Requirement of B Cells for Generating CD4+ T Cell Memory

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B cells can influence T cell responses by directly presenting Ag or by secreting Ab that binds to Ag to form immunogenic complexes. Conflicting evidence suggests that persisting Ag-Ab complexes propagate long-term T cell memory; yet, other data indicate that memory cells can survive without specific Ag or MHC. In this study, the roles of B cells and Ag-Ab complexes in T cell responses to lymphocytic choriomeningitis virus (LCMV) infection were investigated using B cell-deficient or B cell-competent mice. Despite normal lymphocyte expansion after acute infection, B cell-deficient mice rapidly lost CD4+ T cell memory, but not CD8+ T cell memory, during the contraction phase. To determine whether Ag-Ab complexes sustain CD4+ T cell memory, T cell responses were followed in B cell-transgenic (mlg-Tg) mice that have B cells but neither LCMV-specific Ab nor LCMV-immune complex deposition. In contrast to B cell-deficient mice, mlg-Tg mice retained functional Th cell memory, indicating that B cells selectively preserve CD4+ T cell memory independently of immune complex formation. An in vivo consequence of losing CD4+ T cell memory was that B cell-deficient mice were unable to resolve chronic virus infection. These data implicate a B cell function other than Ab production that induces long-term protective immunity. The Journal of Immunology, 2009, 182: 1868–1876.

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Abbreviations used in this paper: FDC, follicular dendritic cell; MHC-II, MHC class II; LCMV, lymphocytic choriomeningitis virus; mlg-Tg, membrane Ig transgenic; SIIN, 4-hydroxy-3-nitropheryl acetyl; CTLp, CTL precursor.

Copyright © 2009 by The American Association of Immunologists, Inc. 0022-1767/09/82/00 3 Abbreviations used in this paper: FDC, follicular dendritic cell; MHC-II, MHC class II; LCMV, lymphocytic choriomeningitis virus; mlg-Tg, membrane Ig transgenic; SIIN, 4-hydroxy-3-nitropheryl acetyl; CTLp, CTL precursor.

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responses. In contrast to these studies, B cell-deficient mice generated CD4 memory responses to influenza A virus infection (41); however, the IL-2-limiting dilution method used in this study may have introduced error by, for example, in vitro expansion of contaminating IL-2 T cells. Given the variable nature of infections, including the cell types infected, the differing nature of APCs at sites of infection, and the chronicity/infections of kinetics, it is difficult to understand in general terms how B cells and Ag-Ab complexes affect CD4 memory.

Some studies that have used noninfectious models of T cell differentiation found reduced levels of CD4 T cell memory in the absence of B cells (17, 39); although, in one case, the reduced levels may have been related to a weak primary response to keyhole limpet hemocyanin immunization (39). In another study, primed CD4+ T cells did not survive when transferred to recipients in the absence of Ag (9), which led to the hypothesis that immune complexes are required for Th cell memory maintenance. Moreover, B cells and dendritic cells were implicated in the maintenance of CD4 memory against soluble keyhole limpet hemocyanin immunization where B cells produced the Abs that associated in persistent Ag-Ab complexes that were in turn acquired by dendritic cells (interdigitating, CD11c+) and presented to memory T cells (17). However, it is uncertain whether these Ag-Ab complexes served to rescue CD4 T cells or whether B cells themselves were responsible for, by example, providing a cytokine or other signal to maintain them. In view of data showing the persistence of memory in the absence of specific Ag and evidence that MHC-II is not required for the survival of memory CD4 T cells (6, 48, 49), it remains unclear whether persisting Ag-Ab complexes are needed for CD4 T cell memory.

