Humoral Immunity Due to Long-Lived Plasma Cells

Mark K. Slifka,* Rustom Antia,[†]
Jason K. Whitmire,* and Rafi Ahmed*[‡]
*Emory Vaccine Center
Department of Microbiology and Immunology
Emory University School of Medicine
[†]Department of Biology
Emory University
Atlanta, Georgia 30322

Summary

Conventional models suggest that long-term antibody responses are maintained by the continuous differentiation of memory B cells into antibody-secreting plasma cells. This is based on the notion that plasma cells are short-lived and need to be continually replenished by memory B cells. We examined the issue of plasma cell longevity by following the persistence of LCMV-specific antibody and plasma cell numbers after in vivo depletion of memory B cells and by adoptive transfer of virus-specific plasma cells into naive mice. The results show that a substantial fraction of plasma cells can survive and continue to secrete antibody for extended periods of time (>1 year) in the absence of any detectable memory B cells. This study documents the existence of long-lived plasma cells and demonstrates a new mechanism by which humoral immunity is maintained.

Introduction

Antibodies provide the first line of defense against infection by microbial pathogens, and it is not coincidental that many of the currently used vaccines that confer long-term protective immunity also induce prolonged humoral responses (Plotkin and Mortimer, 1988; Ahmed and Gray, 1996; Slifka and Ahmed, 1996b). For example, immunization with live viral vaccines such as those against measles, polio, and yellow fever, or even inert vaccines such as those against tetanus or diphtheria toxoid, result in circulating antibody in the serum that can be detected for decades (Plotkin and Mortimer, 1988; Slifka and Ahmed, 1996b). Since the half-life of free immunoglobulin (Ig) is less than 3 weeks (Fahey and Sell, 1965; Talbot and Buchmeier, 1987; Vieira and Rajewsky, 1988), continuous antibody production is necessary to sustain antigen-specific Ig levels in the serum.

The majority of serum antibody is produced by terminally differentiated plasma cells. These nondividing cells differ from memory B cells in many respects. For instance, plasma cells down-regulate surface expression of many typical B cell markers, including major histocompatibility (MHC) class II and surface Ig (Abney et al., 1978; Halper et al., 1978). These changes indicate that,

unlike memory B cells, mature plasma cells are unlikely to participate in antigen processing and presentation. Instead, the main function of a plasma cell is to continuously secrete large quantities of specific antibody. Plasma cell secretion rates have been estimated to be as high as 10,000 molecules/second (Helmreich et al., 1961; Hibi and Dosch, 1986). In contrast, memory B cells do not spontaneously secrete antibody; rather, following appropriate stimulation, these cells proliferate and differentiate into antibody-secreting cells (ASC). Because protection against microbial infection often relies on the level of preexisting antibody in the serum or mucosal surfaces, the number and specificity of preexisting plasma cells are critical components of protective immunity.

The mechanisms underlying long-term antibody production are not fully understood, but the conventional model postulates that the maintenance of serum antibody requires the continuous proliferation and differentiation of memory B cells into antibody-secreting plasma cells (Szakal et al., 1989; Tew et al., 1990; MacLennan et al., 1992; Stites et al., 1994; Gray et al., 1996; Zinkernagel et al., 1996). This model is based on the belief that plasma cells are short-lived. Although an early study (Miller, 1964) had suggested that some plasma cells may survive for several months, current immunological dogma holds that plasma cells have a half-life of only a few days (Cooper, 1961; Schooley, 1961; Makela and Nossal, 1962; Nossal and Makela, 1962; Levy et al., 1987) to at most a few weeks (Ho et al., 1986). Such rapidly dying cells would of course require repopulation by memory B cells in order to sustain ongoing humoral responses.

In this study, we have reinvestigated this issue and show that plasma cells are long-lived and that a substantial fraction of these terminally differentiated cells can survive for the life of the mouse. We have found that long-lived plasma cells are present in the bone marrow as well as in the spleen. Consistent with our findings, a recent study (Manz et al., 1997) that examined plasma cell longevity in the bone marrow showed that plasma cells can survive for more than 90 days without turnover. Our study, as well as that by Manz et al., documents the importance of plasma cells in maintaining long-term antibody production and challenges the presently popular concept that plasma cells are short-lived.

Results

Humoral Response to Viral Infection
The role of plasma cells in maintaining long-term antibody production was examined using the mouse model of infection with lymphocytic choriomeningitis virus (LCMV) (Oldstone and Dixon, 1967; Oldstone et al., 1985). Adult mice infected with LCMV (Armstrong strain) make vigorous antiviral T and B cell responses and clear the virus within 2 weeks (Ahmed et al., 1988; Lau et al., 1994; Slifka et al., 1995). These acutely infected mice then

[‡]To whom correspondence should be addressed (e-mail: ra@microbio.emory.edu).
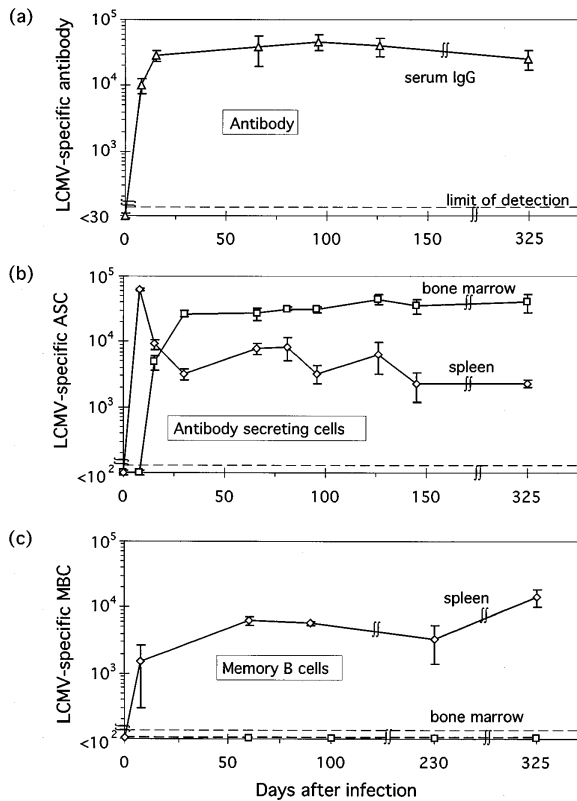
Figure 1. Kinetics of the Humoral Response Following Acute LCMV Infection

Adult BALB/c mice (6–8 weeks old) were infected intraperitoneally with 2×10^5 pfu of LCMV Armstrong and analyzed for the presence of virus-specific antibody in the serum (A) and the number of LCMV-specific ASC (B) or memory B cells (MBC) (C) in the spleen and bone marrow. Virus-specific IgG in the serum was determined by ELISA (Ahmed et al., 1984) and measured in ELISA units. LCMV-specific plasma cells were quantitated by ELISPOT assay (Slifka et al., 1995) and are defined as cells that lack detectable surface Ig and MHC class II molecules (Abney et al., 1978; Halper et al., 1978) and spontaneously secrete large amounts of specific antibody directly ex vivo without restimulation by antigen. Memory B cells were analyzed by limiting dilution analysis (Slifka and Ahmed, 1996a) and are defined as surface Ig^+MHC class II^+ cells that do not spontaneously secrete antibody but proliferate and differentiate into antibody-secreting plasma cells following restimulation with antigen. The data shown represent the average of three to six mice at each timepoint.

continue to maintain high levels of virus-specific antibody in the serum for life (Figure 1A) (Moskophidis and Lehmann-Grube, 1984; Slifka et al., 1995).

