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Temporal Requirements for B Cells in the Establishment of CD4 T Cell Memory

Sarah B. Mollo,* Allan J. Zajac,† and Laurie E. Harrington*

CD4 T cell memory generation is shaped by a number of factors, including the strength and duration of TCR signaling, as well as the priming environment, all of which can be modified by B cells. Studies using B cell–deficient mice indicate B cells play a critical role in generating effector and memory CD4 T cells; however, when and how B cells are acting to promote these responses has not yet been ascertained. In this study, we use anti-CD20 Ab depletion of B cells at different times following Listeria monocytogenes infection to show that B cells are necessary for the induction of optimal CD4 T cell memory, but not for the transition and maintenance of this population. Importantly, the prerequisite of B cells early postinfection is partially dependent on their expression of MHC class II. B cells are not only required during the priming phase, but also necessary for the initiation of robust secondary responses by memory CD4 T cells. Interestingly, the requirement during the recall response is independent of B cell Ag presentation. Overall, these studies demonstrate the temporally and functionally distinct roles for B cells in regulating CD4 T cell responses. *The Journal of Immunology, 2013, 191: 000–000.

Upon activation, CD4 T cells proliferate and differentiate into effector CD4 T cells and, through the production of cytokines, recruit and activate the appropriate cells to efficiently fight infection (1). Following pathogen clearance, the majority of the effector CD4 T cells undergo apoptosis, leaving behind a population of memory CD4 T cells capable of responding faster and more effectively than their naïve counterparts (2, 3). This augmented secondary response is due to the increased precursor frequency of memory CD4 T cells, as well changes in their functional capacity including increased sensitivity to Ag (4), the ability to simultaneously produce multiple cytokines (5), and differential expression of molecules important for survival (6–8) and migration (5, 9–11). These characteristics of memory CD4 T cells provide the host with enhanced protection upon secondary infection, yet the requirements for the generation of CD4 T cell memory remain unclear.

Rituximab, an mAb that depletes CD20-expressing B cells, is used therapeutically in patients with B cell lymphomas and autoimmune diseases. Interestingly, rituximab ameliorates the disease course in patients with autoimmune disorders in which CD4 T cells are thought to be the primary pathogenic cell population, highlighting a potential role for B cells in regulating CD4 T cell responses (12–15). The prevalent use of this Ab underscores the importance of understanding the impact B cells have on the formation and maintenance of CD4 T cell memory, as the loss of B cells could affect both the generation of new memory CD4 T cell responses as well as the ability of existing memory populations to mount recall responses. B cell depletion studies in mice have shown that short-term B cell depletion can result in aberrant CD4 T cell responses (16); however, the effects on CD4 T cell memory development remain to be elucidated.

A number of studies have shown that B cells can shape CD4 T cell responses by multiple mechanisms including cytokine production (17, 18), Ag presentation (18–20), and cellular localization (21). In addition, the absence of B cells during development results in severely disrupted splenic architecture, which could indirectly alter the CD4 T cell response (22, 23). Due to the multifaceted functions of B cells, we postulated that B cells could impact the generation of CD4 T cell memory at different phases throughout the response.

To dissect the temporal requirements of B cells for the formation and maintenance of memory CD4 T cells, we used an anti-CD20 mAb to deplete B cells prior to or at different times postinfection with recombinant Listeria monocytogenes–GP61 (LM-GP61). B cells are required for the priming of optimal memory CD4 T cells, but are not necessary during the contraction and maintenance phases of the response. This is consistent with our finding that mice lacking the ability to present Ag via B cells to CD4 T cells have decreased effector and memory CD4 T cell responses. Importantly, memory CD4 T cells are reliant on B cells for a robust recall response, yet this is independent of MHC class II (MHC II) expression. Together, these data highlight the importance of B cells for promoting protective CD4 T cell responses.