In the current study, the requirement of B cells and Ag-Ab complexes for T cell memory against lymphoproliferative choriomeningitis virus (LCMV) was investigated in B cell-deficient mice and B cell-transgenic (mlg-Tg) mice (18) using assays that do not require extensive in vitro culture and that accurately reflect the numbers of cells in vivo. Moreover, CD4+ T cell responses to specific MHC-II-restricted epitopes were followed, which eliminates potential contamination by CD8 T cells. All of the B cells in the mlg-Tg mice express only a membrane-bound form of IgM that uses a H chain commonly found in the response to the hapten NP (4-hydroxy-3-nitrophenyl-acetyl). These mice have normal proportions of B cells but a restricted repertoire, severely reduced Ab, and undetectable immune complex formation on FDC (18). We report that B cell-deficient mice (which lack B cells and Ag-Ab immune complexes) generated normal primary CD4+ T cell responses, but CD4+ T cell memory was short-lived. In contrast, mlg-Tg mice established and maintained Th cell memory. Because these mice do not form LCMV-Ag-Ab complexes, these findings indicate that a B cell function other than Ab production sustains CD4+ T cell memory.

Materials and Methods

Mice and virus

C57BL/6 (H-2b) mice were purchased from The Jackson Laboratory and were used as controls for the B cell-deficient mice. The μMT+/− (B cell-deficient) mice (50) were obtained from The Jackson Laboratory and bred at Emory University (Atlanta, GA). The mlg-Tg mice have been described elsewhere (51). They are transgenic for a rearranged VDJ-H chain with a μ H chain lacking the secretion sequence. They were maintained on the JH-knockout BALB/c genetic background (18) to prevent any expression of endogenous Ig. The transgene is not on the same chromosome as the other H chain constant regions; therefore, there is no H chain switching. Since the transgene only affects the H chain, any Ig diversity would result from L chain rearrangements. These mice have normal proportions of B cells in the periphery but do not secrete Ab. CB.17 mice were used as controls for the mlg-Tg mice. C57BL/6 and BALB/c carrier mice used in these experiments were generated and bred at Emory University as previously described (52). Adult experimental mice (6–8 wk old) were infected with 2 × 10^9 PFU of the Armstrong CA 1371 strain of LCMV (53). Where indicated, mice were given 2 × 10^9 PFU of LCMV-11b, LCMV-A22, or LCMV-clone 13 to generate chronic infection (54). The level of virus in the serum and tissues was quantitated by plaque assay on Vero cells (55). All animal experiments were approved by the Emory or The Scripps Research Institute Animal Care and Use Committees.

Flow cytometry

Spleen cells were surface stained with Abs from BD Pharmingen that recognize CD8 (clone 53-6.7), CD4 (clone RM4-5), CD44 (clone IM7), and B220 (clone RA3-6B2) using a concentration of 1 mg Ab/10^6 cells. Anti-mouse IgH (H + L) was purchased from Caltag Laboratories and used as recommended by the manufacturer. Tetramer staining was done as described (55) using allophycocyanin-conjugated D633-41, Kb34-41, and D6396-404 made at Emory University.

Quantification of virus-specific IFN-γ-secreting CD8+ and CD4+ T cells

The method for quantifying T cell responses by staining T cells for intracellular IFN-γ has been described previously (56). Spleen cells were stimulated in vitro with medium or with GP61–80 (for CD4 T cells) or GP34–41 (for CD8 T cells) for 5 h with brefeldin A (GolgiPlug; BD Pharmingen). They were then surface stained with anti-CD8 or anti-CD4 and stained for intracellular IFN-γ using a Cytofix/Cytoperm staining kit (BD Pharmingen) as per the manufacturer’s recommended protocol. For intracellular IFN-γ stain, FITC-conjugated monoclonal rat anti-mouse IFN-γ (clone XMG1.2) and its control isotype Ab (rat IgG1) were used. All Abs were purchased from BD Pharmingen. In some experiments, virus-specific CD8+ and CD4+ T cell responses were measured by an IFN-γ ELISPOT assay using whole spleen cells or CD4-purified preparations from mice immunized with LCMV as has been described previously (56). Either carrier mouse spleen cells or purified LCMV peptides (NP, 396–404; GF33–41; and GF276–286 for H-2b mice or NP118 for H-2d mice) that bind to MHC class I were used to stimulate CD8 T cell responses. LCMV peptides that bind to MHC-II (NP309–328 and GF61–80 of Armstrong) were used to stimulate I-Aβ-restricted CD4 T cell responses (56).