To characterize the antibody response further and to examine the dynamics of plasma cells and memory B cells in sustaining long-term antibody production, we quantitated the number of virus-specific plasma cells and memory B cells present in LCMV-infected mice. As shown in Figure 1B, the initial plasma cell response occurred in the spleen and peaked at day 8 postinfection, with 6×10^4 ASC per spleen. Thereafter, the number of virus-specific ASC declined to 3×10^3 ASC/spleen, indicating a 95% reduction in numbers by 30 days postinfection. LCMV-specific ASC in the spleen then persisted at this level for more than 300 days. Similar ASC

kinetics were observed in the lymph nodes (data not shown). In contrast, LCMV-specific plasma cells were not detected in the bone marrow at 8 days postinfection, but as splenic populations declined, an increasing number of plasma cells accumulated in the bone marrow. After day 30 and for the life of the mouse, the bone marrow contained 2×10^4 to 3×10^4 virus-specific ASC. This indicates that bone marrow is the predominant site of antiviral antibody production, with nearly 90% of virus-specific plasma cells residing in this compartment (Slifka et al., 1995). Similar results have been obtained with other viral infections (Bachmann et al., 1994; Hyland et al., 1994) and after booster vaccination with inert antigens (Benner et al., 1981; Dilosa et al., 1991; Smith et al., 1997).

Since memory B cells, by definition, do not spontaneously secrete antibody directly ex vivo, memory B cell numbers were quantitated after 6 days of in vitro stimulation with virus. This induced virus-specific B cells from LCMV-immune mice (but not from naive mice) to proliferate and differentiate into ASC (Slifka and Ahmed, 1996a). Quantitation of LCMV-specific memory B cell numbers was performed in vitro by limiting dilution analysis. In contrast to the migration of ASC to the bone marrow, LCMV-specific memory B cells were identified mostly in the spleen and lymph nodes of infected mice (Figure 1C). The total number of memory B cells per spleen increased from approximately 1.5×10^3 at day 8 postinfection to approximately 5×10^3 by day 60. This indicates that a memory B cell frequency of about 1 per 2×10^4 was maintained for longer than 300 days after infection. Virus-specific memory B cells were also identified in the lymph nodes (frequency 1 per 6×10^3) and peripheral blood (1 per 2×10^5), but were not detected in the bone marrow (<1 per 3×10^5) by limiting dilution analysis.

Memory B Cell Depletion In Vivo

It is evident from Figure 1 that LCMV-immune mice maintain stable populations of both virus-specific memory B cells and plasma cells. The relatively constant pool of ASC provides the high level of virus-specific antibody in the serum, but how are these ASC numbers maintained? Does this maintenance reflect a population of long-lived plasma cells, or are these cells dying rapidly and being replenished by the stimulation of memory B cells? To address this question, we depleted memory B cells in vivo and monitored virus-specific antibody titers and plasma cell numbers when there was no longer a memory B cell pool present to repopulate dying plasma cells.

To deplete memory B cells in vivo, adult LCMV-immune mice (>60 days postinfection) were given 600 rad of total body irradiation. Within 24 hr after irradiation, this dose depleted more than 95% of total lymphocytes from the spleen and the lymph nodes (data not shown). Previous studies have shown that 500–600 rad of whole-body irradiation effectively eliminates antigen-specific memory B cell responses in vivo (Makinodan et al., 1962; Okudaira and Ishizaka, 1981) and that memory B cells and naive B cells are equally radiosensitive (Anderson and Lefkovits, 1980). In our studies, LCMV-specific memory B cell depletion was confirmed by several techniques. Functional analysis, such as limiting dilution experiments, showed that no virus-specific memory B cells

Table 1. In Vivo Depletion of LCMV-Specific Memory B Cells by γ-irradiation

Group	Number of Mice	Days Postirradiation	Reconstitution[a]	Number of Cells/spleen	Frequency of Memory B Cells[b]
LCMV-immune	25	No irradiation	None	8.9×10^7	1 per 2.0×10^4 ($\pm 0.5 \times 10^4$)
Naive	4	No irradiation	None	9.4×10^7	$<1/10^6$
LCMV-immune	3	1	None	0.4×10^7	$<1/10^6$
	3	1	IgH[b]	1.8×10^7	$<1/10^6$
	3	15	IgH[b]	8.4×10^7	$<1/10^6$
	3	30	IgH[b]	7.9×10^7	$<1/10^6$
	3	50	IgH[b]	8.4×10^7	$<1/10^6$
	3	146	IgH[b]	5.3×10^7	$<1/10^6$
	4	240	IgH[b]	5.7×10^7	$<1/10^6$

BALB/c mice (IgH[a]) were infected with 2×10^5 pfu LCMV Armstrong at 6–8 weeks of age. At 60 days postinfection, mice received 600 rad of total-body irradiation to deplete memory B cells.
[a] Within 4 hr postirradiation, mice were reconstituted with allotypic BALB/c IgH[b] spleen and bone marrow cells.
[b] After irradiation, the splenic memory B cell frequency dropped below detection (<1 memory B cell per 10^6 spleen cells) as determined by limiting dilution analysis. Likewise, LCMV-specific memory B cells were no longer detected in the lymph nodes after irradiation, and the absence of memory B cells was confirmed by adoptive transfer/challenge experiments.

(<1 per 10^6 spleen cells) could be detected in irradiated mice (Table 1). In addition, adoptive transfer and challenge experiments indicated that memory B cell numbers had fallen to below the level of detection. Transfer of 2×10^7 spleen cells from irradiated immune mice resulted in undetectable titers by enzyme-linked immunosorbent assay (ELISA) (<30 ELISA units) at day 7 postchallenge, in contrast to transfer of the same number of LCMV-immune spleen cells, which resulted in high ELISA titers (50,400 ELISA units).

To monitor the depletion of host B cells visually, irradiated LCMV-immune BALB/c (IgH[a]) mice were reconstituted with B and T lymphocytes plus bone marrow cells from naive congenic BALB/c IgH[b] mice on the day of irradiation. The lymphoid tissues of these irradiated/reconstituted mice consisted almost exclusively of donor IgH[b]-bearing B cells (Figure 3C). Following irradiation and reconstitution, no LCMV-specific memory B cells were detected (Table 1), indicating that not only were the host's preexisting memory B cells depleted, but naive donor (IgH[b]) B cells were not primed into becoming part of the memory B cell pool. Also, rechallenge of irradiated/reconstituted LCMV-immune mice with the same strain of LCMV failed to induce an anamnestic response, again indicating that memory B cell populations had dropped to below the level of detection (data not shown). Taken together, these results not only demonstrate that host memory B cells were effectively depleted, but also show that irradiated LCMV-immune mice did not contain a depot of viral antigen capable of priming naive B cells.

In contrast to memory B cells, which are easily depleted by irradiation (Makinodan et al., 1962; Anderson and Lefkovits, 1980; Okudaira and Ishizaka, 1981), plasma cells are notably radiation resistant (Makinodan et al., 1967; Okudaira and Ishizaka, 1981). Analysis by the enzyme-linked immunospot (ELISPOT) technique 1 day after irradiation revealed a drop of 2-fold or less in the total number of virus-specific plasma cells in the bone marrow and spleen (Figure 3A). Thus, total body irradiation (followed by allotypic B cell reconstitution) provided a simple in vivo system for monitoring the persistence of virus-specific serum antibody and plasma cell numbers in an environment containing no detectable virus-specific memory B cells and lacking antigen to stimulate naive B cells.

