrophages reversed the impaired Ag trapping by splenic follicular dendritic cells in C3-deficient mice. The Journal of Immunology, 1998, 160: 2619–2625.

Definitive evidence has accumulated demonstrating a crucial role for the complement system in humoral immune responses. Analysis of mice in which either the C3 or the C4 locus was disrupted by gene targeting confirmed and extended earlier studies in complement-deficient guinea pigs (1, 2). Both deficient strains of mice had an impaired Ab response against the T-dependent Ag bacteriophage φX174, and their response was characterized by a reduction in the number and size of germinal centers (GCs) within splenic follicles (3). The nature of the defect was found to lie at the B cells, as T cells were equally primed in both complement-deficient mice and wild-type (WT) littermates. In addition, mice with a disrupted Cr2 locus, which results in disruption of both CD21 and CD35, showed a phenotype similar to the C3−/− and C4−/− animals. Cr2−/− mice had an impaired humoral response to soluble T-dependent Ags such as bacteriophage φX174 (4) or SRBC (5) that was characterized by a reduction in the number and size of GCs (4). Direct evidence that activation products of C3 enhance the B cell response has been demonstrated by coupling of one, two, or three copies of C3d to hen egg lysozyme (HEL) using a recombinant approach (6). HEL bearing one, two, or three copies of C3d was 100-, 1,000-, and 10,000-fold more immunogenic, respectively, than HEL alone, showing an inverse correlation between the numbers of copies of C3d per HEL and the amount of soluble HEL required for induction of a humoral response.

These results raise the question of what is the source of C3 for Ag attachment. While the primary source of C3 in circulation is the liver (7), extrahepatic synthesis has been reported and a variety of cell types including mononuclear cells (8), polymorphonuclear leukocytes (9), umbilical vein endothelial cells (10), pulmonary alveolar type II epithelial cells (11), intestinal (12) and proximal tubular epithelial cells (13, 14), skin fibroblasts (15), epidermal keratinocytes (16), and astroglia cells (17) synthesize and secrete C3. In the spleen, local C3 synthesis has been described in rat-into-mouse bone marrow (BM) chimeras, where rat C3 was found in the spleen of these chimeric mice (18). Synthesis of C3 in various organs can be directly up-regulated by cytokines during an inflammatory response. For example, IFN-γ released by activated T cells can induce macrophages to secrete proinflammatory cytokines, e.g., IL-1γ and IL-6, that can up-regulate the synthesis of C3 and other complement components and acute phase proteins (19, 20). In addition, IFN-γ can induce C3 synthesis directly (21) as well as stabilize C3 mRNA (22). In the human kidney, proximal tubular epithelial cells synthesize C3 in inflammatory disease such as systemic lupus erythematosus (23). In normal mouse kidney, C3 mRNA levels are low, however, following LPS challenge C3 expression is increased in cortical tubular epithelium (24). In aged (18 wk) MRL/lpr/lpr mice, which develop lupus-like nephritis, C3 message was increased and localized to perivascular inflammatory cells surrounding medium size arteries (24).

To investigate the role of C3 produced locally at lymphatic sites during an immune response to T-dependent Ag, we took advantage of recently developed mice in which the C3 locus was disrupted by gene targeting (25). C3−/− mice were reconstituted with BM of MHC-matched littermates. Engraftment not only restored local C3 synthesis but rescued both the impaired humoral response and retention of soluble T-dependent Ags by follicular dendritic cells (FDCs). Analysis of splenic sections of both immunized C3 BM chimeras and WT animals demonstrated a dramatic up-regulation of C3 synthesis by macrophages within the white pulp areas. Expression of C3 message was dependent on immunization with Ag as C3 mRNA was not found in nonimmune C3 BM chimeras or...
WT controls. We provide evidence that C3 synthesized by macrophages locally within the splenic lymphoid compartment can contribute sufficient C3 for complement enhancement of the humoral response.

Materials and Methods

Generation of C3 BM chimeras

C3−/− mice were constructed by gene targeting by homologous recombination in embryonic stem cells (25). BM cells of WT littermates were isolated by flushing femurs and tibias with cold medium (DMEM supplemented with 1% bovine calf serum) using a 26-gauge needle, followed by depletion of erythrocytes with lysis buffer (0.15 M NH4Cl, 10 mM KHC03, and 0.1 mM EDTA) as described earlier (4). In brief, 5 × 10⁶ BM cells were injected i.v. into lethally irradiated (2 × 650 rad) 6- to 8-wk-old C3−/−-deficient mice. Mice were kept under barrier isolation conditions. Studies were performed according to institutional guidelines for animal use and care.