Materials and Methods

Mice and generation of bone marrow chimeras

C57BL/6 (wild-type [WT]), B6.129S2-Igh-6m1Cgnv3 (B cell−/−), and B6.PL-Thy1aCyJ (WT/Thy1.1) mice were purchased from The Jackson Laboratory; C57BL/6NTac (WT) and B6.129-H2-Ab1tm1Gru (MHC II−/−) were purchased from Taconic Farms. Mice were maintained in fully accredited facilities at the University of Alabama at Birmingham. To generate bone marrow chimeric mice, bone marrow was prepared from WT, MHC II−/−, and B cell−/− mice and depleted of T cells using CD5 (Ly-1) microbeads (Miltenyi Biotec). Recipient B cell−/− mice were irradiated with a split dose of 1000 rad and reconstituted with a mixture of 1.5 × 10^6 total CD5-depleted bone marrow cells from WT and B cell−/− (20:80 ratio), MHC II−/− and B cell−/− (20:80 ratio), or B cell−/− mice. Mice were maintained on acidified water containing sulfamethoxazole, trimethoprim,
and neomycin for 6 wk. Chimeras were infected with LM-GP61 between 8 and 10 wk following reconstitution.

**Infections and anti-CD20 mAb treatment**

Mice were infected with either $2 \times 10^5$ CFU LM-GP61 by i.v. injection or $2 \times 10^5$ PFU lymphocytic choriomeningitis virus (LCMV)-Armstrong by i.p. injection. Mice were administered 240 μg anti-CD20 (SD2) mAb or IgG2a isotype control mAb (both kindly provided by Genentech) by i.p. injection at indicated time points. In some experiments, PBS was used in place of the isotype control mAb.

**Cell preparation**

Spleens were disrupted into single-cell suspensions, and erythrocytes were removed by lysis using 0.83% (w/v) NH₄Cl. Cells were resuspended in RPMI 1640 medium supplemented with 10% FCS, 50 μM 2-ME, 100 U/ml penicillin, 100 μg/ml streptomycin, sodium pyruvate, and nonessential amino acids (R10). Lymph nodes were disrupted into single-cell suspensions and resuspended in R10. Lymphocytes were isolated from whole blood by Histopaque 1083 gradient (Sigma-Aldrich). Peritoneal lavage was used to collect cells from the peritoneal cavity.

**Cell-surface staining and flow cytometry**

Splenocytes were first treated with anti-CD16/CD32 (2.4G2; University of Alabama at Birmingham Immunoreagent Core) and then stained with various combinations of the following mAbs: anti-CD4 (RM4-5; eBioscience), anti-CD44 (IM7; eBioscience), anti-CD62L (MEL-14; eBioscience), anti-B220 (RA3.6B2; eBioscience), anti-CD19 (eBio1D3; eBioscience), anti-CD8α (53-6.7; eBioscience), anti–MHC II (M5/114.15.2; eBioscience), anti-IgM (Invitrogen), anti-CD35/21 (76; eBioscience), anti-A4-1 (AA4.1; eBioscience), anti-CD5 (53-7.3; BD Biosciences), and anti-CD11b (M1/70; BioLegend). For tetramer staining, cells were stained with anti-CD4 (RM4-5; eBioscience), anti-CD127 (A7R34; eBioscience), and anti–CCR7 (4B12; eBioscience) mAbs in conjunction with the LCMV I-Ab(GP66–77) tetramer (obtained from the National Institutes of Health tetramer core facility) or the *L. monocytogenes* I-A^B^ (listerialysin O [LLO]190–201) tetramer (kindly provided by Dr. Marc Jenkins, University of Minnesota) as previously described (24). All samples were acquired on an LSRII flow cytometer (BD Biosciences), and data were analyzed using FlowJo software (Tree Star).

**In vitro stimulation and intracellular staining**

Splenocytes were stimulated for 5 h at 37˚C in 5 to 6% CO₂ in the presence of the I-Ab–restricted peptides GP61–80 or LLO190–201 (Biosynthesis) and brefeldin A (GolgiPlug; BD Biosciences). Intracellular cytokine staining was performed using Cytotox/Cytoperm plus the Fixation/Permeablization kit according to the manufacturer’s directions (BD Biosciences). Cells were stained with anti-CD4 (RM4-5; eBioscience), anti–IFN-γ (XMG.12; eBioscience), anti–IL-2 (JES6-5H4; eBioscience), anti–TNF-α (MP6-XT22; eBioscience), anti–IL-4 (11B11; eBioscience), and anti–IL-17A (eBio17B7; eBioscience) mAbs.