Antibody Titer and Plasma Cell Lifespan in the Absence of Memory B Cells
The levels of virus-specific antibody in the sera of irradiated/reconstituted LCMV-immune BALB/c mice were monitored for an 8-month period (Figure 2). Although antibody titers showed a gradual decline, it was striking that high levels of virus-specific antibody were still present in the serum even 8 months after memory B cell depletion. The LCMV-specific antibody was entirely of the host IgH[a] allotype, showing that virus-specific antibody production was maintained solely by preexisting host plasma cells. No virus-specific antibody of the donor IgH[b] allotype could be detected. This provided additional evidence that there was no priming of naive B cells in irradiated/reconstituted LCMV-immune mice (Figure 2A). Persistence of host IgH[a] virus-specific antibody in the serum was not due to residual, preexisting virus-specific Ig, since passive transfer of LCMV-immune serum into naive recipients demonstrated a rapid rate of decline (IgG half-life [$t_{1/2}$] = 11.7 ± 0.5 days).

Analysis of the dynamics of serum antibody titers following irradiation provides a quantitative estimation of the rate of plasma cell loss in vivo. Since irradiation eliminates the memory B cell population and kills only a fraction of plasma cells, we expected the antibody titer to follow a biexponential decay. The initially rapid decay of serum antibody is a consequence of the loss of a fraction of the plasma cells upon irradiation, followed by a slower decay that reflects the rate of loss of surviving plasma cells in the absence of renewal by memory B cells. Using a mathematical model of decay that incorporates these processes (see Experimental Procedures), we obtained estimates of the fraction of plasma cells lost upon irradiation as well as estimates of the lifespan of plasma cells. The LCMV-specific serum IgG data indicated a mean plasma cell $t_{1/2}$ of 138 days. Similar results ($t_{1/2}$ = 141 days) were obtained by fitting a simple exponential decay curve to the antibody titers obtained after the first 30 days following irradiation. To characterize this result further, the half-life of plasma
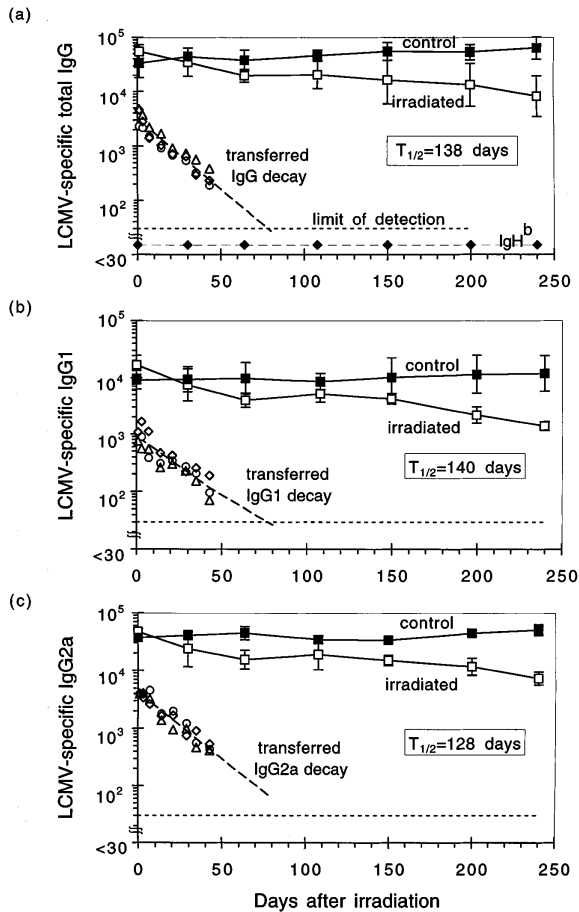
Figure 2. Virus-Specific Antibody Production after Memory B Cell Depletion

At 60 days postinfection, LCMV-immune BALB/c IgH^a mice were irradiated to deplete memory B cells and then reconstituted with naive BALB/c IgH^b B cells. The levels of LCMV-specific IgG (A), IgG1 (B), and IgG2a (C) in the serum of these mice were determined by ELISA (Ahmed et al., 1984). LCMV-specific antibody consisted entirely of the host IgH^a allotype; no donor IgH^b (IgG2a^b) antibody responses were detected. The half-life of plasma cells was obtained by fitting the data for antibody concentration A[t] in individual mice to a biexponential decay described by the function $A(t) = [A(0)/(a - p)][a(1 - f)e^{-pt} + (af - p)e^{-at}]$. The decay rate of free Ig was determined by passively transferring LCMV-immune serum into naive recipients and monitoring the decline in serum antibody levels by ELISA (open triangles, circles, and diamonds represent three individual recipient mice). The mean values ($\pm$ 1 SEM) for the half-lives of transferred Ig were $t_{1/2}$ (total IgG), 11.7 $\pm$ 0.5 days; $t_{1/2}$ (IgG1), 14.4 $\pm$ 0.4 days; and $t_{1/2}$ (IgG2a), 13 $\pm$ 1 days.

cells making LCMV-specific antibody of the IgG1 and IgG2a subclasses was also determined. Using the biexponential decay model, we obtained a mean $t_{1/2}$ of 140 days for IgG1-secreting plasma cells and a $t_{1/2}$ of 128 days for IgG2a-secreting plasma cells. IgG1 production usually indicates a helper T cell type 2 (Th2)–type cytokine response, whereas IgG2a production is indicative of a Th1-type response (Romagnani, 1997). These results therefore show that long-lived plasma cells are generated during both Th1 and Th2 responses and that these subclasses of ASC appear to have remarkably similar half-lives in vivo.

To determine the half-life of LCMV-specific plasma cells directly, we used the ELISPOT technique to quantitate individual IgG-producing virus-specific ASC in the bone marrow and spleens of irradiated/reconstituted mice. The results of this analysis are summarized in Figure 3A. Similar to the estimates obtained from serum antibody titers (Figure 2), direct quantitation of ASC numbers indicated that LCMV-specific plasma cells decay exponentially, with a mean $t_{1/2}$ of 94 days (range 85–105 days) in the bone marrow and 172 days (range 149–202 days) in the spleen. The actual data from the day 240 time point are shown in Figures 3B–3D. Figure 3B shows that LCMV-specific plasma cells in irradiated/reconstituted mice were entirely of the host (IgH^a) allotype even though all of the B cells in these mice were of donor origin (IgH^b). In other words, these mice were essentially (IgH^b) mice in terms of their B cells, but all of the LCMV-specific ASC were of IgH^a allotype (i.e., were present before irradiation and had survived >240 days after irradiation). Besides demonstrating that the long-lived ASC were of host origin, we also determined whether these cells maintained a plasma cell phenotype, as indicated by the presence of little to no surface-bound Ig or MHC class II molecules (Abney et al., 1978; Halper et al., 1978). As shown in Figure 3D, removal of MHC class II– or surface Ig–bearing cells (by adherence to antibody-coated plates) did not result in a reduction in the number of LCMV-specific ASC. Thus, these ASC had a surface phenotype (MHC class II$^{lo/-}$ Ig$^{lo/-}$) characteristic of plasma cells. Finally, no memory B cells (either IgH^a or IgH^b) were detected in these mice (Table 1). These results show that a substantial number of plasma cells can survive for longer than 8 months without replenishment by memory B cells. It is worth emphasizing that the half-life of plasma cells calculated by the ELISPOT assay was not substantially different from the half-life estimated from serum antibody titers. This suggests not only that plasma cells are long-lived, but also that the rate of antibody production per plasma cell does not appear to wane even after several months of continuous antibody production.