Quantitation of virus-specific IL-2-secreting CD4+ T cells

CD4+ T cell production of IL-2 was measured by cytokine-specific ELISA kits purchased from Genzyme Diagnostics and were performed and analyzed as recommended by the manufacturer. The ELISAs were read with a Bio-Rad Microplate Reader 3550 using appropriate filters.

B cell assays

Levels of serum Ab determined by LCMV-specific ELISA and quantitated of the number of LCMV-specific Ab-secreting cells by ELISPOT assay have been described previously (57).

Results

Primary T cell response to LCMV in B cell-deficient mice

The role of B cells in generating antiviral T cell responses was investigated using B cell-deficient mice (μMT+/−) infected with LCMV-Armstrong. At day 8, spleen cells were recovered and direct killing activity was measured on infected cells. Consistent with previous reports (27, 28, 58), normal levels of antiviral CTL developed (60–70% specific lysis at 50:1 E:T ratio) and the virus was eliminated as indicated by plaque assay (both groups had <1.7 log10 PFU/ml serum and <2.7 log10 PFU/gram of brain, kidney, liver, lung, or spleen). The frequency of CD8+ T cells specific to
each epitope was quantified by intracellular staining for IFN-γ. In B cell-deficient mice, 15% of the CD8+ T cells were specific to GP33-41 and in +/+ mice 14% were specific to this dominant epitope (Fig. 1A). These frequencies indicated that B cell-deficient mice had 8 x 10^5 and +/+ had 9 x 10^6 GP33-41-specific CD8+ T cells per spleen (Fig. 1B). The magnitude of the responses to NP396-404, GP276-286, and NP205-212 were similar in both groups of mice (data not shown). These assays confirm earlier limiting dilution analysis of CTL precursor frequencies in these mice (27) and show that expansion of LCMV-specific CD8 T cells specific to dominant (GP33-41, NP396-404) and subdominant (GP276-286, NP205-212) epitopes does not depend upon B cells.

Early LCMV-specific CD4+ T cell responses were followed in B cell-deficient mice. Robust CD4+ T cell responses were found in B cell-deficient mice during the primary phase of the T cell response (Fig. 1C). On the H-2b background, a major component of the CD4 response is directed against the LCMV epitope GP61-80 presented on I-A^b. The frequency of GP61-80-specific splenic CD4+ T cells was 2% in B cell-deficient mice and 5% in +/+ mice, corresponding to 2.3 x 10^5 cells/spleen in B cell-deficient mice and 7 x 10^5 cells/spleen in +/+ mice (Fig. 1D). Analysis of lymph nodes by IL-2 ELISA indicated that CD4+ T cell priming was normal there (data not shown). In uninfected B cell-deficient mice, the number of CD4+ T cells in the spleen (~5 x 10^6/spleen) is one-half to one-third of that found in uninfected wild-type mice. After infection, the B cell-deficient mice generate a virus-specific CD4+ T cell response that continues to be ~30–40% of controls; therefore, the peak antiviral response in the B cell-deficient mice is proportionate to the starting number of CD4+ T cells.

Memory T cell response to LCMV in B cell-deficient mice

Because the B cell-deficient mice generated good early T cell responses, the effects of B cells and Ag-Ab complexes on memory T cell levels could be determined independently of their influences on the primary response. Therefore, LCMV-specific T cells were quantified in immune B cell-deficient mice at later time points. At day 154 postinfection, a large frequency of splenic CD8+ T cells specific for GP33-41 could be found in +/+ mice and B cell-deficient mice (Fig. 1A). Similar frequencies were also observed by tetramer analysis (data not shown). These percentages correspond to ~1 x 10^6 LCMV-specific CD8+ T cells in +/+ and 2 x 10^5 in B cell-deficient mice (Fig. 1B). B cell-deficient mice also showed strong CD8 memory responses to other LCMV epitopes and the level of CD8 memory was found to be stable from 2 mo to >6 mo postinfection (data not shown). These results confirm previous reports that CD8+ T cell memory can form and be maintained in the absence of B cells (27–29).

