Long-Lived Bone Marrow Plasma Cells Are Induced Early in Response to T Cell-Independent or T Cell-Dependent Antigens

Alexandra Bortnick, Irene Chernova, William J. Quinn III, Monica Mugnier, Michael P. Cancro and David Allman

*J Immunol* published online 23 April 2012
http://www.jimmunol.org/content/early/2012/04/23/jimmunol.1102808
Long-Lived Bone Marrow Plasma Cells Are Induced Early in Response to T Cell-Independent or T Cell-Dependent Antigens

Alexandra Bortnick,* Irene Chernova,* William J. Quinn, III,* Monica Mugnier,† Michael P. Cancro,* and David Allman*

The signals required to generate long-lived plasma cells remain unresolved. One widely cited model posits that long-lived plasma cells derive from germinal centers (GCs) in response to T cell-dependent (TD) Ags. Thus, T cell-independent (TI) Ags, which fail to sustain GCs, are considered ineffective at generating long-lived plasma cells. However, we show that long-lived hapten-specific plasma cells are readily induced without formation of GCs. Long-lived plasma cells developed in T cell-deficient mice after a single immunization with haptenated LPS, a widely used TI Ag. Long-lived plasma cells also formed in response to TD Ag when the GC response was experimentally prevented. These observations establish that long-lived plasma cells are induced in both TI and TD responses, and can arise independently of B cell maturation in GCs. The Journal of Immunology, 2012, 188: 000–000.

Antibodies play pivotal roles in host defense and can cause disease when specific for self-antigens. Plasma cells, the main source of Abs, are thought to consist of two pools: short-lived cells that secrete low-affinity IgM Abs and form extrafollicular foci in the spleen or lymph node; and long-lived cells that secrete high-affinity, isotype-switched Abs found mainly in the bone marrow (BM) (1). Whereas short-lived plasma cells die within 3–5 d (2), long-lived plasma cells are thought to persist for months or years in mice and perhaps decades in people (3–6). Sustained serum Ab titers due to long-lived plasma cells are essential for protective immunity against many pathogens (3, 7–9).

Therefore, defining the events underlying the generation of long-lived plasma cells is essential for understanding how Ab-mediated immunity is established and maintained.

Why some plasma cells achieve longevity whereas others are short-lived is not known. One current and widely cited model posits that long-lived plasma cells emanate chiefly from germinal centers (GCs) in response to T cell-dependent (TD) Ags (4, 10). GCs are microanatomical structures enriched with Ag-stimulated B cells (GCs) in response to T cell-dependent (TD) Ags (4, 10). GCs are microanatomical structures enriched with Ag-stimulated B cells undergoing class switch recombination, somatic hypermutation (SHM), and affinity-based selection (11). Indeed, it was proposed recently that long-lived plasma cells are generated preferentially within the GC to favor the continuous secretion of high-affinity, and therefore more protective, Abs (12). This viewpoint is supported by data showing that the bulk of BM plasma cells induced by TD Ag secrete high-affinity class-switched Abs (13). However, the notion that long-lived plasma cells must derive from GCs appears incompatible with recent data showing that low-affinity IgM Abs can mediate long-term protection against certain microbial pathogens (14).

Classically, Ags that do not engage Th cells are thought to generate transient IgM responses that reflect the activity of short-lived plasma cells (2, 15–17). These TI cell-independent (TI) Ags may also generate short-lived GCs that fail to engender SHM and affinity maturation (18, 19). However, several recent studies challenge the long-standing paradigm that TI Ab responses are always short-lived. For instance, immunization of T cell-deficient mice with the spirochete Borrelia hermsii induces long-term IgM-dependent protection (20, 21). IgM Abs induced by the intracellular bacteria Francisella tularensis and Ehrlichia muris also mediate long-term protection; however, these responses may reflect the continuous generation of short-lived Ab-secreting cells (22, 23). More recently, E. muris and the encapsulated bacterium Streptococcus pneumoniae were shown to elicit a long-standing pool of Ag-specific BM plasma cells in T cell-sufficient mice (24–26). Although the latter work provides evidence that typical TD Ags are not unique in their ability to induce long-term Ab responses, these studies did not address whether long-lived plasma cells can be generated in the absence of T cells. Therefore, whether T cell-derived signals are strictly required to generate long-lived plasma cells remains unclear.

