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CD40 Ligand-Deficient Mice Generate a Normal Primary Cytotoxic T-Lymphocyte Response but a Defective Humoral Response to a Viral Infection

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CD40 ligand is expressed on activated T cells and interacts with CD40 on B cells and monocytes. It is not known what role CD40 ligand plays in the generation of immune responses to viral infection. To address this issue, we examined virus-specific T- and B-cell responses in CD40 ligand-deficient (CD40L−/−) mice following infection with lymphocytic choriomeningitis virus (LCMV). We found that primary anti-LCMV specific antibody responses were severely impaired in CD40L−/− mice, with the defect being most striking for antibody of the immunoglobulin G1 (IgG1) isotype. Interestingly, low levels of LCMV-specific antibodies of the IgG2a, IgG2b, and IgG3 isotypes were made in the CD40L−/− mice, showing that IgG1 responses are totally dependent on CD40L but that at least some IgG2a, IgG2b, and IgG3 responses can be CD40L independent. However, unlike CD40L+/+ mice, CD40L−/− mice were unable to sustain virus-specific antibody responses and showed a gradual decline in serum antibody levels over time. The CD40L−/− mice were also deficient in the generation of memory B cells. In contrast to the severely impaired humoral responses, CD40L−/− mice generated potent virus-specific $CD8^+$ cytotoxic T-lymphocyte responses after LCMV infection and were able to clear the virus. These results show that CD40L does not play a role in generating primary virus-specific $CD8^+$ cytotoxic T-lymphocyte responses but does affect the primary antibody response and the generation of memory B cells.

CD40 ligand is expressed transiently at high levels on activated $CD4^+$ T cells and interacts with CD40, which is present on B cells and monocytes/macrophages (26). CD40L stimulation of B cells results in their activation and induces survival signals which permit further development. Evidence of a role for CD40 as a necessary signal for T-dependent (TD) humoral responses was found in vivo by blocking the CD40-CD40L interaction by treating mice with soluble CD40-immunoglobulin (sCD40Ig) (14) or monoclonal antibody against CD40L (11) or by genetically disrupting these genes (9, 17, 25, 33). Serum levels of immunoglobulin G (IgG) antibodies to TD antigens is blocked when this interaction is inhibited, but antibody responses to T-independent (TI) antigens are normal. Consistent with these observations, defects in CD40L cause the human disease X-linked hyperimmunoglobulin syndrome (HIM) (3, 6, 10, 13, 18). This disease is characterized by high levels of serum IgM and very low amounts or no IgG, IgA, or IgE (24). Unable to class switch to the gamma heavy chain without CD40 signaling, antigen-specific, activated B cells from these individuals produce IgM only, resulting in a relative increase in serum IgM.

In addition to stimulation of CD40 signaling pathways in B cells and monocytes, CD40-CD40L interactions may also influence T cells via CD40L signaling. HIM patients have normal numbers of T cells, which indicates that CD40L-CD40 interactions are not necessary for T-cell development, and their T cells are capable of proliferating in vitro in response to phytohemagglutinin, concanavalin A, pokeweed mitogen, and alloantigens (24). However, individuals with HIM suffer from *Pneumocystis carinii* pneumonia, *Giardia lamblia*, and *Cryptosporidium* infections of the intestinal tract (24), which suggests that this interaction could be important for developing some T-cell effector functions. A recent study using CD40L−/− mice has shown that priming of antigen-specific $CD4^+$ T cells requires CD40L signaling (15). Without CD40L signaling, $CD4^+$ T cells do not proliferate or secrete cytokines when stimulated with recall antigen. It is also reported that at least some $CD8^+$ T cells express CD40L (4, 19, 21, 29), but the role of CD40L stimulation in the priming of $CD8^+$ T cells has not been resolved.

It is not known what role CD40L plays in the context of a viral infection. The purpose of these studies was to investigate the role of CD40L in the generation of antiviral antibodies and in the development of antiviral cytotoxic T lymphocytes (CTL). Following lymphocytic choriomeningitis virus (LCMV) infection, significantly fewer antibody-secreting cells were found in the spleens and bone marrow of infected mice, as demonstrated by ELISPOT assay, and no memory B cells were detected. Furthermore, CD40-ligand deficient mice were found to have significantly lower antiviral serum IgG levels than mice with CD40 ligand. Analysis of virus-specific IgG subclasses demonstrated that while low levels of IgG2a, IgG2b, and IgG3 were present, IgG1 levels were below our limits of detection. However, the mice were able to clear the infection by 8 days postinfection and had normal CTL responses compared with CD40L+/+ mice. These results indicate that CD40L is required for developing normal antiviral antibody responses but

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is not required for generating a primary antiviral CTL response.

MATERIALS AND METHODS

Mice. C57BL/6 (*H-2*^{b}) mice were purchased from Jackson Laboratory, Bar Harbor, Maine. The CD40-ligand-deficient mice (C57BL/6 background) used in these experiments were created by targeted gene disruption which abrogates surface expression of the CD40 ligand molecule (33). C57BL/6 carrier mice used in these experiments were bred at the University of California at Los Angeles.