In these very same mice, memory CD4 responses were analyzed by the same assays. Although 0.5–1.0% of CD4 T cells were specific for GP61-80 in +/+ mice, B cell-deficient mice had levels at or below the detection limit (<0.01%) by flow cytometry (Fig. 1C). In immune mice, there were 8.5 x 10^5-specific CD4+ T cells in +/+ mice, but there was a >95% reduction to ~2 x 10^5-specific CD4+ T cells in B cell-deficient mice (Fig. 1D). Similar results were obtained with IFN-γ ELISPOT analysis. The results shown in Fig. 1 demonstrate a dichotomy between CD8 and CD4 memory: when analyzed in the same mice and by the same assays, B cell-deficient mice had approximately normal levels of CD8 memory but lost CD4 memory.
Since the most prominent defect in these mice was the loss of CD4 memory, it was of interest to determine when this occurred (Fig. 2). Although initial virus-specific IL-2 and IFN-γ-producing CD4+ T cells were normal, these responses began to wane as early as the second week after infection, and most of the CD4+ T cell response was gone by day 30. This rapid decay might explain discrepancies in previous studies regarding B cell-dependent priming of CD4+ T cells: a response may or may not be found depending on the time point studied. These data indicate that B cell function is revealed during the contraction phase of the response in promoting the establishment and maintenance of CD4 T cell memory.

Long-term CD8 and CD4 memory in mlg-Tg mice

Because B cell-deficient mice lack serum Ab, one hypothesis is that absence of Ag-Ab complexes leads to the loss of CD4 memory. To investigate this possibility, 0.3 ml of hyperimmune serum from LCMV-immune mice was transferred into B cell-deficient mice at days 5 and 15 after infection to form immune complexes. In principle, the anti-LCMV Ab in the serum should bind to Ag-Ab complexes on FDC (18). Fig. 3 shows that mlg-Tg mice have FDC, but they produce no secreted Ab and, therefore, do not deposit Ag-Ab complexes on FDC (18). These mice contain normal numbers of B cells and face (18, 51). These mice contain normal numbers of B cells and permit normal mouse IgM synthesis and expression on the surface.

To determine whether direct effector responses were affected by the absence of Ab, CTL responses were measured by a 51Cr release assay in mlg-Tg mice. These mice had normal CTL responses and the infection was controlled in the serum, liver, lung, and kidney by day 8 after infection (Table I). The early CD8+ T cell response was quantified by IFN-γ ELISPOT (Fig. 4A) and these data indicate that there was a normal expansion of LCMV-specific CD8+ T cells after infection, which resulted in 8 × 10^5 NP118–126-specific cells per spleen in the mlg-Tg mice. Furthermore, elevated numbers of LCMV-specific CD8 T cells persisted during the memory phase with 1–5 × 10^5/spleen in mlg-Tg mice and ∼1 × 10^5/spleen in +/- mice. The generation of LCMV-specific CTP precursors (CTLp) was not affected in mlg-Tg mice, as both +/- and mlg-Tg mice produced ∼1 × 10^5/spleen at day 8 (Fig. 4B). Moreover, high numbers of CTLp remained during the memory phase with both groups containing 3–4 × 10^5/spleen. Consistent with what was seen in B cell-deficient mice, CD8+ T cell memory was not affected by the absence of serum Ab and Ag-Ab complexes.

CD4+ T cell responses were followed in the spleens of mlg-Tg mice at various times after infection. There was expansion of LCMV-specific CD4+ T cells at day 8 (Fig. 4C). In +/- mice, there were 2–3 × 10^5 LCMV-specific CD4+ T cells per spleen at day 8 and mlg-Tg mice had 9 × 10^5-specific splenic CD4+ T cells. This result confirms that Ag-Ab complexes are not needed for early responses, consistent with what was seen in B cell-deficient mice. To determine whether these immune complexes are needed for Th cell memory, virus-specific CD4+ T cell responses were analyzed at later times in the mlg-Tg mice. At days 30 and 60 after infection, there remained 3–4 × 10^5 LCMV-specific CD4+ T cells

Table I. Normal antiviral CTL responses in mlg-Tg mice

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in +/+ mice and 5 × 10³ in mlg-Tg mice. Virus-specific CD4⁺ T cells from mlg-Tg mice made normal levels of IL-2 at day 8 and 2 mo later (Fig. 4D). These results show that immune complexes with LCMV Ag are not required to establish and maintain CD4⁺ T cell memory and, in the absence of these complexes, memory CD4⁺ T cells retain their capacity to secret IFN-γ and IL-2.