Immunization protocol and measurement of the immunologic response

C3−/− mice, C3 BM chimeras (4 to 6 wk post-transplantation) and WT littermates were immunized i.v. with 50 μg of soluble NP-KLH and challenged with an equal dose 3 wk later. Serum samples were taken each week and sera were used for ELISA according to standard protocols.

Measurement of serum C3

Analysis of protein in the serum was performed by ELISA (sensitive to about 5 ng/ml) using a rat mAb to mouse C3 and a polyclonal rabbit anti-mouse C3 serum (Cappel, Organon Teknika Corp., West Chester, PA) for detection as described earlier (3).

Immunohistochemistry

Tissue sections were prepared for immunohistology as described (4). For two-color immunolabeling, splenic tissues were incubated with a panel of purified mAbs that recognize different monocyte-macrophage determinants such as MOMA-2 (26), ERTR-9 (27), MOMA-1 (28) (kindly provided by Dr. G. Kraai, Free University, Amsterdam, The Netherlands), 2F8 (29) (kindly provided by Dr. S. Underwood, Division of Cancer Research, National Cancer Institute, USA) and 4F80 (PharMingen, San Diego, CA) followed by a sequential incubation step with biotinylated rabbit anti-rat IgG (Vector Laboratories, Burlingame, CA) and streptavidin-alkaline phosphatase (AP) (ExtraAvidin-AP, Sigma, St. Louis, MO). Tissue sections were counterstained with pea agglutinin (PNA, EY Laboratory, San Mateo, CA) to label macrophages, and B220+ B cells using an anti-B220 mAb (PharMingen) followed by an anti–FITC mAb (Boehringer Mannheim, Indianapolis, IN) coupled to AP. CD3-positive cells were visualized with Fast Blue B (Sigma, St. Louis, MO) for detection as described earlier (3).

Isolation of RNA and PCR amplification of cDNA specific for C3 of total splenocytes purified MOMA-2+ splenic macrophages and B220+ splenic B cells

C3−/− mice, C3 BM chimeras, and WT mice were immunized two times with 50 μg of soluble NP-KLH as described, and on day 7 after the second immunization splenocytes were taken and splenic mononuclear cells were isolated as described (3). Splenocytes were incubated with either MOMA-2 mAbs or B220 mAbs (PharMingen), and cells were purified by positive magnetic immunoselection using goat anti-rat IgG-coated beads (Dynal beads M 450, Dynal, Oslo, Norway) at a final concentration of approximately 1 × 10⁶ beads/1 × 10⁶ cells. Purity of cells (macrophages 95% and B cells >97%) was confirmed by flow cytometry using mAbs specific for Mac-1 and CD19, respectively. Total cellular RNA was isolated from total splenocytes, purified MOMA-2+ macrophages, and B220+ B cells using Trizol reagent (Life Technologies, Gaithersburg, MD) according to the manufacturers protocol. Equal amounts of total RNA, quantified at 260 nm, were reverse transcribed into cDNA by first strand synthesis employing SuperScript preamplification system for first-strand cDNA synthesis (Life Technologies). The cDNA was directly amplified on a thermocycler (Ampliton II, Barnstead/Thermolyne, Dubuque, IA) using Taq DNA polymerase (Promega, Madison, WI) and oligonucleotide primer pairs for m3 (sense: GCGTGACTCTGTTGTTGGT; antisense: TCTCTGGTTCCTTCACTCT and mg3PDH (glyceraldehyde 3-phosphate dehydrogenase control amplimer set, Clontech, Palo Alto, CA; sense: TGAAG GTGTTGAAAGCAGGTTGCG; antisense: CTATGGACCATGAGTCCAC CAC). The amplification profile involved 40 cycles of denaturation at 95°C for 1 min, primer annealing at 55°C for 1 min, and primer extension at 72°C for 2 min. Aliquots of PCR-generated products were fractionated on 0.9% ethidium bromide-agarose gels and validated by the predicted size.