Virus. The Armstrong CA 1371 strain of LCMV was used in this study (2). Mice 6 to 12 weeks old were infected with 2×10^5 PFU of the Armstrong strain intraperitoneally.

Virus titration. Infectious LCMV in serum and tissues was quantitated by plaque assay on Vero cell monolayers as previously described (2).

CTL assay. Major histocompatibility complex class I-restricted LCMV-specific CTL activity was determined by ^{51}Cr release assay as previously described (2). One lytic unit is the number of effector cells which gives 30% lysis in a CTL assay.

Flow cytometry. The number of CD4$^+$ and CD8$^+$ T cells in the spleen and lymph nodes was determined by staining with specific monoclonal antibodies followed by fluorescence-activated cell sorting (FACS) analysis as previously described (1). For FACS analysis, phycoerythrin-conjugated anti-CD8, biotinylated anti-mouse CD4, streptavidin-phycoerythrin, and fluorescein isothiocyanate-conjugated anti-mouse CD44 were purchased from Pharmingen, La Jolla, Calif.

ELISA. LCMV-specific antibody titer was determined by solid-phase enzyme-linked immunosorbent assay (ELISA) as described previously (2, 28). To determine the isotypes of anti-LCMV serum antibody, ELISAs were performed with biotinylated goat anti-murine IgG1, IgG2a, IgG2b, IgG3 from Caltag, San Francisco, Calif. After additional washes to remove unbound biotinylated antibody, horseradish peroxidase-avidin-D (Vector Laboratories, Burlingame, Calif.) was added to each well of the ELISA plate. Color development of the ELISA was done as described previously (2).

ELISPOT assay. Quantitation of LCMV-specific antibody-secreting cells in the spleen and bone marrow was performed as previously described (28).

Limiting dilution analysis of memory B cells. The assay is a modification of the limiting dilution assay for quantitating CTL precursors (30) as developed by Slifka and Ahmed (27).

RESULTS

Virus-specific antibody responses in CD40L−/− mice. Others have shown that in vivo treatment of mice with a monoclonal antibody specific for CD40L (11, 31) or sCD40Ig (14) inhibits primary and secondary TD humoral responses to nonreplicating antigens. Furthermore, mice lacking CD40L or CD40 do not generate normal TD antibody responses (9, 17, 25, 33). To investigate whether CD40-CD40L interaction is required for humoral responses after a live viral infection, sera, spleen cells, and bone marrow were collected from CD40L−/− and CD40L+/+ mice infected with 2×10^5 PFU of the Armstrong strain of LCMV and analyzed for anti-LCMV IgG by ELISA and ELISPOT assays.

Figure 1 shows that there is a profound defect in the IgG antibody response to LCMV in CD40L−/− mice. At all time points tested (days 8 to 162), the level of virus-specific antibody was significantly less in mice lacking CD40 ligand than in +/+ mice. At 8 days postinfection, the CD40L−/− mice produced approximately 30-fold less LCMV-specific IgG than CD40L+/+ mice. At day 15, this difference was 50-fold, and in immune mice at days 100 and 160 postinfection, there was a 600-fold difference in antibody level. This result demonstrates that CD40 ligand is required for generating high levels of class-switched IgG antibody. Not only was the magnitude of the anti-LCMV antibody response significantly less in the absence of CD40 ligand, but the size of the response waned with time, unlike in +/+ mice. In the CD40L+/+ mice, a peak in serum anti-LCMV IgG was reached by day 15 (ELISA titer, ≈100,000). The level of antibody remained at this magnitude ≥1 year postinfection (Fig. 1 and data not shown). In striking contrast, in CD40L−/− mice, there was an immediate decrease in serum LCMV-specific antibody after day 15. The titer dropped 3- to 6-fold by day 30 and 6- to 14-fold by day 60 compared with the level found at day 15 (Fig. 1). The virus-specific IgG subtypes also displayed a similar decline with time (Fig. 2a).

However, in contrast to previous studies using TD immunization protocols, CD40L-deficient mice make low levels of virus-specific IgG. Previous reports indicated that when the CD40-CD40L interaction is abrogated, no TD IgG antibody responses are generated (9, 17, 25, 33). Figure 1 shows that at day 8 postinfection, antiviral antibody titers were as high as 900, 30-fold above the limit of detection of the ELISA. By day 15, some of the infected CD40L−/− mice had titers in the range of 1,000 to 5,000. While the antibody levels of these mice are significantly less than those of infected +/+ mice, low levels of antiviral IgG are made in the absence of CD40L.