As a more rigorous test of memory, +/+ and mlg-Tg mice immunized 90 days earlier were rechallenged with a high-dose infection (1 × 10⁶ PFU of LCMV Armstrong). Three days after the rechallenge, levels of virus were quantified by plaque assay (Fig. 5A). Although naive mice had very high levels of virus in the liver, immune +/+ and mlg-Tg mice controlled the infection quickly and reduced the viremia to levels below detection. A direct ex vivo ⁵¹Cr release assay indicated that immune +/+ and mlg-Tg mice were capable of producing IFN-γ in the liver of naive mice before reinfection and the bars showed the number found after reinfection. Bars represent the average of two to three mice. CD4⁺ T cells from two to three mice were purified and the number of LCMV-specific CD4⁺ T cells found in immune mice before reinfection. Purified CD4⁺ T cells from each group were also analyzed by ELISA for IL-2 secretion following virus stimulation in vitro. The limit of detection for this assay was 15 pg/ml. Dashed lines depict limits of detection for each assay.

LCMV-specific CD8⁺ T cells expanded from 4 × 10⁵ in immune mlg-Tg mice to 5 × 10⁷/spleen at day 3 after reinfection. In contrast, naive mice generated only 1 × 10⁵/spleen by this time. There was also secondary expansion of memory CD4⁺ T cells in immune +/+ and mlg-Tg mice. CD4⁺ T cells were purified from immune mice before and after challenge infection and analyzed by IFN-γ ELISPOT (Fig. 5D). The number of Ag-specific CD4⁺ T cells expanded from 4 × 10⁵ to 1.6 × 10⁶/spleen 3 days after the infection in +/+ mice. Similarly, the number of Ag-specific CD4⁺ T cells in mlg-Tg mice expanded from 5 × 10⁵ to 3.5 × 10⁶/spleen. By comparison, naive mice generated only 500 specific cells during the same period. The responding CD4⁺ T cells were also capable of producing IL-2. These data show that memory CD8⁺ and CD4⁺ T cells were functionally intact in mlg-Tg mice.

FIGURE 4. Primary and memory T cell responses in mlg-Tg mice. MLCM-specific CD8⁺ T cells per spleen of infected mice. A, Numbers of LCMV-specific CD8⁺ T cells were quantified by IFN-γ ELISPOT assay following stimulation with LCMV peptide NP₁₁₈₋₁₂₆. The averages of two to three mice per time point are shown. B, CTLp were quantified by limiting dilution assay at day 8 (primary) or days 34, 60, and 68 (memory). The bars represent the average of four to seven mice per group. C, CD4⁺ T cells were colabeled with and then stimulated with LCMV-carrier spleen cells in an IFN-γ ELISPOT assay. The line graphs show the total number of CD4⁺ T cells per spleen that produced IFN-γ in this assay. The averages of two to three mice per time point are shown. D, Purified CD4⁺ T cells were cultured with virus-infected carrier spleen cells for 24 h. ELISAPA was used to measure the levels of IL-2 in the supernatants. The bars represent the average of four to seven mice per group. Dashed lines indicate the limits of detection for each assay and the error bars represent SDs.