Generation of the mC3 RNA probe and in situ hybridization

The pBSTmC3-442 plasmid was constructed by cloning the cDNA of mC3 (30) into the NorI site of the pBluescript II KS+ vector (Stratagene, La Jolla, CA) at position 670, digestion with KpnI (KpnI cuts the mC3 cDNA at position 442 and the pBluescript II KS+ vector at position 759) and subsequent religation, leaving the first 442 bp of the mouse C3 cDNA in the plasmid. Antisense digoxigenin (DIG)-labeled mC3 transcripts were produced by linearizing the pBSTmC3-442 plasmid with NorI and transcribing with a T3 RNA polymerase. DIG-labeled sense transcripts were produced by linearizing the plasmid with EcoRI and transcribing with T7 RNA polymerase. DIG-labeled RNA probes were produced according to the instructions of the manufacturer (Genius System, Boehringer-Mannheim, Indianapolis, IN). RNase-free tissue sections were prepared and in situ hybridization was performed as described (31). To detect hybridized DIG-labeled RNA, tissues were incubated in 2% rabbit serum in buffer 1 (100 mM Tris-HCL, pH 7.5, and 150 mM NaCl) for 20 min, and then incubated with sheep anti-DIG AP F(ab')₉ (Boehringer-Mannheim), diluted 1:500 in buffer 1 containing rabbit serum, for 1 hr. The slides were rinsed once in buffer 1, followed by a 10-min rinse in buffer 2 (100 mM Tris-HCL (pH 7.5), 100 mM NaCl, and 50 mM MgCl₂ (pH 9.5)), and finally, in the substrate solution containing 450 μg/ml of nitro blue tetrazolium salt and 175 μg/ml of 5-bromo-4-chloro-3-indolylophosphate toluidine salt (Boehringer Mannheim), and 1.25 mM levamisole (Sigma) in buffer 2 was added to the tissue. After sufficient substrate development that occurred during 16 to 48 h, slides were washed rigorously in Tris-EDTA (10 mM Tris-HCL (pH 8.0) and 1 mM EDTA (pH 8.0)), counterstained with methyl green (Sigma) according to standard protocols, and mounted with Permount. Cells producing mC3 mRNA within the white pulp of the spleen detected by in situ hybridization were counted and the average number of positive cells per white pulp was calculated. Each symbol represents one individual mouse analyzed at the time point indicated.

Statistics

Student’s t test was used to evaluate differences between the Ab and GC responses of C3−/− vs WT controls and C3 BM chimeras vs WT controls. Differences were considered statistically significant when p < 0.05.

Results

Reconstitution of C3-deficient mice with WT BM rescues the impaired immune response to T-independent Ag

To determine whether BM graft would rescue the impaired response of C3−/− mice, deficient mice were lethally irradiated and subsequently engrafted with BM of WT MHC-matched littermates. Four to six weeks after transplant, chimeric mice were immunized with 50 μg of soluble NP-KLH i.v. at days 0 and 21 and were bled each week following primary immunization. A total of 50 μg soluble NP-KLH was given i.v. in the absence of adjuvants to avoid circumventing a role for complement in the humoral response. As reported previously (3), C3−/− mice fail to make a normal Ab response to T-dependent Ags. C3 BM chimeras made a normal primary and secondary immune response (Fig. 1). Numbers of germinal centers (GCs) (Table I) were comparable between chimeric mice and WT controls 7 days following secondary immunization, indicating that the impaired immune response to T-dependent Ag in C3−/− mice can be reconstituted by engraftment with WT BM.

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C3 synthesis within the splenic white pulp is regulated

We used in situ hybridization, immunohistochemistry, and reverse-transcribed PCR (RT-PCR) to investigate the location and the type of C3-producing cells in the spleen. To detect cells expressing C3 mRNA DIG-labeled antisense and sense RNA probes specific for mC3 were developed. When hybridized to splenic tissues from immunized WT mice, the antisense C3 RNA probe strongly labeled cells within the white pulp of the spleen (Fig. 2a). As expected, the C3 sense RNA probe did not hybridize (data not shown). In splenic sections of immunized WT mice, cells expressing C3 message were equally distributed within the T and B cell zone of the white pulp (Fig. 2a); however, not all cells appeared to express the same level of C3 mRNA. A panel of mAbs that recognize different monocyte-macrophage subsets (MOMA-1, MOMA-2, 2F8, 4F80, and ERTO-9) (26–29) was used to identify the cells producing C3. From the mAbs used, only cells stained by MOMA-2 (Fig. 2a) colocalize with cells expressing C3 message (Fig. 2d) within the T and B cell zone of the splenic white pulp. MOMA-2 mAb has previously been described to stain predominantly macrophages within the splenic white pulp and the medulla of lymph nodes (26). As expected, the C3 antisense RNA probe did not hybridize to splenic tissues of immunized C3−/− mice (Fig. 2b), although normal numbers of MOMA-2+ macrophages were present (Fig. 2e). Interestingly, in the immunized C3 BM chimeras, C3-producing MOMA-2+ macrophages were clustered