Figure 2 shows the LCMV-specific serum IgG subtype profiles of both CD40L+/+ and CD40L−/− mice. The predominant subtype was IgG2a at all time points in both sets of mice and constituted 70 to 90% of the total anti-LCMV IgG in CD40L-deficient mice and 60 to 75% in +/+ mice at day 15 postinfection (Fig. 2b). This contrasts with the finding of Kawabe et al. (17), who showed that no isotype of IgG is made following dinitrophenol (DNP)-ovalbumin (OVA) immunization of CD40 knockout mice, and with those of Renshaw et al. (25), who show that no isotypes of IgG are made to trinitrophenol (TNP) in TNP-keyhole limpet hemocyanin (KLH) (in alum)-immunized CD40L−/− mice (25). Furthermore, we also find IgG2b and IgG3 antibody specific for LCMV. IgG2b contributed between 2 and 6% of the total anti-LCMV serum antibody, and IgG3 contributed 9% of the IgG at day 15 (Fig. 2b). In +/+ mice, IgG2b contributed 8 to 12% of the virus-specific IgG, and IgG3 represented ≈12% at this time. However, we find that the anti-LCMV IgG1 response is severely altered in CD40L-deficient mice. In infected +/+ mice, IgG1 constituted 7 to 12% of the serum antiviral IgG, but in CD40L-deficient mice, IgG1 was below the level of detection and constituted <0.5% of the total antiviral IgG. The IgG1 level was at least 170-fold less than the IgG2a level in the CD40L−/− mice and was at least 20-fold less than IgG2b and IgG3 levels.

The number of plasma cells producing anti-LCMV IgG antibody was enumerated by ELISPOT assay. Table 1 shows the frequency and total number of antibody-secreting cells per spleen of mice at day 8 postinfection. There are significantly more antibody-secreting cells in CD40L+/+ mice than in CD40L−/− mice. The frequency of anti-LCMV IgG plasma cells at day 8 postinfection in the CD40L+/+ mice ranged between 570 and 915 antibody-secreting cells per 10^6 spleen cells, whereas in the CD40L-deficient mice, the frequency was only 4 to 10. Previous studies in our laboratory have shown that long-term antibody production of serum antibody occurs in the bone marrow after acute LCMV infection (28), and so the numbers of anti-LCMV plasma cells in the spleen and bone marrow were quantitated at later time points postinfection. As can be seen from Table 1, the frequency of anti-LCMV plasma cells in the spleen of immune +/+ mice ranged between 12 and 54 per 10^6 spleen cells and between 43 and 55 per 10^6 bone marrow cells. In contrast, the frequency of IgG anti-LCMV plasma cells in the spleens of CD40L−/− mice was below the level of detection (<2/10^6 cells or <100 plasma cells per total spleen) and in the bone marrow was ≤1 plasma cell per 10^6 bone marrow cells (or <200 plasma cells per total bone marrow), indicating that there are few plasma cells and there is little to no migration of plasma cells to the bone marrow in the absence of CD40L. Therefore, the low level of anti-LCMV IgG serum antibody is due to a dearth of LCMV-specific antibody-secreting cells in the spleen at day 8 and the relative absence of

plasma cells in the spleen and bone marrow at later time points. Thus, these results show that CD40L is necessary for the efficient generation of antiviral IgG-secreting plasma cells.

The number of virus-specific memory B cells was quantitated in an in vitro limiting dilution memory B-cell assay (27). The results of this assay indicate that there was less than one LCMV-specific IgG memory B cell per 10^6 spleen cells in day 60 immune CD40L-deficient mice, whereas in control +/+ mice, the frequency was one memory B cell per 10^4 immune spleen cells (Fig. 3). Therefore, CD40 ligand is required to generate normal numbers of antiviral memory B cells.

CD40L−/− mice clear an acute viral infection. Infection of adult +/+ mice with the Armstrong strain of LCMV causes an acute infection which is resolved by LCMV-specific cytotoxic $CD8^+$ T lymphocytes by day 8 postinfection. The importance of these virus-specific CTL in the clearance of LCMV has been documented by numerous investigators (1, 2, 7, 8, 16, 20, 22, 23, 34). Since there are some reports of CD40L expression on $CD8^+$ T cells (4, 19, 21, 29) and because there may be stimulation of cytokine secretion and/or costimulatory molecules following CD40 stimulation of antigen-presenting cells, the importance of CD40L in generating a primary CTL response was investigated following infection of CD40L-deficient mice with LCMV.

At day 8 postinfection, the amounts of infectious virus in the sera, livers, and spleens of CD40L−/− and CD40L+/+ mice were quantitated by plaque assay. Table 2 shows that both CD40L+/+ and CD40L−/− mice cleared the infection by day 8, as the number of plaques was below the level of detection in all tissues and sera analyzed.

To investigate the role of CD40L in generating antiviral CTL effectors, the primary anti-LCMV CTL response was quantitated by CTL assay using spleen cells harvested at day 8 postinfection. Table 2 shows that there was no difference in the abilities of spleen cells harvested from CD40L−/− and from CD40L+/+ mice to kill LCMV-infected target cells. Furthermore, the numbers of lytic units per spleen were similar between the two groups of mice: CD40L-deficient mice had 250 to 320 lytic units per spleen, and the CD40L+/+ mice had 300 lytic units per spleen. These data indicate that CD40L expression is not required for generating primary antiviral CTL.