To extend our studies, we also looked at plasma cell longevity in another mouse strain, C57BL/6 Thy1^a IgH^a mice. Similar to the BALB/c strain, C57BL/6 Thy1^a IgH^a mice cleared acute LCMV Armstrong infection within 2 weeks (data not shown). At 80 days postinfection, these mice were depleted of memory B cells by administration of a high dose (800 rad) of γ-radiation. This treatment was followed by reconstitution with naive allotypic C57BL/6 (IgH^b) B cells, T cells, and bone marrow. Similar to the previous irradiation/reconstitution experiments, no donor (IgH^b) LCMV-specific antibody was detected, indicating again that there was no viral antigen remaining that was capable of eliciting a detectable antibody response by naive B cells (data not shown). In addition, host memory B cell frequencies dropped to below the level of detection (<1 memory B cell per 10^6 spleen cells) following irradiation. After depletion of the memory B cell pool, virus-specific serum antibody titers were maintained for more than 500 days, and although serum antibody titers declined over time ($t_{1/2}$ = 80 days, range = 70–93 days), an impressive level of virus-specific antibody was still observed nearly 1.5 years after irradiation
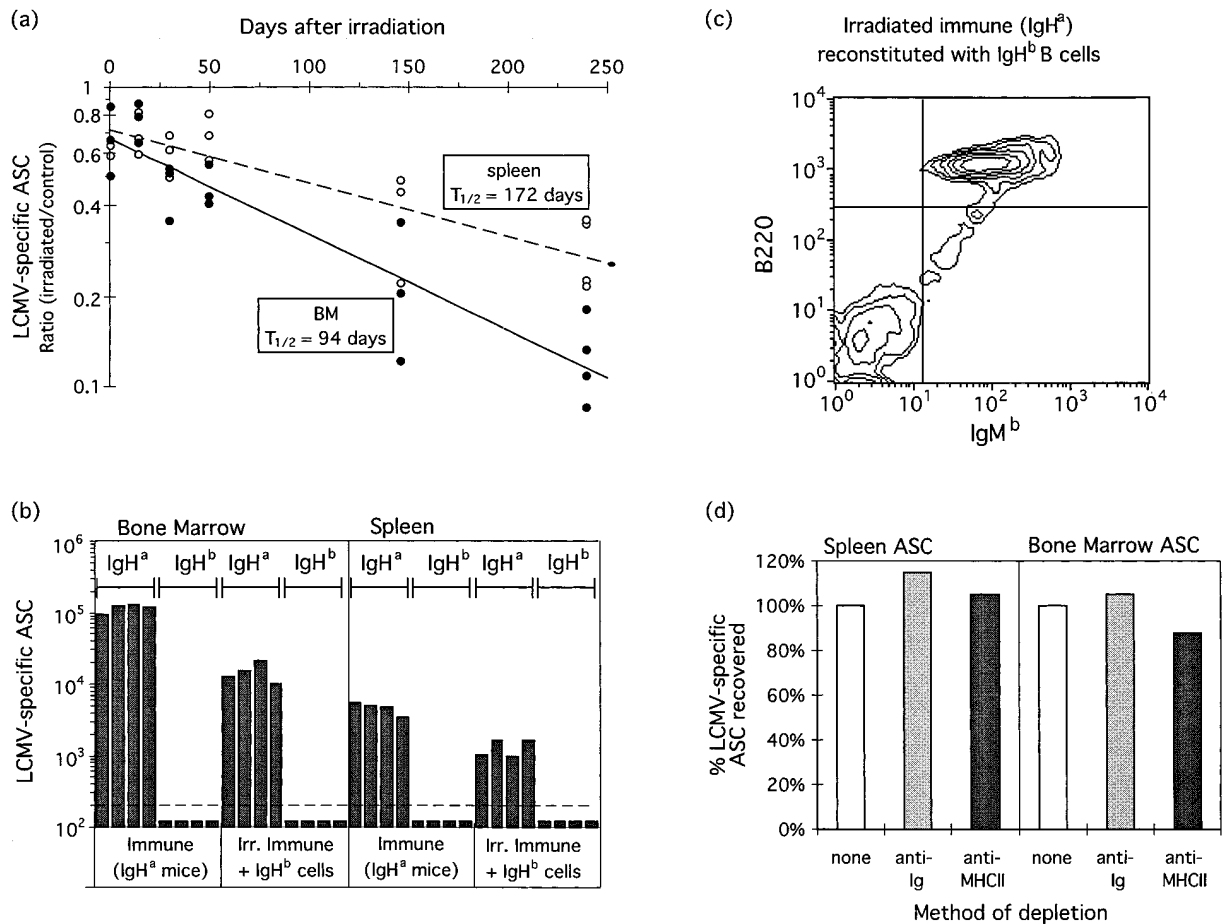
(a) Days after irradiation

(c) Irradiated immune (IgH^a)
reconstituted with IgH^b B cells

(b)

(d)

Figure 3. Plasma Cell Survival after Memory B Cell Depletion

(A) Plasma cells were quantitated in the spleen and bone marrow of irradiated/reconstituted LCMV-immune mice and compared to nonirradiated immune mice using an ELISPOT assay (Slifka et al., 1995). The data are presented as a ratio of the number of LCMV-specific ASC observed in irradiated mice divided by the average number of ASC observed in nonirradiated control mice (three or four control mice per time point). The ratio of virus-specific ASC in the spleen (open circles) and bone marrow (BM) (closed circles) of individual irradiated mice are shown at the indicated time points. The decay rate of plasma cell numbers was obtained from the slope of the log-linear plot shown and indicated a $t_{1/2}$ of 94 days for plasma cells in the bone marrow and 172 days for plasma cells in the spleen.

(B) The total number of virus-specific plasma cells in the spleen and bone marrow of irradiated/reconstituted LCMV-immune BALB/c mice were compared to nonirradiated controls at 240 days postirradiation. Each bar represents the number of virus-specific plasma cells in the spleen or bone marrow of individual mice. After reconstitution with naive IgH^b B cells, no LCMV-specific IgH^b-secreting plasma cells were detected by the ELISPOT assay.

(C) After irradiation, LCMV immune mice (IgH^a) were reconstituted with allotypic (IgH^b) spleen and bone marrow cells. At 240 days postirradiation/ reconstitution, flow cytometry showed that the host IgH^a-bearing B cells were completely replaced by naive allotypic IgH^b-bearing B cells. Each sample was double-stained for the B cell marker B220 (vertical axis) and the allotypic (donor) marker IgM^b (horizontal axis). The contour plot represents 30,000 gated events.

(D) Spleen and bone marrow cells from irradiated LCMV-immune mice (240 days postirradiation) were depleted of Ig$^+$ or MHC class II$^+$ cells by panning on antibody-coated plates. This procedure depleted more than 95% of mature B cells but did not decrease the number of ASC scored by the ELISPOT assay, indicating that these LCMV-specific ASC have the phenotype of plasma cells.

(Figure 4). These results show that a substantial number of plasma cells can survive for more than a year without replenishment by memory B cells and that at least a subpopulation of plasma cells can survive and continue to secrete antibody for the natural lifespan of the immune host.

Plasma Cell Survival Following Adoptive Transfer
Adoptive transfer of bone marrow containing LCMV-specific plasma cells served as an additional approach for determining plasma cell longevity. Virus-specific

plasma cells (1.5 × 10^4 per recipient) were adoptively transferred into naive nonirradiated mice, and the kinetics of the resulting serum antibody responses were monitored. There are few to no memory B cells in the bone marrow (Shepherd and Noelle, 1991; Paramithiotis and Cooper, 1997), but as a precaution, bone marrow cells were treated with an antiproliferative agent, mitomycin C, prior to transfer. Mitomycin C causes a permanent loss in proliferation by means of its covalent interactions with DNA (Tomasz et al., 1974) and has been shown to block LCMV-specific memory B cell function (Slifka and
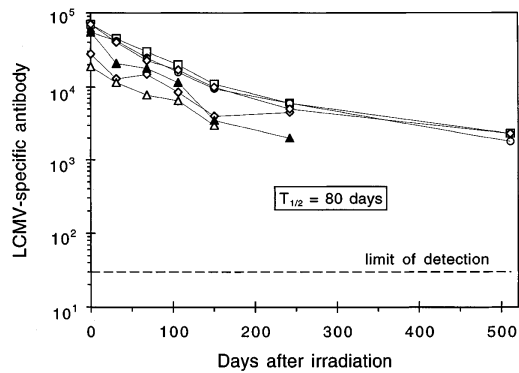
Figure 4. Persistence of LCMV-Specific Antibody Production by Long-Lived Plasma Cells

At 80 days postinfection, LCMV-immune C57BL/6 Thy1[a] IgH[a] mice were given 800 rad of total-body irradiation followed by an intravenous injection of naive C57BL/6 (IgH[b]) B cells, T cells, and bone marrow. After irradiation, LCMV-specific memory B cells were undetectable by limiting dilution analysis (<1 memory B cell per 10[6] spleen cells), and no LCMV-specific donor (IgH[b]) antibody was detected in the serum (data not shown). LCMV-specific serum IgG titers were monitored longitudinally in individual mice (each symbol denotes a mouse) by ELISA for more than 500 days postirradiation, and the survival of virus-specific plasma cells was determined by the mathematical model described in Figure 2.