FIGURE 5. Anamnestic responses in immune mlg-Tg mice. Wild-type and mlg-Tg mice immunized 90 days earlier with LCMV were rechallenged with 10⁶ PFU of LCMV (strain Armstrong) and, at day 3 after the reinfction, levels of virus and T cell responses were quantified. A, The level of virus infection in the liver was determined by plaque assay. The amount of infection in the liver of naive mice is shown for comparison. B, Levels of CTL in each group were measured by direct ex vivo ⁵¹Cr release assay. Average specific lysis for immune mlg-Tg, +/+ and naive mice are shown with SD error bars. C, The number of LCMV-specific CD8⁺ T cells was determined by IFN-γ ELISPOT following NP₁₁₈₋₁₂₆ stimulation. Horizontal lines represent the number of memory cells found in immune mice before reinfection and the bars show the number found after reinfection. Bars represent the average of two to three mice. D, CD4⁺ T cells from two to three mice were purified and the number of LCMV-specific CD4⁺ T cells was determined by IFN-γ ELISPOT following stimulation with LCMV-infected carrier spleen cells. The horizontal lines represent the number of memory CD4⁺ T cells found in immune mice before reinfection. Purified CD4⁺ T cells from each group were also analyzed by ELISA for IL-2 secretion following virus stimulation in vitro. The limit of detection for this assay was 15 pg/ml. Dashed lines depict limits of detection for each assay.
and that they were able to respond to challenge infection by increasing in number and producing cytokine. These findings establish that serum Ab and LCMV-Ag-Ab complexes are not required to maintain CD4 or CD8 memory.

**B cell-deficient mice are impaired in their ability to resolve chronic LCMV infection**

Previous studies revealed an important role for CD4 T cells during chronic viral infection. When CD4 T cell responses are missing due to gene ablation, transient depletion with Ab or lack of costimulation, CD8-dependent clearance of chronic virus infections becomes impaired (60–62). Similar associations of CD4+ T cell loss with diminished antiviral CTL are seen after HIV, Hepatitis B Virus, Human CMV, and Hepatitis C Virus infections in humans (63–65).

Previous studies in mice examined situations in which no CD4 response (or a severely impaired response) was present during all phases of the chronic infection. Therefore, it is unclear whether...
CD4+ T cells function early on, perhaps to qualitatively or quantitatively enhance CTL development and memory CD8 development (66–68), or whether they function later, perhaps to maintain antiviral CTL (69). Since B cell-deficient mice generate strong primary antiviral CD8 and CD4 responses, but then lose the CD4 response over the following 2–3 wk, it was of interest to examine whether loss of Th cell responses would compromise CTL-mediated clearance of chronic LCMV infection. Therefore, B cell-deficient mice were infected with various strains of LCMV that normally persist in the serum in +/- mice for 3 wk to 4 mo, depending on the variant. These variants spread throughout the body, but the viral load was eventually reduced in mice capable of maintaining strong CTL responses (54, 55, 70). Although +/- mice eventually controlled these infections, B cell-deficient mice never reduced the level of virus in the blood, liver, kidney, or lung (Fig. 6A and data not shown). Even when the level of virus was similar in magnitude early on (clone13, A22), only the +/- mice cleared the infection in the blood.

To determine whether the lack of viral control in B cell-deficient mice correlated with the reduced frequency of LCMV-specific T cells, T cell responses were analyzed in a separate set of mice that were given LCMV-11b infection 44 days before. Although wild-type mice contained an elevated frequency of GP33, +/+ specific CD8+ T cells, infected B cell-deficient mice had a very low frequency (Fig. 6B). The total number of epitope-specific CD8+ T cells in B cell-deficient mice that were able to make IFN-γ was 1% of that found in +/- mice (Fig. 6C, top). Tetramer staining confirmed that there were fewer epitope-specific CD8+ T cells in the B cell-deficient mice compared with the infected wild-type mice (Fig. 6C, bottom). During this chronic virus infection, LCMV-specific CD4 responses were also lost in B cell-deficient mice (Fig. 6, D and E). In contrast to +/- mice, IL-2 production by CD4+ T cells was severely diminished in B cell-deficient mice (Fig. 6E, bottom). The defective antiviral T cell responses seen in the B cell-deficient mice correlated with elevated viral loads in the livers, lungs, and kidneys of the same mice; the wild-type mice that contained functional T cell responses had a lower viral load at this time (Fig. 6F). Hence, chronic virus infection in mice devoid of B cells results in the loss of virus-specific Th cells and the exaggerated deletion of virus-specific CD8+ T cell responses.