Table I. Comparison of number of PNA+ germinal centers (GCs) within splenic follicles of C3−/− mice, WT littermates, and C3 BM chimeras either immunized or nonimmune

<table>
<thead>
<tr>
<th></th>
<th>Immunized</th>
<th>Nonimmune</th>
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<tbody>
<tr>
<td></td>
<td>No. of GCs</td>
<td>No. of GCs</td>
</tr>
<tr>
<td>WT</td>
<td>6 ± 12</td>
<td>6 ± 9</td>
</tr>
<tr>
<td>C3−/−</td>
<td>4 ± 2</td>
<td>2 ± 1.2</td>
</tr>
<tr>
<td>C3 BM chimera</td>
<td>12 ± 2.3</td>
<td>1.2 ± 0.5</td>
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a Number of GCs based on counting of splenic follicles stained with PNA.
b Mean ± SD.

As expected, the C3 sense RNA probe did not hybridize (data not shown). In splenic sections of immunized WT mice, cells expressing C3 message were equally distributed within the T and B cell zone of the white pulp (Fig. 2a); however, not all cells appeared to express the same level of C3 mRNA. A panel of mAbs that recognize different monocyte-macrophage subsets (MOMA-1, MOMA-2, 2F8, 4F80, and ERTO-9) (26–29) was used to identify the cells producing C3. From the mAbs used, only cells stained by MOMA-2 (Fig. 2a) colocalize with cells expressing C3 message (Fig. 2d) within the T and B cell zone of the splenic white pulp. MOMA-2 mAb has previously been described to stain predominantly macrophages within the splenic white pulp and the medulla of lymph nodes (26). As expected, the C3 antisense RNA probe did not hybridize to splenic tissues of immunized C3−/− mice (Fig. 2b), although normal numbers of MOMA-2+ macrophages were present (Fig. 2e). Interestingly, in the immunized C3 BM chimeras, C3-producing MOMA-2+ macrophages were clustered

![FIGURE 1. Engraftment of C3−/− mice with bone marrow of MHC-identical WT mice corrected the defective humoral immune response. C3 BM chimeras (constructed by engraftment of C3−/− mice with bone marrow of MHC-identical WT littermates, closed triangles), C3−/− mice (open triangles), and WT littermates (open circles) were injected i.v. with 50 µg soluble NP-KLH on days 0 and 21 and bled at the times indicated. Levels of specific NP-IgG Abs were determined by ELISA as described in Materials and Methods. All results are given as means ± SD.](http://www.jimmunol.org/)

![FIGURE 2. In situ localization of C3 mRNA in splenic tissues of immunized C3 BM chimeras, C3−/− mice, and WT littermates. C3 BM chimeras, C3−/− mice, and WT littermates were immunized as described in Figure 1. Spleens were taken 7 days after secondary immunization and cryosections were made as described (4). Cells expressing C3 mRNA were detected by in situ hybridization using an mC3 antisense RNA probe and subsequent detection with sheep anti-DIG-AP. Hybridization was visualized by substrate development nitro blue tetrazolium-5-bromo-4-chloro-3-indolylphosphate toluidine salt (producing a blue/black precipitate) for 16 to 48 h. Results from hybridizing with the C3 sense probe were negative (data not shown). Arrowheads in a and c indicates C3 mRNA synthesis. Alternatively, serial sections were double stained as described. MOMA-2+ macrophages are given in blue and PNA+ GC B cells are designated in crimson red. Results from staining with isotype control Ab were negative (results not shown). Arrowheads in d, e, and f indicates MOMA-2+ cells. Magnification, x100.)
within the follicles in or near PNA⁺ GCs (Fig. 2, c and f) in contrast to that observed within WT mice.

To provide further evidence that MOMA-2⁺ macrophages in the white pulp were the cells that produce C3 mRNA, splenic mononuclear cells were isolated, RNA was extracted, and RT-PCR specific for C3 was performed (Fig. 3). A band representing mC3 message was found at the predicted size (706 bp) in splenic cell preparations of immune WT mice and C3 BM chimeras (Fig. 3). Under similar conditions no specific band was found using RNA isolated from total splenocytes from nonimmune WT mice or immunized C3⁻/⁻ mice (Fig. 3). To further define the cell type that produces C3, MOMA-2⁺ macrophages and B220⁺ B cells were purified by positive magnetic immunoselection, RNA isolated, and RT-PCR specific for C3 was performed. Only the RNA isolated from MOMA-2⁺ macrophages gave the predicted C3 cDNA band (Fig. 3). Thus, MOMA-2⁺ macrophages represent a major source of locally produced C3 in immune mice.