$CD8^+$ T cells were also analyzed by flow cytometry. Figure 4a shows a representative profile of $CD8^+$ T cells at day 8 postinfection. Both CD40L+/+ and CD40L-deficient mice developed activated $CD8^+$ T cells compared with uninfected controls. This finding indicates that CD40L is not required for $CD8^+$ T-cell activation. Furthermore, there was expansion of $CD8^+$ T cells in both CD40L+/+ and CD40L−/− mice (Fig. 4b), all of which was in the activated, $CD44^{hi}$ subset. The number of activated $CD8^+$ T cells increased 5-fold in the infected +/+ mice and 12-fold in the knockout mice compared with the respective uninfected control mice. The total number of activated $CD8^+$ T cells was also not affected by the presence or absence of CD40 ligand (Fig. 4b). The CD40L+/+ mice had 57×10^6 activated $CD8^+$ T cells, and the CD40L−/− mice had

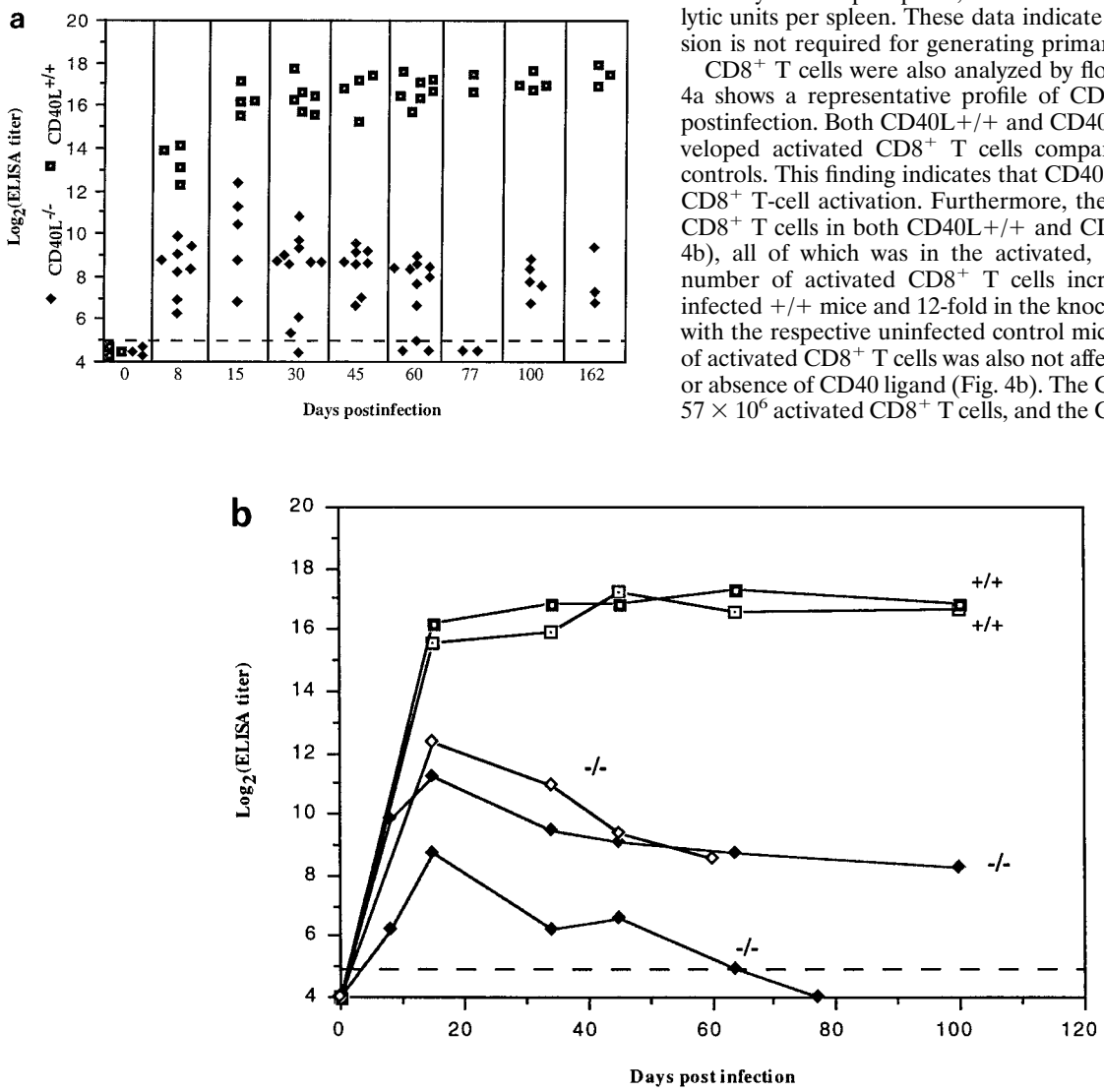

FIG. 1. LCMV-specific serum IgG in CD40L+/+ and CD40L-deficient mice. Serum antibody was measured in mice at the indicated time points after infection with LCMV. Anti-LCMV IgG antibody was measured by ELISA as described previously (2). (a) Each point represents the serum titer for one mouse. (b) LCMV-specific antibody levels in individual mice. The data shown are representative of many mice analyzed.