A subset of CD8+ T cells can be programmed early to differentiate into memory cells (71–74). It is unknown what signals are required for this differentiation event that occurs within the first week of infection, although CD4+ T cells contribute to this process (66, 67, 69). A primary CD4+ T cell response is made in LCMV-Armstrong-infected B cell-deficient mice during this programming period (Fig. 1), and they generate normal numbers of memory CD8 T cells that can resolve a rechallenge infection (Refs. 27 and 30 and Fig. 1). To test whether the virus-specific memory CD8+ T cells found in acutely infected B cell-deficient mice are capable of controlling a widespread infection, spleen cells from LCMV-Armstrong-immunized B cell-deficient mice were transferred into LCMV-carrier mice that are congenitally infected and immunologically tolerant to the virus; virus reduction in these LCMV-carrier mice is due to the transferred cells (52, 75, 76). Consistent with an earlier report (75), the level of virus in the blood of recipient mice was reduced to below the limits of detection by splenocytes from the immune +/- mice; however, the cells from the immunized B cell-deficient mice (Fig. 6G) did not do so. This difference in viral load was reflected by the number of virus-specific CTLp surviving later (Fig. 6H): carrier mice that received wild-type immune cells and controlled the infection had 4 × 103 CTLp/spleen, whereas carrier mice that received immune B cell-deficient spleen cells had levels of CTLp that were below detection (<400/spleen). Even though Armstrong immune +/- and B cell-deficient mice had memory CTL (27), those present in immune B cell-deficient mice could not confer protection to infected mice. The results indicate that B cells and memory CD4 T cells play important roles for maintaining CTL in conditions of chronic infection and that having CD4 help early on is not sufficient to limit these infections.

Discussion

CD8 memory is maintained in the absence of specific Ag and even selecting MHC molecules, and consistent with these findings, CD8 memory does not require B cells or Ag-Ab complexes for maintenance (Refs. 27, 29, and 30 and Figs. 1 and 4). Evidence also indicates that CD4+ memory T cells survive in the absence of MHC (6, 48). However, CD4+ T cell memory maintenance requires B cells in some systems, but not in others; no clear picture has emerged to explain these differences. Importantly, in cases where B cells affect CD4+ T cell memory, the mechanisms have not been elucidated. In this study, we examined the role of B cells and Ag-Ab complexes in the induction and maintenance of T cell responses to LCMV, including the relevance of such responses to viral clearance and secondary protection. We learned that a primary CD4 response is formed to LCMV in the absence of B cells; however, it decays rapidly and is essentially undetactable by 30 days after infection. Also, the role of B cells in this process, contrary to predictions, was independent of Ab secretion.

A major finding of this study is that B cells play a pivotal role during the contraction phase to generate CD4 memory. It was previously thought that immune complexes on FDC were acquired by B cells (11) or by dendritic cells (17), which then process the complex and present antigenic peptide via MHC-II for memory T cells. However, our results with mlg-Tg mice show that CD4 memory does not depend upon these complexes, since these mice do not secrete the Ab required for their formation. Other evidence indicates that CD4 memory cells survive in the absence of specific Ag and MHC-II (6, 48), although in one case they became non-functional without these signals (49). We find that not only do memory cells persist in the absence of Ag-Ab complexes in mlg-Tg mice, these cells are functional in cytokine production and fully capable of undergoing secondary recall responses in vivo (Fig. 5). An important caveat is that although the B cells in these mice have a transgene coding for the H chain of Ig, it is possible that variation in the L chain of Ig leads to virus-specific B cells, since this H chain in combination with various L chains can contribute to a variety of autoantibodies (51) and responses to a variety of proteins (Ref. 18 and L. G. Hannum and M. J. Shlomchik, unpublished data). This raises an interesting possibility, for which there is no precedent: virus-specific B cells could serve as APCs by retaining Ag via BCR-Ag complexes and then re-presenting antigenic peptide via MHC-II. B cells can retain processed Ag for 1 day (77), but whether they can retain Ag longer remains to be seen. B cells can clearly present Ags to CD4+ T cells and they can also transport particulate Ags to the follicles (15).