Our work addresses the capacity of TI and TD Ags to induce long-lived plasma cells during the earliest phases of plasma cell differentiation. We show that haptenated LPS, a classic type 1 TI Ag, readily induces a long-standing pool of BM plasma cells in mice that lack T cells. These Ab-secreting cells are detected for >100 d after a single immunization, exhibit a t1/2 of 45–55 d, and arise despite an inability to detect Ag-induced GC B cells. These data challenge the long-standing notion that type 1 TI Ags fail to induce the formation of long-lived plasma cells, and suggest that long-lived plasma cells need not arise from GCs. Similarly, we show that long-lived plasma cells also form in response to a standard TD Ag without undergoing maturation and selection in
GCs, as plasma cells secreting low-affinity IgM Abs persisted for $\geq 100$ d in mice in which we prevented GC formation early in these responses. These findings indicate that maturation in GCs is not requisite for achieving longevity in the plasma cell lineage and also suggest that competence to enter long-lived plasma cell pools is achieved early in TI and TD Ab responses.

Materials and Methods

**Mice**

C57BL/6 (B6), B6.TcR$\alpha^{-/-}\beta^{-/-}$, and MD4 Ig transgenic (anti-HEL) females (age 8–10 wk) were obtained from Jackson Laboratories. AID$^{-/-}$ mice were provided by Dr. Nina Papavasiliou (The Rockefeller University). All animal procedures were approved by the University of Pennsylvania Office of Regulatory Affairs.

**Chimeras**

Hosts were exposed to whole-body radiation (900 R) 5 d post immunization. Hosts were exposed to whole-body radiation (900 R) 5 d post immunization. Depletion of CD3$\text{+}$ cells using anti-CD3 mAb coated on MACS DCs (Miltenyi) prior to injection was used to deplete CD3$\text{+}$ cells from the chimera recipients. All data from chimera recipients were compared against those of B6 controls, which were composed of hematopoietically reconstituted B6 BM cells that were not depleted of CD3$\text{+}$ cells.

**Immunizations**

Mice 8–10 wk old were immunized i.p. with 50 $\mu$g NP$_{33}$-CGG in alum or 50 $\mu$g NP$_{33}$-CGG in PBS.

**ELISPOT**

MultiScreenHTS plates (Millipore) were coated with 10 $\mu$g per well of either goat anti-mouse Ig (H+L) (Southern Biotech), or NP$_{33}$-BSA or NP$_{33}$-BSA (BioSearch), in sodium bicarbonate buffer, and then blocked with 2% BSA/PBS. Cells were serially diluted across the plate and then incubated for 4–6 h at 37°C. Biotin–anti-IgG, goat–anti-IgM, or goat–anti-IgG1 (Southern Biotech) diluted in block buffer was added, followed by three washes with 0.1% Tween 20 detergent, and a secondary incubation with ExtrAvidin-alkaline phosphatase (Sigma-Aldrich). Spots were detected using BCIP/NBT (Sigma-Aldrich) and scanned and counted with an ImmunoSpot Analyzer (Cellular Technology).

Flow cytometry and cell sorting

Spleen and BM cells were harvested and stained with optimal dilutions of the indicated Abs, as described (28). NFAT reagents were purchased from eBioscience: FITC–anti-IgA, 1–3 (R26-46; Pharmingen) and peanut lectin (Sigma-Aldrich); PE–anti-CD138 (281-2; Pharmingen); PE–Texas Red–anti-B220 (RA3-6B2); PE–Cy7–anti-CD4 (RM4-5), anti-CD8$\text{a}$ (53-6.7), anti–Gr-1 (RB6-8C5), anti-F4/80 (BM8), and MD4 Ig transgenic (anti-HEL) (Southern Biotech) diluted in block buffer was added, followed by three washes with 0.1% Tween 20 detergent, and a secondary incubation with ExtrAvidin-alkaline phosphatase (Sigma-Aldrich). Spots were detected using BCIP/NBT (Sigma-Aldrich) and scanned and counted with an ImmunoSpot Analyzer (Cellular Technology).

BrdU labeling

B6 mice were immunized with NP-LPS and fed BrdU in the drinking water (0.5 mg/ml with 1% dextrose) for 3 d beginning immediately after immunization. Spleen and BM cells were stained with appropriate Abs, fixed, and permeabilized (Fix & Perm; Invitrogen), treated with 225 $\mu$g/ml DNase (DN25; Sigma-Aldrich), stained intracellularly with FITC–anti-BrdU Abs and NP-APC, and washed twice before analysis on an LSR2 flow cytometer. Multiple files per sample were concatenated before data analysis using FlowJo 8.8.

**CD40/CD154 blockade**

On days 5, 7, and 9 post immunization, NP-CGG/alum–immunized mice were given i.v. inoculations (300 $\mu$g per injection) of anti-CD154 (MR-1) or control Hamster IgG (both from BioXcell), as described by Takahashi et al. (29).

**Statistical analysis**

Significant differences in plasma cell frequencies between two experimental groups were evaluated with the unpaired two-tailed t test, using Excel software.