Figure 5. Adoptive Transfer of LCMV-Specific Plasma Cells Results in Prolonged Antibody Production

Bone marrow from LCMV-immune mice (containing 1.5×10^4 LCMV-specific plasma cells) was adoptively transferred into naive BALB/c recipients, and virus-specific serum IgG levels were measured by ELISA. The bone marrow cells were treated with an antiproliferative agent, mitomycin C, prior to transfer. Plasma cell half-life estimates in individual mice were fit by the model $A(t) = [k/(a - p)][e^{-pt} - e^{-at}]$. The open circles show antibody levels in individual mice that received mitomycin C–treated cells; the filled circles show the lack of a detectable antibody response (<10 ELISA units) in mice that received sonicated bone marrow cells. This demonstrated that the antibody response observed after bone marrow transfer was due to the transfer of plasma cells and not due to passively transferred antibody or viral antigen.

Ahmed, 1996a). As shown in Figure 5, LCMV-specific IgG levels initially increased until reaching equilibrium about 15 days after transfer. Antiviral antibody titers then gradually declined over the following 4-month period of observation while remaining at readily detectable levels. The decay rate of plasma cells was estimated using a simple mathematical model describing the adoptive transfer system (see Experimental Procedures), and the results of this experiment indicated a mean plasma cell $t_{1/2}$ of 74 days.

Antibody production was not due to passively transferred antibody or transferred viral antigen, because if bone marrow cells were sonicated prior to transfer, no LCMV-specific antibody could be detected in the recipients (<10 ELISA units). In addition, if mice received mitomycin C–treated LCMV-immune bone marrow (or spleen cells) and were later challenged with LCMV, no memory B cell responses could be detected (data not shown). Collectively, these data indicate that in the absence of memory B cells, adoptively transferred plasma cells have an extended lifespan and sustain prolonged antibody production in otherwise naive recipients. These results also suggest that plasma cells continue to secrete antibody for extended periods of time in the absence of detectable antigen.

Discussion

This quantitative study of plasma cell longevity demonstrates that plasma cells can survive for periods longer than 1 year. In addition, this study also relates the lifespan of antigen-specific plasma cells located in different anatomical sites to the level of specific antibody observed in the serum. Two experimental approaches were used in these studies; irradiation/reconstitution of immune mice and adoptive transfer of virus-specific plasma
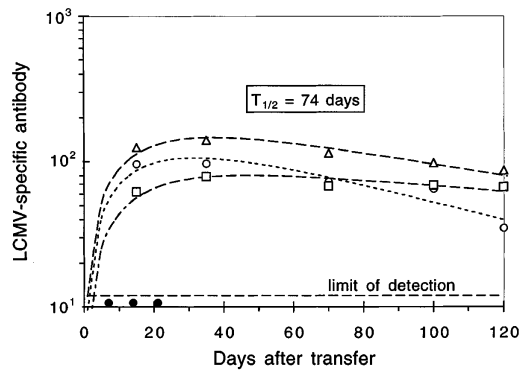
cells into naive mice. These results show that plasma cells survive for much longer than previously believed and are an important constituent of long-term humoral immunity. Although some studies have proposed that plasma cells may survive longer than a few days or weeks (Miller, 1964; Okudaira and Ishizaka, 1981; Ho et al., 1986; Manz et al., 1997), the current consensus has remained that plasma cells have a rapid turnover rate (Szakal et al., 1989; Tew et al., 1990; MacLennan et al., 1992; Stites et al., 1994; Gray et al., 1996; Zinkernagel et al., 1996).

Why do our conclusions differ from previously published observations (Cooper, 1961; Schooley, 1961; Makela and Nossal, 1962; Nossal and Makela, 1962), which show that plasma cells are short-lived? The previously established paradigm is based on studies that analyzed the lifespan of plasma cells during the acute phase of the humoral response (the first 2 weeks after vaccination), a time when B cells are rapidly expanding, differentiating into ASC, and being depleted during clonal selection and affinity maturation (Kelsoe, 1996). During this period there also appears to be migration of plasma cells from the spleen and lymph nodes to the bone marrow compartment. If we restrict our analysis to this early period (days 8–30 postinfection), when the number of LCMV-specific plasma cells are rapidly declining in the spleen (Figure 1), then we also observe an apparently short half-life for plasma cells ($t_{1/2}$ = 5.7 days). This is in contrast to the estimated plasma cell half-life in the spleen from days 60–300 postinfection ($t_{1/2}$ = 172 days), as shown in Figure 3A. Thus, the previous studies were correct in determining the lifespan of plasma cells at early time points after antigenic stimulation, but they did not accurately reflect the lifespan of plasma cells found during

the later stages of a humoral immune response, once homeostasis is achieved. It makes intuitive sense that plasma cells generated during the early stages of the immune response are short-lived since it would not be advantageous to have long-lived antibody responses of poor specificity and/or low affinity. On the other hand, it would be more advantageous for ASC that survive the clonal selection and affinity maturation process to be long-lived, since these cells would provide a powerful first line of defense against microbial pathogens.

It is well documented that microbial infections often induce prolonged serum antibody responses (Plotkin and Mortimer, 1988; Ahmed and Gray, 1996; Slifka and Ahmed, 1996b). The sustained antibody production against microbes has commonly been attributed to repeated exposure to the pathogen or to low-grade persistence of the microbe (Slifka and Ahmed, 1996b). In the case of nonreplicating antigens, such as diphtheria or tetanus toxoid, long-lasting antibody responses are also induced. For example, people vaccinated against either diptheria or tetanus can maintain circulating antibody levels for more than 25 years (Gottlieb et al., 1964; Kjeldsen et al., 1985). Although antibody titers decline over time in these individuals, it is extraordinary that circulating serum antibody can still be detected for decades after immunization with a noninfectious antigen. It has been proposed that long-term antibody responses are due to continuous stimulation of memory B cells by antigen–antibody complexes that persist on follicular dendritic cells (Szakal et al., 1989; MacLennan et al., 1992; Gray, 1993). Though plausible, this theory would require an extremely high rate of memory B cell proliferation and differentiation into plasma cells (see below). It is also difficult to understand how antigen trapped on follicular dendritic cells would persist in an immunogenic form for such long periods of time and why it would not be consumed by the continuous stimulation of memory B and T cells. Thus the existing models, all of which assume that plasma cells are short-lived, do not offer a satisfactory explanation of how long-term antibody responses are maintained.