B cells serve a vital role during persistent viral infections, as B cell-deficient mice are less able to eliminate these infections (Fig. 6 and Refs. 28 and 58). However, our interpretation of why B cell-deficient mice are impaired in their responses to chronic infection differs from that proposed by earlier studies that did not quantify CD4 responses and perhaps for this reason favored the conclusion that neutralizing Ab is vital for the eventual resolution of chronic infection. In view of the fact that B cell-deficient mice lose CD4 memory and that depletion of CD4+ T cells even transiently during the acute phase of the response impairs the ability of CTL to resolve infection (54, 78, 79), an additional mechanism is
that CTL responses are lost during chronic infection due to short-lived virus-specific CD4 help. Perhaps CD4-dependent IL-2 production or CD4 induction of costimulatory molecule expression on APCs allows CD8+ T cells to endure high Ag load over time. Our results highlight the importance of B cells and CD4 memory for sustaining CD8 CTL during chronic infection, which is particularly relevant to successful vaccine design in humans. Another clinical implication from our study is that B cell-depleting drugs may inadvertently lead to reduced memory T cell responses and increase patient susceptibility to viral infections. Indeed, there is an increased incidence of viral, bacterial, and fungal infections in patients that have received B cell-depleting drugs (80).

CD8+ and CD4+ T cell memory are differentially regulated (1). The expansion of the CD8 response is much greater (~20-fold) than the CD4 response. In addition, the contraction phase of the CD8 response is quick, occurring mostly within the second week after infection, whereas that of the CD4 response is protracted and occurs over 1 mo (81, 82). The resulting levels of memory remain greater for CD8+ T cells than for CD4+ T cells. Once established, CD8 memory is stable over the lifetime of the mouse. CD4 memory is generally found to be long-lived, although some evidence indicates that it slowly declines in geriatric mice (36, 82). Hence, there are regulatory mechanisms that differentially affect the homeostatic levels of CD8 and CD4 memory over the long term (1). Without B cells, the contraction phase of the CD4 response accelerates and becomes more severe, resulting in a complete loss of memory CD4 T cells 2 wk after the peak response (Fig. 2), whereas CD8 memory was maintained stably up to 400 days (the last time point measured) after infection in the same mice. Hence, during the generation of CD4+ T cell memory, B cells function early, at least by the contraction phase of the response; however, they may act even earlier than this by programming T cell survival during the cognate interactions that stimulated T cell proliferation. Even signals received during the first few days of a response may be sufficient to activate and differentiate effector and memory CD8 cells (71, 72) and influence contraction (83); therefore, it may be that B cells further these earliest differentiation signals to improve CD4 memory development. Qualitative differences in the way they stimulate CD4 T cells, for example, via cytokines (84) and/or costimulatory molecule expression, could be crucial for selecting a pool of CD4 T cells that survive on into the memory phase. In this scenario, the unique roles of B cells may be due to B cell-specific combinations of local cytokines (e.g., lymphotoxin α, lymphotoxin β, TNF) as well as costimulatory molecules (e.g., B7-RP, OX40L (85, 86)) that act directly on CD4 memory cells; indeed, B cells differ from dendritic cells markedly in these respects. B cells might act through indirect ways to augment CD4+ T cell responses. B cells facilitate the movement of Ag-presenting dendritic cells within lymphoid organs (87, 88), which may impact the quality of Ag presentation to CD4+ T cells. B cells also influence T cell trafficking and accumulation within the lymphoid organs (88). B cell effects on stromal cell number or organization might affect T cell survival or the basal IL-7 levels that are key for CD4 memory (3, 4). Thus, B cells most likely contribute to the quality of CD4 T cell memory via multiple mechanisms. Our data indicate that optimal vaccine strategies will recruit B cells to engage CD4+ T cells, which in turn will directly affect CD4 T cell memory and indirectly affect CD8 T cell effector function, particularly in the face of chronic infection.

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Disclosures

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References