**CD4 T cell purification and transfer**

Single-cell suspensions of splenocytes were prepared from WT/Thy1.1 mice infected 26–38 d previously with LCMV-Armstrong. CD4 T cells were isolated using the Dynabeads FlowComp Mouse CD4 purification kit.

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**FIGURE 1.** B cells are essential for the priming of optimal CD4 T cell effector and memory responses. WT mice treated 7 d previously with IgG2a (or PBS) or anti-CD20 mAb, as well as B cell−/− mice, were infected with LM-GP61. On day 7 (A–C) or days 84–116 (D–F) postinfection, splenocytes were stimulated with either LLO190–201 or GP61–80 peptides and stained for intracellular IFN-γ. (A and D) Representative flow cytometry plots showing LLO190–201-stimulated gated CD4+ cells. The frequencies (B, E) and numbers (C, F) of IFN-γ+ CD4 T cells following LLO190–201 or GP61–80 stimulation are shown. Data are compiled from three experiments with 8–11 mice/group (A–C) or five experiments with 7–20 mice/group (D–F). *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.
according to the manufacturer’s directions (Invitrogen). CD4 T cells (3 to 4 \times 10^8) were transferred into bone marrow chimeric mice by i.v. injection.

**Statistical analysis**

Statistical significance was determined using a one-way ANOVA with Tukey multicomparison test or unpaired Student t test on Prism software (GraphPad). Statistical significance is denoted as *p < 0.05, **p < 0.01, ***p < 0.001, and ****p < 0.0001.

**Results**

**Decreased Listeria-specific effector CD4 T cell response in mice depleted of B cells**

To dissect the temporal requirement of B cells for the development and maintenance of CD4 T cell memory, we used B cell–depleting anti-CD20 mAb, which depletes all nonplasma cell subsets of B cells (Supplemental Fig. 1A–C, 1E, 1G). We first investigated if B cells were required during the priming of a Th1 CD4 T cell effector response. C57BL/6 (WT) mice were injected with anti-CD20 mAb or IgG2a isotype control (or PBS) 7 d prior to infection with recombinant LM-GP61, which expresses the I-A\(^{b}\)-restricted GP61-80 epitope from LCMV. B cell knockout (\(^{-/-}\)) mice were also infected with LM-GP61 to compare CD4 T cell responses in mice that permanently lack B cells with those specifically depleted of B cells during the induction period. Mice depleted of B cells during the priming phase had a lower frequency and number of cytokine-producing CD4 T cells compared with control mice; the number of LLO190- and GP61-specific IFN-\(\gamma\)-producing CD4 T cells were decreased 2.9- and 3.4-fold, respectively, in the anti-CD20 mAb treated mice at day 7 postinfection (Figs. 1A–C, 2). However, B cell \(^{-/-}\) mice exhibited an even greater reduction in the number of LLO190- and GP61-specific CD4 T cells compared with the anti-CD20–treated WT mice (6.6- and 10.4-fold; Fig. 1C). The number of activated CD4 T cells was also decreased, suggesting that the defect is not due to reduced IFN-\(\gamma\) production specifically but an overall reduced CD4 T cell response (Supplemental Fig. 2). Consistent with this, neither IL-4 nor IL-17A production was detected, indicating that the absence of B cells did not shift the effector phenotype of the response (data not shown). These data demonstrate that B cells are required during the priming phase of the response and that permanent genetic deletion of B cells has additional effects on the generation of CD4 T cell responses.