Local C3 synthesis in macrophages appears to be regulated since C3 message was not detected in spleens of nonimmune WT mice by in situ hybridization (data not shown) or by RT-PCR (Fig. 3). Results obtained by in situ hybridization and RT-PCR could be confirmed by measuring C3 in the peripheral blood. C3 was not detectable in the peripheral blood of C3⁻/⁻ mice (Fig. 4a) or nonimmune C3 BM chimeras (data not shown). However, C3 was identified in the peripheral blood of chimeric mice following primary and secondary immunization with soluble NP-KLH. C3 protein levels in the blood peaked 7 days following Ag challenge, and C3 was at least fivefold higher after the secondary than the primary injection (Fig. 4a). Examination of spleens of chimeric mice for macrophage C3 mRNA synthesis (Fig. 4b) showed a correlation with peak C3 levels in the blood. However, only one chimeric mouse was available following primary immunization and therefore additional mice need to be examined. Following secondary immunization, the increase in C3-producing cells that was observed in spleens of C3 BM chimeras (Fig. 4b) would account for at least part of the serum C3. Although, other sources such as peritoneal and lymph node macrophages can also contribute.

C3 produced locally in the spleen is sufficient to enhance trapping of Ag by FDCs

Splenic tissues from WT, C3⁻/⁻ mice, and C3 BM chimeras were analyzed by three-color immunohistochemistry for the presence of C3 (blue color) on day 7 after secondary immunization (Fig. 5, a, b, and c). Histologic sections were counterstained with PNA (a marker for GC B cells) (crimson) and CD3 (distinguishes the T cell zone) (pink). Interestingly, dense deposits of C3 were identified in the FDC-rich region of the GC of immune WT mice (Fig. 5a). Less intense staining for C3 was detected in a dendritic pattern throughout the white pulp. As expected, C3 was not detected in splenic tissues of immunized C3⁻/⁻ mice (Fig. 5b). C3 staining of immune C3 BM chimeras was similar but less intense than observed for WT mice (Fig. 5c).

To investigate whether Ag colocalizes with C3 within the follicles, immune mice were injected with labeled Ag (NP-BSA-biotin) and spleens were harvested 20 h later. Ag was visualized by incubating splenic cryosections with streptavidin AP (Fig. 5, g, h, and i; blue). In splenic tissues of immunized WT mice, the labeled...
Ag (Fig. 5g) appeared to colocalize with C3 (Fig. 5a) in the FDC-rich region of the follicle. Staining of sections with Ab specific for FDCs (FDC-M1) suggested that both C3 and Ag were deposited on FDC surfaces (Fig. 5d). This would support previous results that localization and efficient retention of Ag on FDC surfaces is dependent on C3 (32, 33). Binding of C3 by FDCs is mediated by complement receptors 2 (CD21) and 1 (CD35) (34). Mice deficient in both CD21 and CD35, constructed by disruption of the Cr2 locus by gene targeting (4), fail to trap Ag sufficiently. Likewise, labeled Ag was not identified in splenic follicles of immunized C3<sup>2/2</sup> mice (Fig. 5h), although FDCs were present (Fig. 5e). To examine whether BM-derived cells could provide sufficient amounts of C3 for efficient Ag trapping, immune C3<sup>2/2</sup> mice were reconstituted with BM of WT MHC-identical littermates. BM-derived cells repopulated the lymphatic sites following immunization with soluble NP-KLH and produced C3 locally in the spleen (Fig. 2) and lymph node (data not shown). MOMA-2<sup>1</sup> macrophages were identified as the major cell type that produced C3 based on immunohistology (Fig. 2) and RT-PCR (Fig. 3). In splenic tissues of immune WT mice, MOMA-2<sup>1</sup> macrophages appeared randomly distributed within the T cell and B cell zones of the white pulp. In contrast, C3-producing cells were not identified in the red pulp area, which includes high numbers of macrophages. An apparently different pattern of C3-producing cells was observed in splenic tissues of immune C3 BM chimeras. C3-producing cells appeared to be clustered in the follicles in or near GCs (Fig. 2c). Only a few MOMA-2<sup>1</sup> macrophages outside the follicles produced high levels of C3 message at day 7 following secondary immunization (Fig. 2f). GCs are areas of rapidly dividing cells, and one explanation for the clusters of C3-producing cells is that this region is more accessible for the grafted cells to repopulate. Alternatively, the GC region is rich in lymphokines produced by activated T and B lymphocytes that might provide additional signals to induce C3 synthesis.