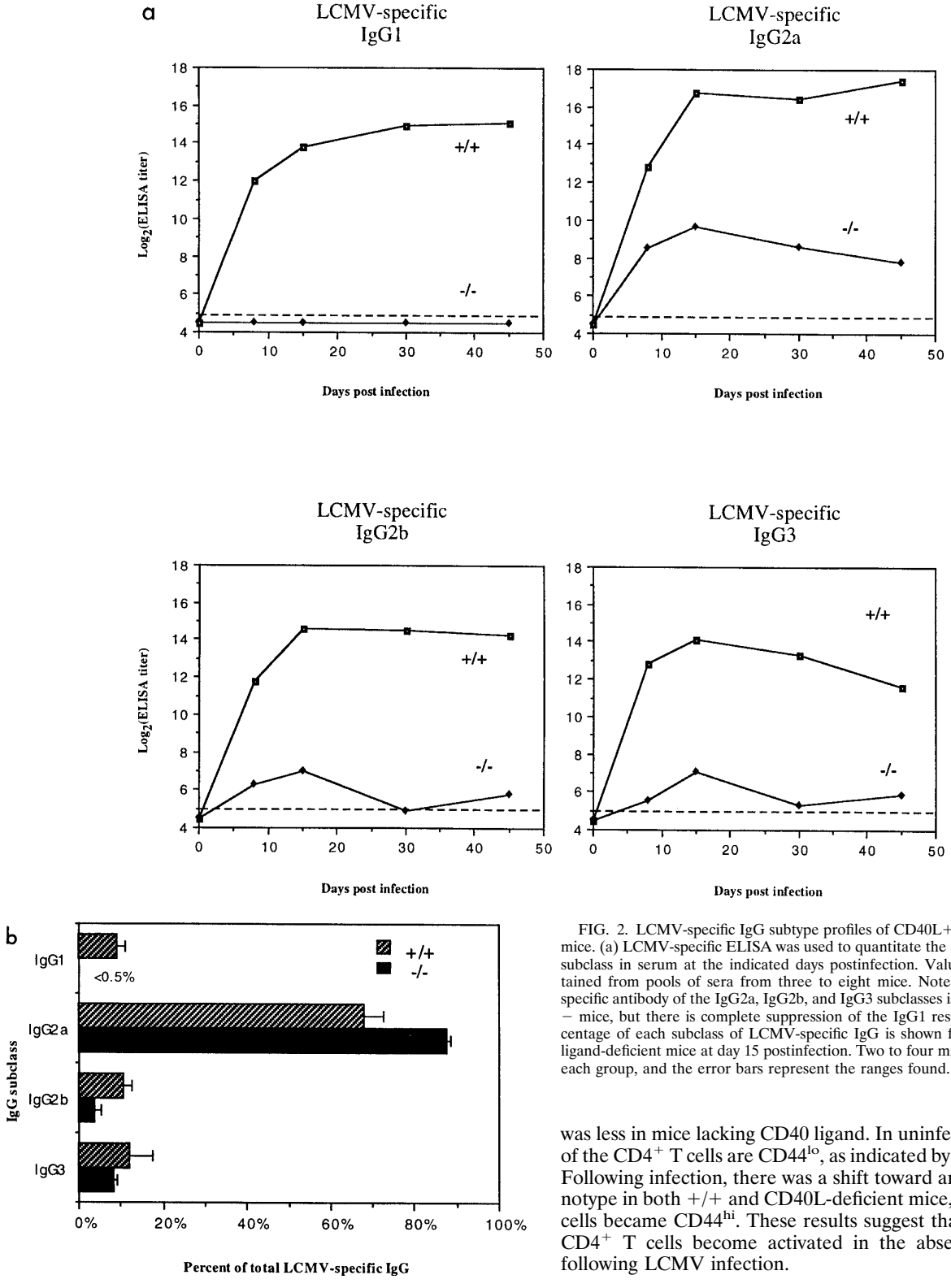

FIG. 2. LCMV-specific IgG subtype profiles of CD40L+/+ and CD40L−/− mice. (a) LCMV-specific ELISA was used to quantitate the amount of each IgG subclass in serum at the indicated days postinfection. Values shown were obtained from pools of sera from three to eight mice. Note that some LCMV-specific antibody of the IgG2a, IgG2b, and IgG3 subclasses is made in CD40L−/− mice, but there is complete suppression of the IgG1 response. (b) The percentage of each subclass of LCMV-specific IgG is shown for +/+ and CD40-ligand-deficient mice at day 15 postinfection. Two to four mice were analyzed in each group, and the error bars represent the ranges found.