**B cells act during the priming phase to promote CD4 T cell memory**

To assess if the absence of B cells during priming results in a defect in the memory CD4 T cell pool, mice treated with anti-CD20 mAb prior to LM-GP61 infection were analyzed >80 d postinfection. By this time point, the B cell compartment was completely replenished (Supplemental Fig. 1D). In B cell–depleted mice, the frequency and number of \(L.\) \textit{monocytogenes}–specific IFN-\(\gamma\)–, IL-2–, and TNF-\(\alpha\)–producing CD4 T cells were lower than in the WT cohorts (Figs. 1D–F, 2). In contrast, the cytokine-producing CD4 T cells were almost completely ablated in B cell \(^{-/-}\) mice at this time point. In addition, there was also a decrease in the number of I-A\(^{b}\)(LLO190)-tetramer binding CD4 T cells (Supplemental Fig. 3). Importantly, LLO190–specific memory CD4 T cells generated in mice that lacked B cells during priming expressed molecules associated with conventional memory cells, CD127 and CCR7 (Supplemental Fig. 3). Interestingly, comparison of the number of Ag–specific CD4 T cells on day 7 versus day 20 indicated that there is an enhanced contraction of the effector cells in the anti-CD20 mAb–treated mice; a 5.6-fold decrease in the number of IFN-\(\gamma\)– LLO190–specific cells was noted in the WT mice compared with a 13.5-fold decrease in the anti-CD20 mAb–treated mice (Fig. 2A). Together, these data reveal a role for B cells in the priming of both effector and memory CD4 T cells.

**Depletion of B cells at later phases does not impact CD4 T cell memory**

To ascertain the requirements of B cells for the transition of an effector CD4 T cell into a memory cell, as well as for the maintenance of the CD4 T cell memory pool, mice were treated with anti-CD20 mAb on days 7 (contraction phase) or 27–32 (maintenance phase) after LM-GP61 infection. B cell depletion was verified in the peripheral blood (Supplemental Fig. 1E, 1G). CD4 T cell responses were subsequently analyzed during the memory phase (>95 d postinfection), at which point the B cell compartment was reconstituted (Supplemental Fig. 1F, 1H). In contrast to depleting B cells prior to priming, no defect in the CD4 T cell memory response was noted when B cells were depleted during the contraction and maintenance phases. The frequency and number of LLO190– and GP61–specific CD4 T cells producing IFN-\(\gamma\) were similar in both cohorts of mice (Fig. 3), indicating that by day 10 postinfection, B cells are not essential for the transition of CD4 T cells into the memory compartment and that once formed, memory CD4 T cells do not require B cells for their survival.

**Ag presentation by B cells is important for CD4 T cell memory to Listeria**

To determine if B cells impact CD4 T cell responses via their ability to present Ag, mixed bone marrow chimeric mice were infected with LM-GP61; experimental chimeras contained B cells that lack...
MHC II, whereas control chimeras either contained WT B cells or no B cells. Importantly, chimeric mice with MHC II−/− B cells possessed an intact CD4 T cell compartment (Supplemental Fig. 4). On day 7 postinfection, a reduction in the LLO190- and GP61-specific IFN-γ–producing CD4 T cells were detected in mice with MHC II−/− B cells compared to controls with WT B cells; the response was further reduced in mice that lacked B cells completely (Fig. 4A–C). Similar findings were observed by analysis of IL-2 and TNF-α production (data not shown). Moreover, memory LLO190- and GP61-specific CD4 T cells were also decreased in

**FIGURE 3.** B cells are not necessary during the contraction or maintenance phases for memory CD4 T cell survival. WT mice were treated with IgG2a (or PBS) or anti-CD20 mAb on day 7 (A, B) or on days 27 or 32 (C, D) post–LM-GP61 infection. The frequencies (A, C) and numbers (B, D) of IFN-γ+ CD4 T cells after LLO190-201 or GP61-80 stimulation were enumerated. Data shown are from three experiments with 14 to 15 mice/group (A, B) or two experiments with 8 to 9 mice/group (C, D).