Local C3 synthesis appears to be regulated, since C3 mRNA was not detectable in nonimmune animals by either in situ hybridization (data not shown) or by RT-PCR (Fig. 3). However, immunization with a T-dependent Ag induced detectable levels of C3 mRNA. C3 in the peripheral blood of C3 chimeras could be observed following

**FIGURE 5.** C3 is Critical for Ag retention on the surface of FDCs. Serial cryosections of splenic tissues taken form C3<sup>2/2</sup> chimeras, C3<sup>2/2</sup> mice, and WT littermates 7 days after secondary immunization were triple stained with either Abs to mC3 (a,b,c), FDCs (FDC-M1, d,e,f) given in blue and counterstained with PNA to visualize GC B cells (crimson red) and CD3 to show T cell areas (pink). Alternatively, labeled Ag (NP-BSA-biotin, g,h,i) trapped on the surface of FDCs was visualized (blue) by incubation with streptavidin AP and counterstained with PNA (crimson red) and CD3 (pink) as described in Materials and Methods. Results from staining with isotype control Ab were negative (results not shown). Magnification, ×100.
primary and secondary immunization with soluble NP-KLH, indicating that bone marrow-derived cells contributed to C3 in the peripheral blood. Interestingly, C3 protein in the circulation (Fig. 4a) and C3 mRNA (Fig. 4b) in spleens of C3 BM chimeras was higher after the secondary than primary immunization. IFN-γ released by activated T cells can induce C3 synthesis (21) as well as stabilize C3 mRNA (22), and this would provide potential mechanisms for increased synthesis of C3 by macrophages. Furthermore, IFN-γ induces macrophages to produce IL-6 and IL-1α, which enhances transcription of C3 (19, 20). Support for a critical role of IFN-γ in the regulation of local C3 synthesis in the spleen comes from the observation that more IFN-γ-producing cells were found at day 3 following secondary immunization than on day 3 following primary immunization (data not shown).

Other cytokines that could potentially regulate C3 expression are TNF-α, IL-1β, IL-8 (35), and TGF-β (36). Evidence that specific immune inducible up-regulation of BM-derived cells to synthesize C3 has been reported. For example, BM-derived cells secrete significant levels of C3 locally following transplantation, during a period characterized by inflammatory stimuli, including graft-vs-host disease and infection (37). However, BM-derived C3 was not detectable 6 wk following transplantation.

Previous studies have shown that transient depletion of C3 with cobra venom factor (32, 33) or with anti-C3 mAb (38) inhibits localization and retention of Ag on the surface of splenic FDCs. We found that BM-derived cells can provide sufficient C3 for enhancement of FDC uptake of Ag (Fig. 5). Interestingly, Ag uptake by FDCs occurred although the C3 BM chimeras had low levels of C3 (less than 20 ng/ml) in the peripheral blood. One function of local C3 synthesis would be to provide an increased concentration of C3 for covalent attachment to Ag within the lymphoid compartment. Ag localization to FDCs is not a passive process but is thought to involve active transport to the surface of the FDC, a process that is delayed until the onset of Ab production after immunization (39). In our model, sufficient Ag retention by FDCs surfaces occurred despite low levels of C3 in the circulation of C3 BM chimeras. A subset of homozygous C3-deficient humans has been described that had a similar phenotype as our C3 BM chimeras. Macrophages of these patients produced approximately 25% of the normal rate of C3 when cultured in vitro (40) and serum contained <1% of normal C3 concentration. Interestingly, their Ab response to routine immunization was normal (41).

In summary, we have found that engagement of C3−/− mice with WT BM can reconstitute the impaired immune response in C3-deficient mice. MOMA-2+ macrophages within the white pulp of the spleen were identified as the major cell type that produced C3, and synthesis appears to be regulated since C3 mRNA was not maintained at a normal rate of C3 when cultured in vitro (40) and serum contains 1% of normal C3 concentration. Interestingly, their Ab response to routine immunization was normal (41).

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References