53×10^6 activated $CD8^+$ cells. Therefore, CD40L is not required for $CD8^+$ T-cell activation and expansion in vivo following a viral infection.

There was also some activation of $CD4^+$ T cells in CD40L-deficient mice (Fig. 4a). However, the amount of activation was less in mice lacking CD40 ligand. In uninfected mice, most of the $CD4^+$ T cells are $CD44^{lo}$, as indicated by flow cytometry. Following infection, there was a shift toward an activated phenotype in both +/+ and CD40L-deficient mice, as many $CD4^+$ cells became $CD44^{hi}$. These results suggest that at least some $CD4^+$ T cells become activated in the absence of CD40L following LCMV infection.

DISCUSSION

This study characterizes primary antiviral responses in mice in which CD40-CD40L interaction is abrogated. The primary $CD8^+$ T-cell response is normal following an acute infection. There was expansion of activated $CD8^+$ T cells (Fig. 4), and

TABLE 1. LCMV-specific antibody-secreting cells in CD40L+/+ and CD40L-deficient mice

Group	Day post-infection	Antibody-secreting cells[a] in:			
		Spleen		Bone marrow	
		Per 10^6 cells	Total	Per 10^6 cells	Total
+/+	8	743	80,000	<1	<160
	30	54	2,400	55	5,800
	60	12	246	43	5,900
−/−	8	7	680	<1	<160
	30	<2	<100	<1	<160
	60	<2	<100	1	160

[a] The number of LCMV-specific IgG-secreting cells was quantitated at the indicated times postinfection by ELISPOT assay as described previously (28).

TABLE 2. Mice lacking CD40L eliminate an acute LCMV infection and generate a primary effector CTL response[a]

Mouse	LCMV-specific CTL in spleen (% specific ^{51}Cr release at indicated effector/target ratio)							
	Infected targets				Uninfected targets			
	50:1	16.7:1	5.6:1	1.9:1	50:1	16.7:1	5.6:1	1.8:1
−/−	59	34	19	11	88	76	76	54
−/−	49	29	16	8	87	66	44	54
+/+	68	43	22	14	55	54	54	54

[a] Adult CD40L+/+ and CD40L−/− (C57BL/6) mice were injected intraperitoneally with 2×10^5 PFU of the Armstrong strain of LCMV. At day 8 postinfection, virus titers and CTL activity were measured. Virus titers, determined by plaque assay on Vero cells as described previously (2), were, for all mice, <1.7 $\log_{10}$ PFU/ml of serum, <2.5 $\log_{10}$ PFU/g of liver, and <1.7 $\log_{10}$ PFU/10^6 spleen cells. LCMV-specific CTL activity in the spleen was measured by direct ex vivo CTL assay as described previously (2).

the magnitude of the anti-LCMV CTL response appeared normal as determined by CTL assay (Table 2). Furthermore, the mice cleared LCMV infection in the spleen, liver, and serum (Table 2).

The antibody response was severely altered, however. The quantity of serum anti-LCMV IgG was much lower in the knockout mice: 30-fold less at day 8, 50-fold less at day 15, and 200- to 600-fold less at later time points postinfection. Few antibody-secreting cells were found in the spleen at day 8, and the number of plasma cells in the bone marrow in immune mice was at or below our level of detection. No virus-specific memory B cells were found.

Previous studies demonstrate that abrogation of the CD40-CD40L interaction in mice prevents TD antibody responses. Presence of sCD40Ig during the immunization of mice with DNP-OVA reduces the amount of serum IgG1 and IgG2a specific to DNP (14) compared with control treated mice. In addition, treatment of mice with anti-gp39 (CD40 ligand) also inhibits the development of primary and secondary responses to TD antigens (11). Furthermore, mice which lack CD40 (9, 17) or CD40L (25, 33) do not generate normal TD humoral responses but have normal TI antibody responses. Deletion of CD40 eliminates IgG antibodies specific to KLH (a TD antigen) in TNP-KLH-immunized mice (9). Kawabe et al. (17) demonstrated that mice incapable of expressing CD40 do not generate any of the IgG isotypes to DNP in DNP-OVA immunized mice, indicating that immunoglobulin class switching does not take place in response to TD antigens. Furthermore, mice lacking CD40L are unable to undergo isotype switching to IgG1 in response to immunization with KLH in complete Freund's adjuvant (33). Renshaw et al. (25) also demonstrated that CD40L−/− mice do not generate any of the IgG isotypes to TNP following immunization with TNP-KLH and generate only weak secondary IgM antibodies to TNP. We found that mice lacking CD40 ligand did not make anti-LCMV IgG1 antibodies but did make IgG2a, IgG2b, and IgG3 antibodies. The fact that any IgG is made indicates that there are additional pathways for making IgG, but these alternative pathways are not sufficient to generate as much IgG as in mice capable of expressing CD40L. One possible explanation for the presence of IgG is that virus-specific antibody-secreting cells were generated in a T-independent fashion. Future studies will explore whether LCMV can generate a TI antibody response which matches the isotype profile found here.