**FIGURE 4.** B cell expression of MHC II is critical to induce optimal CD4 T cell responses. Bone marrow chimeric mice with WT B cells (WT/B cell−), MHC II−/− B cells (MHCII−/−B cell−), or no B cells (B cell−−) were infected with LM-GP61. On day 7 (A–C) or days 42–53 (D–F) postinfection, splenocytes were stimulated with LLO190-201 or GP61-80 peptide and then stained for IFN-γ. (A and D) Representative plots show LLO190-201-stimulated gated CD4 T cells. The frequencies (B, E) and numbers (C, F) of IFN-γ+ CD4 T cells following LLO190-201 and GP61-80 stimulation are shown. Data are compiled from three experiments with 6–10 mice/group (A–C) and three experiments with 9–13 mice/group (D–F). *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.
FIGURE 5. Memory CD4 T cells require B cells for robust recall responses. LCMV-infected WT mice were treated with anti-CD20 or IgG2a control mAb (or PBS) 70–72 d postinfection and then challenged with LM-GP61 7 d later. (A–E) Splenocytes were stimulated with GP61–80 peptide on day 6 post-LM-GP61 challenge. (A) Representative flow cytometry plots gated on CD4 T cells. The frequencies of IFN-γ+ (B) and IFN-γIL-2+ (D) as well as the numbers of IFN-γ+ (C) and IFN-γIL-2+ (E) CD4 T cells are shown. (F–H) Cells were stained ex vivo with the I-Ab(GP66–77) tetramer. (F) Representative flow cytometry plots gated on CD4 T cells. Compiled data show the frequency (G) and numbers (H) of I-Ab(GP66–77)+ CD4 T cells. Data shown are from three separate experiments with 8–14 mice/group. In (C), (E), and (H), the fold increase over unchallenged controls is noted. (I–M) To assess primary CD4 T cell responses, splenocytes were stimulated with LLO190–201 peptide on day 6 post-LM-GP61 challenge. (I) Representative flow cytometry plots gated on CD4 T cells. The frequencies of IFN-γ+ (J) and IFN-γIL-2+ (L) as well as the numbers of IFN-γ+ (K) and IFN-γIL-2+ (M) CD4 T cells are shown. Data shown are from two separate experiments with two to seven mice per group. **p < 0.01, ***p < 0.001, ****p < 0.0001.
mice that lacked MHC II on B cells, and these responses were even further curtailed in the chimeras that completely lacked B cells (Fig. 4D–F and data not shown). Together, these data demonstrate the importance of MHC II expression by B cells in the formation of effector and memory CD4 T cell responses and further implicate a requirement for the presence of B cells to promote CD4 T cell populations.

Robust secondary CD4 T cell responses require B cells, but not MHC II expression

To elucidate if memory CD4 T cells require B cells to elicit an optimal secondary response, we employed a heterologous prime/boost strategy. LCMV-infected WT mice were administered anti-CD20 or isotype control mAbs at >70 d postinfection and 1 wk later challenged with LM-GP61. As expected, control-treated mice mounted a robust GP61-specific recall response, with an ∼22-fold expansion in the number of IFN-γ-producing CD4 T cells and an ∼13-fold expansion in the number of cells that co-produce IFN-γ and IL-2 (Fig. 5A–E). Importantly, mice lacking B cells during rechallenge exhibited a blunted secondary expansion, with only a 6.4- and 3.3-fold increase in the number of IFN-γ and IFN-γ/IL-2–producing CD4 T cells, respectively (Fig. 5A–E). Furthermore, there was reduced expansion in the number of I-Aβ (GP33) tetramer-binding CD4 T cells in the anti-CD20 mAb–treated group compared with control mice (Fig. 5F–H). Notably, mice treated with the anti-CD20 mAb prior to LM-GP61 challenge were still able to elicit a primary LLO190-specific CD4 T cell response, indicating that Ag presentation is intact in these mice (Fig. S1–M). These data show a requirement for B cells to drive the full secondary expansion of the memory CD4 T cell response.