In agreement with our data describing long-lived plasma cells, a recent study (Manz et al., 1997) has used bromodeoxyuridine (BrdU) incorporation during booster vaccination to monitor plasma cell survival in the bone marrow. Manz et al. provide convincing evidence for long-lived plasma cells and show that more than 60% of BrdU^{+} plasma cells in the bone marrow survive at least 90 days without turnover. Although a specific half-life was not calculated, the existence of plasma cells that survive for longer than 3 months was clearly documented. In our study, we examined plasma cell longevity for the life of the mouse ($\sim$2 years), and our experimental strategy, in conjunction with mathematical modeling, allowed us to obtain a precise estimate of the half-life of antigen-specific plasma cells. In addition, we examined plasma cell lifespan in both spleen and bone marrow compartments. This is an important point, because an earlier study suggested that bone marrow plasma cells may have a lifespan of 3–4 weeks whereas splenic plasma cells appeared to have a half-life of only a few days (Ho et al., 1986). In contrast, our studies show unequivocally that plasma cells in both spleen and bone marrow are long-lived (Figure 3). Since the lifespan of mice is only 2–3 years, antibody production for 1–1.5 years by long-lived plasma cells constitutes a significant portion of the animal's life (Figure 4). It remains to be seen how these numbers can be extrapolated to plasma cell longevity in humans, but it is tempting to speculate that the prolonged antibody responses seen after human vaccination with inert antigens are due to long-lived plasma cells.

Our findings on plasma cell longevity address some interesting issues of aging. In our experiments monitoring plasma cell survival, we found that the rate of plasma cell decline was similar irrespective of age and that the half-life of plasma cells in young mice, at 2–4 months of age, was essentially the same as that in old mice, at 12–26 months of age (Figure 4). This new finding strongly suggests that plasma cell longevity is not affected by aging. Since it is well established that the ability to generate new immune responses is greatly impaired in aged individuals, our results highlight a dichotomy between the maintenance of preexisting plasma cells and the ability to generate new ones as a function of age. What this means is that if an individual is well vaccinated at an early age and generates a large number of long-lived plasma cells, then this mechanism may be sufficient to maintain long-term humoral immunity later in life, when the ability to mount new immune responses might be impaired.

In addition to documenting the existence of long-lived plasma cells, our study also defines the quantitative relationship between antigen-specific memory B cells and plasma cells. For instance, we can estimate the rate at which memory B cells must proliferate in order to maintain the stable numbers of LCMV-specific memory B cells and plasma cells observed in mice following immunization (Figure 1). To maintain a stable population of memory B cells (M), the rate at which new memory B cells are generated by proliferation (r M) must equal the rate at which they are lost due to their differentiation into plasma cells and the natural death rate of memory B cells. To maintain a stable population of plasma cells, the rate of generation of plasma cells by differentiation of memory B cells must, in turn, equal the rate at which they die (p P). Assuming that the natural death rate of memory B cells is small, we estimate the amount of memory B cell proliferation required to maintain the plasma cell population from the equation r M = d P (this is a lower bound to the rate of memory B cell proliferation because we have neglected the natural death rate of this population). Converting to half-lives for the turnover rates of memory B cells (Tm) and plasma cells (Tp), we obtain Tm = Tp M/P. From Figure 1, we obtain an estimate of the total number of plasma cells (P = 4 × 10^4) and memory B cells (M = 1.5 × 10^4), assuming that half the memory B cells are in the spleen and half are in the lymph nodes. Inserting these values into the equation above, we find that Tm = (0.37)Tp. If plasma cells were short-lived (t$_{1/2}$ = 3–5 days), then each LCMV-specific memory B cell must divide approximately every 1.5 days in order to sustain the observed plasma cell populations. However, if plasma cells are long-lived (t$_{1/2}$ = 100 days), as we have shown in this study, then memory B cells would need to divide only once every

35 days in order to maintain the observed plasma cell numbers. This latter result is more consistent with published observations of memory B cell turnover (Schittek and Rajewsky, 1990; Vieira and Rajewsky, 1990) and demonstrates that plasma cell longevity is critical in maintaining immunological homeostasis as well as persistent antibody production.

It will be interesting to determine what conditions influence the longevity of antigen-specific plasma cells. It is possible that the anatomical microenvironment plays a role in determining the lifespan of plasma cells. For example, serum antibody responses are generally maintained for a much greater period of time than mucosal antibody responses (Slifka and Ahmed, 1996b). This may be due to the support of serum antibody production primarily by plasma cells in the bone marrow, whereas mucosal antibody levels mostly reflect the number of local plasma cells found in mucosal tissues. Short-lived mucosal antibody responses may be due to the migration of plasma cells to other anatomical sites (such as the bone marrow), or it is possible that plasma cells at mucosal sites are short-lived. By identifying the factors that prolong plasma cell survival, we may be able to increase the potency of suboptimal vaccines and thereby decrease the number of booster vaccinations required to sustain protective immunity. Understanding the mechanisms of plasma cell survival will not only provide fundamental information about these critical cells of the immune system, but will also have practical implications for vaccine development.

Experimental Procedures

Mice
BALB/c Byj IgH[a], BALB/c IgH[b] SMN (formerly known as CB.17 mice), C57BL/6 Thy1[a] IgH[a], and C57BL/6 Thy1[a] (IgH[b]) mice were purchased from the Jackson Laboratory (Bar Harbor, ME). The congenital BALB/c and C57BL/6 carrier colonies were derived from neonatally infected mice and bred at Emory University.

Virus
The Armstrong CA 1371 strain of LCMV was used in this study (Ahmed et al., 1984). LCMV-immune mice were obtained by injecting 5- to 8-week-old BALB/c mice intraperitoneally with 2×10^5 plaque-forming units (pfu) of LCMV Armstrong. C57BL/6 Thy1[a] IgH[a] mice were infected with the same dose of LCMV at 20 weeks of age.

Irradiation and Reconstitution
At 60 days postinfection, LCMV-immune mice received total-body irradiation (96 rad/min from a Gammacell-40 irradiator) [Nordion International] for a total dose of 600 rad) to deplete LCMV-specific memory B cells. BALB/c mice were reconstituted with 3×10^7 allotypic BALB/c IgH[b] spleen cells and 2×10^7 bone marrow cells within 4 hr after irradiation by intravenous injection. C57BL/6 Thy1[a] IgH[a] mice received 800 rad total-body irradiation followed by reconstitution with 3×10^7 spleen cells and 1×10^7 bone marrow cells from naive C57BL/6 mice. At 3 months after irradiation/transfer, LCMV-immune mice were fully reconstituted with mature B cells and T cells, but if rechallenged with the same strain of LCMV (10^6 pfu of virus injected intraperitoneally), no anamnestic antibody responses were detected.

ELISA
At the indicated time points, serum was collected from individual mice by retroorbital bleeding, and LCMV-specific serum antibody titers were determined by a solid-phase ELISA as described previously (Ahmed et al., 1984). Allotype-specific responses were analyzed using IgG2a[a]- and IgG2a[b]-specific antibodies (PharMingen,

San Diego, CA). The half-life of IgG molecules was determined by transferring 0.5 ml of pooled LCMV-immune serum (after clarification by centrifugation at 14,000 rpm, for 5 min) intraperitoneally into naive BALB/c recipients.