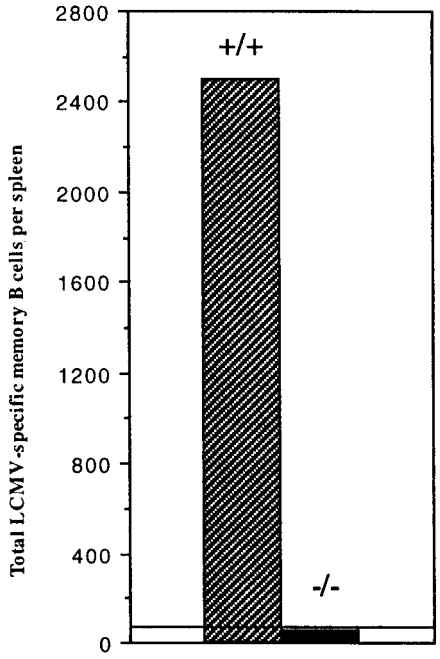

FIG. 3. Total LCMV-specific memory B-cell precursors. A limiting dilution memory B-cell assay was used to quantitate the number of splenic memory B cells present in immune +/+ and CD40L-deficient mice at day 60 postinfection.

A potential explanation for why IgG1 is affected the most by the absence of CD40L is that CD40 differentially affects $CD4^+$ T-helper cells so that Th2 cells (a source of interleukin-4 [IL-4] and IL-10) are inhibited more than Th1 cells, permitting few to no B cells to develop into IgG1-secreting cells. Since both mice generate large numbers of activated $CD8^+$ T cells, gamma interferon levels will be similar whereas levels of IL-4 (which is made only by Th2 $CD4^+$ T cells) may be relatively low. Secretion of IgG2a antibody, a Th1-type response, may occur in the absence of Th2-type cells and might occur even in the absence of fully functional Th1 cells if gamma interferon levels are high. Future experiments will quantitate the amounts of gamma interferon, IL-10, and IL-4 that are made following infection and which cells are making them.

Not only was the magnitude of the LCMV-specific IgG response less in the CD40L-deficient mice, but there was also a decrease after day 15 in antibody levels, whereas in CD40L+/+ mice, the antibody response remained at high levels for long periods of time (Fig. 1 and reference 28). The initial peak at day 15 may be due to activated B cells or short-lived plasma cells. In CD40L+/+ mice, many plasma cells are formed during this period and so the overall serum titer is high, but in the