Because MHC II expression by B cells is critical to prime strong effector and memory CD4 T cell responses to L. monocytogenes, we tested the requirement for B cell Ag presentation during the recall response. LCMV-primed GP61-specific memory CD4 T cells from WT/Thy1.1 mice were transferred into mixed bone marrow chimeric mice with B cells that either expressed or lacked MHC II, and recipient mice were subsequently infected with LM-GP61. In contrast to the prerequisite for Ag presentation by B cells during the primary response, MHC II expression by B cells was dispensable during the secondary response. There were similar numbers of donor CD4 T cells, as well as GP61–specific cytokine-producing CD4 T cells, in both cohorts of bone marrow chimeras following secondary infection (Fig. 6). These results highlight the distinct requirements for B cells in promoting primary and secondary CD4 T cell responses.

Discussion

Collectively, the data presented in this study show that B cells function via multiple mechanisms to influence the generation of CD4 T cell memory and secondary CD4 T cell responses. We demonstrate that B cells are required specifically during the priming phase of LM-GP61 infection for the full induction of the CD4 T cell memory, whereas the response is unperturbed when B cells are depleted during the contraction and maintenance phases. There was a reduction in the CD4 T cell effector response when B cells were absent at the onset of infection; however, there was also an enhanced contraction in these mice, collectively resulting in a lower CD4 T cell memory pool. These data highlight the importance of B cell–CD4 T cell collaboration in forming CD4 T cell memory responses. These results, in conjunction with the observation that CD4 T cell responses are diminished when B cells do not express MHC II, indicate a specific role for B cell Ag presentation to prime optimal CD4 T cell memory.

Our data and that of others (19, 25–28) have shown that B cells can impact the development of CD4 T cell effector and memory responses. However, there have been conflicting reports on the necessity of B cells for the priming of CD4 T cells (29–34). It has been postulated that CD4 T cells are more dependent on B cells for their differentiation when Ag is limiting. Supporting this hypothesis are studies showing a dose-dependent requirement of B cells for the priming of CD4 T cells by protein Ags (16, 35–37). Recent studies exploring the requirements of T follicular helper cells have suggested that B cells provide a mechanism for long-term stimulation of CD4 T cells that can impact their differentiation (36). This, combined with the concept that CD4 T cell memory formation is supported by high signal strength and prolonged interaction with Ag (38–41), leads us to speculate that B cells may provide the extended stimulation that CD4 T cells require for their full expansion and differentiation into memory CD4 T cells.

Our data illustrate a further reduction in CD4 T cell memory in B cell−/− mice compared with mice transiently depleted of B cells by anti-CD20 mAb treatment or mice in which B cells lack MHC II expression. This indicates an additional role for B cells in this process; however, it is unclear if the permanent genetic deletion of B cells is directly or indirectly impacting the CD4 T cell response. Under steady-state conditions, B cell−/− mice have lower numbers of CD4 T cells (22). Therefore, one possibility is that the resultant decreased response is attributed to a lower precursor frequency of CD4 T cells. Additionally, dendritic cell numbers are also reduced in B cell−/− mice, which in turn could impact the size of the ensuing CD4 T cell response (22).

In addition to the importance of B cells for the primary CD4 T cell response, we show that memory CD4 T cells also require B cells for a robust secondary response. This is somewhat surprising, as memory CD4 T cells are thought to require less antigenic stimulation and be less reliant on costimulation than naive CD4 T cells (4, 5, 42). However, this is consistent with a report demonstrating that optimal recall responses required both dendritic cells and B cells (43). Importantly, our data reveal that Ag
presentation by B cells is not essential for the recall response of memory CD4 T cells, which is in sharp contrast to the primary response. This establishes that B cells use distinct mechanisms to regulate naive and memory CD4 T cell responses. One possibility is that cytokine secretion by B cells provides an additional signal that is necessary for the optimal secondary expansion of memory CD4 T cells (17, 18). It is also plausible that the release of specific chemokines by B cells acts either directly on CD4 T cells or indirectly on another cell type, such as a dendritic cell, to coordinate the physical interaction of these cells, resulting in robust recall responses (21, 44). Likewise, B cells are critical for the development and maintenance of specialized stromal cell and macrophage populations in the spleen and the loss of B cells may impact these cells (22, 45), which could in turn compromise the ability of memory CD4 T cells to respond to antigenic challenge.