Plasma Cell Lifespan Estimation from the Decay of Serum Antibody Levels
Irradiation results in the elimination of memory B cells and the loss of a small fraction, f, of plasma cells. Using a simple mathematical model that incorporates these processes (provided that the rate of secretion of antibody per plasma cell remains constant and that the population of plasma cells decays exponentially with rate p), the decay in antibody will follow a biexponential decay described by the function $A(t) = [A(0)/(a - p)][a(1 - f)e^{-pt} + (af - p)e^{-at}]$. A(0) and a were set to the preirradiation antibody level and the rate constant for the decay of transferred antibody, respectively. The biexponential decay has an initially rapid decay rate due to the loss of a fraction, f, of the plasma cells upon irradiation, followed by a slower decay rate due to the loss of plasma cells at rate p. The rate of antibody decay, a, was determined in an independent experiment (Figure 3), allowing estimates for the p and f to be obtained for each individual mouse by fitting their serum antibody concentration to the above function. The rates of decay obtained from fitting the data for total IgG from individual BALB/c mice to the biexponential decay model were p = 0.0011, 0.0046, 0.0028, and 0.011 day^{-1}, which resulted in a mean p of 0.005 day^{-1} (SEM = 0.0023). The average rate of plasma cell decline and SEM were then transformed to half-lives ($t_{1/2} = \ln(0.5)/p$), which are reported in Results. Similar results were obtained by ignoring the initial decline in antibody titers during the first 30 days after irradiation and fitting the later time points to a simple exponential decay with rate equal to that for the loss of plasma cells.

ELISPOT
LCMV-specific ASC were quantitated by the ELISPOT technique using nitrocellulose-bottomed 96-well plates (Millipore, San Francisco, CA) coated with virus (Slifka et al., 1995).

Quantitation of Memory B Cells
Virus-specific memory B cells were restimulated with LCMV in vitro and quantitated by limiting dilution analysis as previously described in detail (Slifka and Ahmed, 1996a). In addition to limiting dilution analysis, adoptive transfer and challenge studies were performed. Spleen cells were tested for memory B cell activity by transferring 2×10^7 total spleen cells intravenously into irradiated (600 rad) naive recipients followed by challenge with 2×10^5 pfu of LCMV on the day of transfer. Serum samples were collected by retroorbital bleeding and tested for virus-specific serum antibody by ELISA. Using this assay, spleen cells from irradiated/reconstituted LCMV-immune mice did not confer an anamnestic antibody response upon challenge. Instead, transfer of spleen cells from irradiated/reconstituted immune mice resulted in the kinetics of a primary antibody response that was indiscernible from that after transfer of an equal number of naive spleen cells.

Evaluation of B Cell Reconstitution by Flow Cytometry
Flow cytometry was performed as previously described (Ahmed et al., 1988) using anti-B220-cychrome, anti-IgM[a]-phycoerythrin (data not shown), and anti-IgM[b]-fluorescein isothiocyanate antibodies (PharMingen) and gating on live cells. Each flow cytometry profile shows 30,000 gated events and is representative of three individual mice assayed separately.

In Vitro B Lymphocyte Depletion
Plates used for B cell depletion were prepared by coating polystyrene Petri dishes (Falcon) with either anti-IgG, anti-IgM, anti-IgA (5 μg/ml, Cappell) or anti–MHC class II (PharMingen, 8 μg/ml) in 0.05 M Tris-HCl, 0.15 M NaCl (pH = 9.5) overnight at 4°C. Plates were washed to remove unbound antibody and blocked 5–10 min with phosphate-buffered saline (PBS)–5% fetal calf serum (FCS) prior to addition of 3 ml of cells suspended in PBS–5% FCS (10–25 $\times$ 10^6 cells/ml). Cells were allowed to adhere for 90 min at 4°C. Nonadherant cells were removed from the plates by gentle pipetting and used

in ELISPOT assays for quantitating LCMV-specific ASC. More than 95% of mature B cells were removed by this procedure. Depletion was determined by staining for $B220^+Ig^+$ cells and quantitating by flow cytometry.

Adoptive Transfer of LCMV-Specific Plasma Cells

Bone marrow from LCMV-immune mice was used for the adoptive transfer studies because bone marrow contains a large number of antiviral plasma cells (Slifka et al., 1995) (Figure 1A) and few mature B cells. Femoral bone marrow (4×10^7 cells) from LCMV-immune BALB/c mice (4 months after infection) containing 1.5×10^4 antiviral plasma cells were adoptively transferred into naive nonirradiated BALB/c recipients. The number of ASC transferred was determined by ELISPOT analysis at the time of injection. Bone marrow cells and spleen cells were treated with mitomycin C (Sigma, St. Louis, MO) at a concentration of 50 μg/ml in PBS with a final cell concentration of 2.5×10^7 cells/ml at 37°C for 20 min. Cells were washed three times, counted, and injected intravenously into the recipient mice. Small serum samples were taken at the indicated time points and tested for LCMV-specific IgG by ELISA. To determine whether immunogenic viral antigen was transferred with the bone marrow cells, two naive nonirradiated BALB/c mice received 5×10^7 sonicated LCMV-immune bone marrow cells. LCMV-specific serum antibody remained below detection in these mice.

Estimation of Plasma Cell Longevity Following Adoptive Transfer

Assuming that a fixed number of plasma cells are transferred and that their number decays thereafter, the initial increase in antibody titers would occur as a result of the antibody secretion by the transferred plasma cells. The later decay in antibody levels would occur as a result of the decrease in plasma cell numbers over time. Using a simple mathematical model that incorporates these processes, the change in antibody concentration A(t) following transfer can then be described by the function $A(t) = [k/(a - p)][e^{-pt} - e^{-at}]$, where k equals the product of the number of transferred plasma cells and the rate at which they secrete antibody, and a and p are the rate constants for decay of antibody and plasma cells, respectively. The rate of antibody decay, a, was determined in an independent experiment (Figure 3), allowing estimates for the p and k for each individual recipient mouse by fitting the observed serum antibody titers to the function above. The rate constants for the loss of plasma cells obtained from individual mice were p = 0.014, 0.0046, and 0.0094 day^{-1}, giving a mean p of 0.0094 day^{-1} (SEM = 0.0028), and indicating a mean plasma cell $t_{1/2}$ of 74 days.

Acknowledgments

References

Abney, E.R., Cooper, M.D., Kearney, J.F., Lawton, A.L., and Parkhouse, R.M.E. (1978). Sequential expression of immunoglobuin on developing mouse B lymphocytes: a systematic survey that suggests a model for the generation of immunoglobulin isotype diversity. J. Immunol. 120, 2041–2049.

Ahmed, R., and Gray, D. (1996). Immunological memory and protective immunity: understanding their relation. Science 272, 54–60.

Ahmed, R., Salmi, A., Butler, L.D., Chiller, J.M., and Oldstone, M.B. A. (1984). Selection of genetic variants of lymphocytic choriomeningitis virus in spleens of persistently infected mice: role in suppression of cytotoxic T lymphocyte response and viral persistence. J. Exp. Med. 160, 521–540.

Ahmed, R., Butler, L.D., and Bhatti, L. (1988). T4$^+$ T helper cell function in vivo: differential requirement for induction of antiviral cytotoxic T-cell and antibody responses. J. Virol. 62, 2102–2106.

Anderson, R.E., and Lefkovits, I. (1980). Effects of irradiation on the in vitro immune response. Exp. Cell. Biol. 48, 255–278.

Bachmann, M.F., Kundig, T.M., Kalberer, C.P., Hengartner, H., and Zinkernagel, R.M. (1994). How many specific B cells are needed to protect against a virus? J. Immunol. 152, 4235–4241.

Benner, R., Hijmans, W., and Haaijman, J.J. (1981). The bone marrow: the major source of serum immunoglobulins, but still a neglected site of antibody formation. Clin. Exp. Immunol. 46, 1–8.

Cooper, E.H. (1961). Production of lymphocytes and plasma cells in the rat following immunization with human serum albumin. Immunology 4, 219–231.

Dilosa, R.M., Maeda, K., Masuda, A., Szakal, A.K., and Tew, J.G. (1991). Germinal center B cells and antibody production in the bone marrow. J. Immunol. 146, 4071–4077.

Fahey, J.L., and Sell, S. (1965). The immunoglobulins of mice: the metabolic (catabolic) properties of five immunoglobulin classes. J. Exp. Med. 122, 41–58.