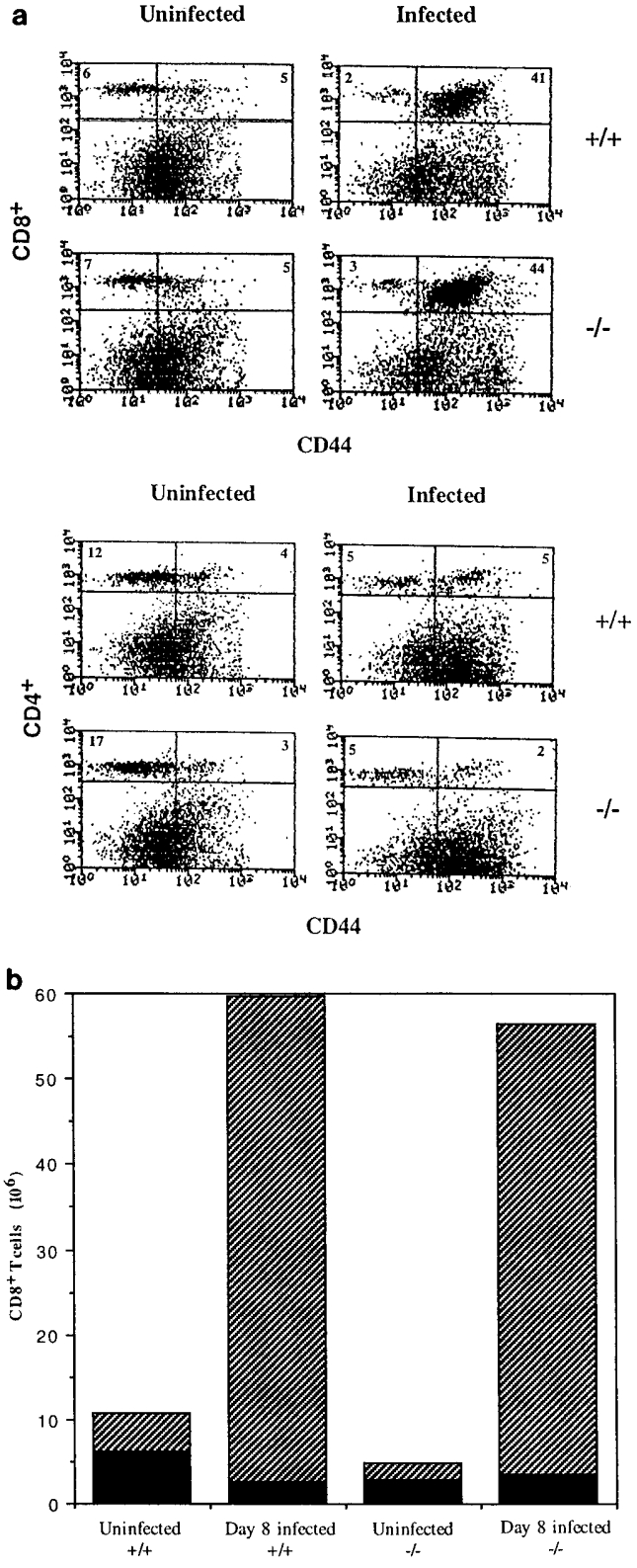

FIG. 4. CD40L is not required for $CD8^+$ T-cell activation and expansion. (a) At day 8 postinfection, spleen cells from CD40L+/+ and CD40L−/− mice were analyzed by flow cytometry using antibodies specific to CD4, CD8, and CD44. CD44 represents an activation marker for T cells. Profiles of uninfected CD40L+/+ and CD40L−/− mice are shown for comparison. Numbers within each quadrant represent the percentages of the total number of cells analyzed. (b) Cell numbers were calculated by multiplying the percentage of cells of each category as determined by flow cytometry by the total number of viable cells per spleen as determined by trypan blue exclusion. At day 8 postinfection, equivalent numbers of activated $CD8^+$ T cells are found in both CD40L+/+ and CD40L−/− mice. The total numbers of $CD8^+$ T cells increased 5-fold in CD40L+/+ mice and 12-fold in CD40L−/− mice compared with the number of $CD8^+$ T cells found in uninfected mice. Cross-hatched columns represent activated, $CD44^{hi}$ $CD8^+$ T cells; solid columns represent $CD44^{lo}$ cells.

absence of CD40L, few plasma cells form, and as the activated B cells or short-lived plasma cells die, there is an overall decrease in serum IgG titer. The antibody found at late time points may be due to a few long-lived plasma cells formed in these mice or some undetectable number of memory B cells which form and differentiate into antibody-secreting plasma cells in a CD40L-independent fashion.

CD40-CD40L interaction has been implicated in the generation of antigen-specific memory B cells (12, 14). Consistent with these observations, we found that in the absence of CD40L, the frequency of virus-specific memory B cells was less than 1 memory B-cell precursor per 10^6 spleen cells in CD40L−/− mice ($n = 4$). CD40L+/+ mice, by comparison, had memory B-cell precursor frequencies which averaged $1/3 \times 10^4$ spleen cells. Therefore, CD40L is required for developing normal precursor frequencies of virus-specific memory B cells.

There was strong activation of $CD8^+$ T cells at day 8 postinfection in the CD40L-deficient mice that was similar to that of CD40L+/+ mice (Fig. 4a). The percentage of $CD8^+$ T cells expressing CD44 increased from 5% in uninfected mice to 40% at day 8 postinfection in both sets of mice. In addition, there was very high proliferation of these T cells (Fig. 4b). Furthermore, the primary CTL responses were not affected by the absence of CD40L (Table 2). There are reports that at least some $CD8^+$ T cells express CD40L (4, 19, 21, 29). While our results do not contradict these findings, there does not appear to be a change in the $CD8^+$ T-cell response in mice lacking CD40 ligand following infection with LCMV.

Armitage et al. (5) report that CD40L-expressing CV1/EBNA cells stimulate CD3- and phytohemagglutinin-activated T cells, indicating that T cells may also have CD40 molecules and that CD40L-CD40 interactions may be important for T cell-T cell interactions. In view of our results, primary $CD8^+$ CTL do not require this interaction and are not affected by the absence of CD40 ligand since there was normal expansion and activation of $CD8^+$ T cells and the anti-LCMV CTL response was normal in the absence of CD40 ligand.

$CD4^+$ T cells are activated during LCMV infection in the absence of CD40L, as indicated by their expression of the activation marker, CD44 (Fig. 4). Future experiments will investigate the degree of activation of $CD4^+$ T cells functionally and elucidate the role of CD40-CD40L interaction in $CD4^+$ T-cell function during primary viral infection and long-term recall responses.

In summary, CD40 ligand is not required to generate antiviral CTL and is not required for $CD8^+$ T-cell activation and expansion. CD40 ligand is required to permit high levels of antiviral IgG. Since low levels of antiviral IgG2a, IgG2b, and IgG3 are found in the sera of LCMV-infected CD40L-deficient mice, there may be CD40L-independent mechanisms for generating small populations of B cells which secrete these antibodies. In addition, CD40L is required for immunoglobulin class switching to the IgG1 isotype, as this isotype is absent from the sera of infected knockout mice but is present in sera of infected wild-type mice.

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