There have been limited studies examining the impact of rituximab treatment on the function of CD4 T cells and cell-mediated immune responses (46–49). It has been shown that patients receiving this form of chemotherapy have a decrease in expression of activation markers on CD4 T cells (47–49). This is consistent with our findings demonstrating that B cells are critical for strong primary and secondary CD4 T cell responses. Importantly, our data suggest that rituximab therapy is likely to have an impact on the induction of CD4 T cell immunity, during both primary and secondary challenges, but that pre-existing CD4 T cell memory should remain intact.

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Disclosures

The authors have no financial conflicts of interest.

References


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SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1. Efficient B cell depletion by anti-CD20 mAb treatment. (A-D) WT mice were injected with anti-CD20 or IgG2a isotype control mAb (or PBS) and 7 days later infected with LM-gp61. (A) Lymphocytes from the blood, inguinal LN, and spleen were stained ex-vivo on day 7 post LM-gp61 infection for B220 and CD19 expression. (B) Lymphocytes obtained from peritoneal cavity on day 7 post LM-gp61 infection were stained ex-vivo for B220 and IgM cells (top), or CD11b and CD5 (bottom, gated on B220+IgM+ cells). (C) Quantitation of the B220+CD19+ cells in the spleens on day 7 post LM-gp61 infection. (D) Enumeration of the B220+CD19+ cells in the spleens on days 83-116 post-LM-gp61 infection. (E, F) WT mice were infected with LM-gp61 and treated with anti-CD20 or IgG2a isotype control mAbs (or PBS) on day 7 post-infection. (E) Peripheral blood lymphocytes were stained ex-vivo for B220 and CD19 expressing cells 3 days after mAb administration. (F) Quantitation of B220+CD19+ cells in the spleens on days 97/99 post-infection. (G, H) WT mice were infected with LM-gp61 and treated with anti-CD20 or IgG2a isotype control mAbs (or PBS) on day 27 post-infection. (G) Peripheral blood lymphocytes were stained ex-vivo for B220 and CD19 expressing cells 7 days after mAb administration. (H) Quantitation of B220+CD19+ cells in the spleens on days 116/119 post-infection.

Supplemental Figure 2. Lower frequency of activated CD4 T cells at peak of the effector response to LM-gp61 in the absence of B cells. WT mice treated with IgG2a isotype control (or PBS) or anti-CD20 mAbs, as well as B cell-/− mice, were infected with LM-gp61. Day 7 post LM-gp61 infection, splenocytes were stained with mAbs.
specific for CD4, CD44 and CD62L. (A) Representative plots showing frequencies of CD44+CD62L- cells (gated on CD4 T cells). The frequencies (B) and numbers (C) of CD44+CD62L- CD4 T cells are shown. Data is compiled from 3 experiments with 2-11 mice per group.

**Supplemental Figure 3. LM-specific memory CD4 T cells in the absence of B cells during priming.** WT mice treated with IgG2a isotype control (or PBS) or anti-CD20 mAb, as well as B cell-/- mice, were infected with LM-gp61 and analyzed on days 84-116 post-infection. (A) Splenocytes were stained ex vivo with the I-A^b(LLO_{190}) tetramer in conjunction with mAbs specific for CD4 (top), CD127 (middle), and CCR7 (bottom); representative plots are gated on CD4+ cells. Compiled data of the frequencies (B) and numbers (C) of I-A^b(LLO_{190})+ CD4 T cells is shown. Data is cumulative from 3 experiments with 7-20 mice per group.

**Supplemental Figure 4. Bone marrow chimeric mice contain B cells devoid of MHC class II expression but have an intact T cell compartment.** Separate cohorts of bone marrow chimeric mice were bled 8 weeks post bone marrow transplant. Peripheral blood leukocytes were stained ex-vivo with mAbs specific for: (A) CD4 and CD8, (B) B220 and CD19, and (C) B220 and MHC II. Data is representative from 3 experiments with 8-14 mice per group.
Supplemental Figure 2
Supplemental Figure 3