Gottlieb, S., McLaughlin, F.X., Levine, L., Latham, W.C., and Edsall, G. (1964). Long-term immunity to tetanus: a statistical evaluation and its clinical implications. Am. J. Pub. Health 54, 961–971.

Gray, D. (1993). Immunological memory: a function of antigen persistence. Trends Microbiol. 1, 39–41.

Gray, D., Siepmann, K., van Essen, D., Poudrier, J., Wykes, M., Jainandunsing, S., Bergthorsdottir, S., and Dullforce, P. (1996). B-T lymphocyte interactions in the generation and survival of memory cells. Immunol. Rev. 150, 45–61.

Halper, J., Fu, S.M., Wang, C.Y., Winchester, R., and Kunkel, H.G. (1978). Patterns of expression of human Ia-like antigens during terminal stages of a B cell development. J. Immunol. 120, 1480–1484.

Helmreich, E., Kern, M., and Eisen, H.N. (1961). The secretion of antibody by isolated lymph node cells. J. Biol. Chem. 236, 464–473.

Hibi, T., and Dosch, H.M. (1986). Limiting dilution analysis of the B cell compartment in human bone marrow. Eur. J. Immunol. 16, 139–145.

Ho, F., Lortan, J.E., MacLennan, I., and Khan, M. (1986). Distinct short-lived and long-lived antibody-producing cell populations. Eur. J. Immunol. 16, 1297–1301.

Hyland, L., Sangster, M., Sealy, R., and Coleclough, C. (1994). Respiratory virus infection of mice provokes a permanent humoral immune response. J. Virol. 68, 6083–6086.

Kelsoe, G. (1996). The germinal center: a crucible for lymphocyte selection. Semin. Immunol. 8, 179–184.

Kjeldsen, K., Heron, I., and Simonsen, O. (1985). Immunity against diphtheria 25–30 years after primary vaccination in childhood. Lancet 1, 900–902.

Lau, L.L., Jamieson, B.D., Somasundaram, T., and Ahmed, R. (1994). Cytotoxic T-cell memory without antigen. Nature 369, 648–652.

Levy, M., Vieira, P., Coutinho, A., and Freitas, A. (1987). The majority of "natural" immunoglobulin-secreting cells are short-lived and the progeny of cycling lymphocytes. Eur. J. Immunol. 17, 849–854.

MacLennan, I.C., Liu, Y.J., and Johnson, G.D. (1992). Maturation and dispersal of B-cell clones during T cell-dependent antibody responses. Immunol. Rev. 126, 143–161.

Makela, O., and Nossal, G.J.V. (1962). Autoradiographic studies on the immune response. J. Exp. Med. 115, 231–245.

Makinodan, T., Kastenbaum, M.A., and Peterson, W.J. (1962). Radiosensitivity of spleen cells from normal and pre-immunized mice and its significance to intact animals. J. Immunol. 88, 31–37.

Makinodan, T., Nettesheim, P., Morita, T., and Chadwick, C.J. (1967). Synthesis of antibody by spleen cells after exposure to kiloroentgen doses of ionizing radiation. J. Cell. Physiol. 69, 355–366.

Manz, R.A., Thiel, A., and Radbruch, A. (1997). Lifetime of plasma cells in the bone marrow. Nature 388, 133–134.

Miller, J.J. (1964). An autoradiographic study of plasma cell and lymphocyte survival in rat popliteal lymph nodes. J. Immunol. 92, 673–681.

Moskophidis, D., and Lehmann-Grube, F. (1984). The immune response of the mouse to lymphocytic choriomeningitis virus: IV. enumeration of antibody-producing cells in spleens during acute and persistent infection. J. Immunol. 133, 3366–3370.

Nossal, G.J.V., and Makela, O. (1962). Autoradiographic studies on the immune response: I. the kinetics of plasma cell proliferation. J. Exp. Med. *115*, 209–230.

Okudaira, H., and Ishizaka, K. (1981). Reaginic antibody formation in the mouse: XI. participation of long-lived antibody-forming cells in persistent antibody formation. Cell. Immunol. *58*, 188–201.

Oldstone, M.B.A., and Dixon, F.J. (1967). Lymphocytic choriomeningitis: production of antibody by "tolerant" infected mice. Science *158*, 1193–1195.

Oldstone, M.B.A., Ahmed, R., Byrne, J., Buchmeier, M.J., Riviere, Y., and Southern, P. (1985). Virus and immune responses: lymphocytic choriomeningitis virus as a prototype model of viral pathogenesis. Br. Med. Bull. *41*, 70–74.

Paramithiotis, E., and Cooper, M.D. (1997). Memory B lymphocytes migrate to the bone marrow in humans. Proc. Natl. Acad. Sci. USA *94*, 208–212.

Plotkin, S.A., and Mortimer, E.A. (1988). Vaccines (Philidelphia: W.B. Saunders).

Romagnani, S. (1997). The Th1/Th2 paradigm. Immunol. Today *18*, 263–266.

Schittek, B., and Rajewsky, K. (1990). Maintenance of B-cell memory by long-lived cells generated from proliferating precursors. Nature *346*, 749–751.

Schooley, J.C. (1961). Autoradiographic observations of plasma cell formation. J. Immunol. *86*, 331–337.

Shepherd, D.M., and Noelle, R.J. (1991). The lack of memory B cells in immune bone marrow. Transplantation *52*, 97–100.

Slifka, M.K., and Ahmed, R. (1996a). Limiting dilution analysis of virus-specific memory B cells by an ELISPOT assay. J. Immunol. Meth. *199*, 37–46.

Slifka, M.K., and Ahmed, R. (1996b). Long-term humoral immunity against viruses: revisiting the issue of plasma cell longevity. Trends Microbiol. *4*, 394–400.

Slifka, M.K., Matloubian, M., and Ahmed, R. (1995). Bone marrow is a major site of long-term antibody production after acute viral infection. J. Virol. *69*, 1895–1902.

Smith, K.G.C., Light, A., Nossal, G.J.V., and Tarlinton, D.M. (1997). The extent of affinity maturation differs between the memory and antibody-forming cell compartments in the primary immune response. EMBO J. *16*, 2996–3006.

Stites, D.P., Terr, A.I., and Parslow, T.G. (1994). Basic and Clinical Immunology (Norwalk, CT: Appleton and Lange).

Szakal, A.K., Kosco, M.H., and Tew, J.G. (1989). Microanatomy of lymphoid tissue during the induction and maintenance of humoral immune responses: structure function relationships. Annu. Rev. Immunol. *7*, 91–111.

Talbot, P.J., and Buchmeier, M.J. (1987). Catabolism of homologous murine monoclonal hybridoma IgG antibodies in mice. Immunology *60*, 485–489.

Tew, J.G., Kosco, M.H., Burton, G.F., and Szakal, A.K. (1990). Follicular dendritic cells as accessory cells. Immunol. Rev. *117*, 185–211.

Tomasz, M., Mercado, C.M., Olson, J., and Chatterjie, O.N. (1974). The mode of interaction of mitomycin C with deoxyribonucleic acid and other polynucleotides in vitro. Biochemistry *13*, 4878–4887.

Vieira, P., and Rajewsky, K. (1988). The half-lives of serum immunoglobulins in adult mice. Eur. J. Immunol. *18*, 313–316.

Vieira, P., and Rajewsky, K. (1990). Persistence of memory B cells in mice deprived of T cell help. Int. Immunol. *2*, 487–494.

Zinkernagel, R.M., Bachmann, M.F., Kundig, T.E., Oehen, S., Pirchat, H., and Hengartner, H. (1996). On immunological memory. Annu. Rev. Immunol. *14*, 333–367.