**Results**

**Persistence of LPS-induced TI plasma cells**

We immunized C57BL/6 (B6) or T cell-deficient B6.TcR$\alpha^{-/-}\beta^{-/-}$-adults with the hapten (4-hydroxy-3-nitrophenyl)acetyl (NP) conjugated to LPS or CGG. We studied the Ab response to NP in B6 background mice for two reasons. First, the kinetics of both plasma cell differentiation and GC-dependent somatic hypermutation and selection in response to NP are well established (29, 30). Second, NP-responsive B-lineage cells are readily identified by flow cytometry with NP-conjugated fluorescent proteins, and such cells can be resolved in conventional inbred mice without using Ig transgenes to increase frequencies of Ag-responsive B cells (31).

We quantified NP-specific plasma cell frequencies in the spleen and BM at several time points after a single inoculation of B6 or B6.TcR$\alpha^{-/-}\beta^{-/-}$ mice with NP-LPS. Because the Ab response to NP-conjugates in B6-background mice is dominated by $\lambda^\text{+}$ B cells (30, 32), we assayed frequencies of plasma cells secreting $\lambda^\text{+}$ NP-binding Abs. Although, as expected, splenic NP-specific plasma cell frequencies increased and then decreased exponentially between days 1 and 5 (15), NP-specific plasma cells were readily observed in the spleen and BM for $\geq 200$ d in B6 mice and $\geq 91$ d in B6.TcR$\alpha^{-/-}\beta^{-/-}$ mice (Fig. 1, A, B). Furthermore, although in the spleen background levels increased substantially for mice immunized with NP-LPS $>300$ d previously, obscuring hapten-specific splenic plasma cells, in the BM we easily detected $\lambda^\text{+}$ NP-specific plasma cells well above background in mice that were immunized $>400$ d previously (Supplemental Fig. 1). Of note, as shown in Fig. 1B, on day 91 postimmunization frequencies of NP-specific plasma cells appeared to be greater in B6.TcR$\alpha^{-/-}\beta^{-/-}$ mice than in B6 mice, although whether this

**FIGURE 1.** Kinetics of the plasma cell response after NP-LPS immunization. (A) NP-specific $\lambda^\text{+}$ plasma cells (PCs) in the spleen and BM were quantified by ELISPOT after B6 adults were immunized once with NP-LPS in PBS. (B) B6 (circles, solid line) or B6.TcR$\alpha^{-/-}\beta^{-/-}$ (squares, dashed line) adults were immunized and evaluated as in (A). For (A) and (B) all symbols represent data from an individual mouse. Note: On day 91, BM PC frequencies were significantly different between B6 and B6.TcR$\alpha^{-/-}\beta^{-/-}$ mice, as indicated by * ($p < 0.002$). At all other time points, no significant differences in PC frequencies were found between these groups. (C) Representative ELISPOT wells from the BM (d 0, d 90) or spleen (d 3) of B6.TcR$\alpha^{-/-}\beta^{-/-}$ mice.
difference is meaningful is, at present, unclear. We conclude that a single immunization with NP-LPS is sufficient to induce the generation of hapten-specific BM plasma cells that are readily detected >400 d later.

**Decay rate of radioresistant TI plasma cells**

Because residual Ag may induce naive B cells to contribute to plasma cell pools after immunization, we sought to estimate the decay rate of hapten-specific BM plasma cells following radiation-induced ablation of the functional naive B cell pool. In this regard, although past experiments show that long-lived plasma cells in the BM are resistant to apoptosis induced by ionizing radiation (IR) (6), it was unclear whether IR resistance is also a property of early plasma cells in the spleen. Significantly, whereas naive B cells readily died upon direct exposure to 200 R, the vast majority of very early splenic plasma cells were resistant to IR-induced apoptosis, regardless of whether they were induced by immunizing with NP-LPS or NP-CGG and exposed to doses up to 800 R (Fig. 2). It should be noted, consistent with the past observation that cultured GC B cells die rapidly without stimulation of CD40 (33), in our hands GC B cells died immediately in culture, with or without IR (not shown). To assess the capacity of NP-LPS-induced plasma cells to persist without input from naive B cells, we quantified NP-specific plasma cells out to 190 d after NP-LPS–immunized B6 mice were exposed to 900 R whole-body IR. Notably, past experiments using this approach indicate that the half-life of BM plasma cells induced after acute infection with lymphocytic choriomeningitis virus, a complex TD viral Ag, is 94 d (range, 85–105 d) (6). Fig. 3 shows that NP-specific plasma cells in NP-LPS–immunized and irradiated/reconstituted mice declined with an estimated $t_{1/2}$ of 55 d (range 49–63 d). Therefore, by these measurements the half-life of NP-LPS–induced plasma cells is only modestly less than that observed during acute LCMV infection.

Given that whole-body IR may change microenvironments in the BM and elsewhere, potentially altering plasma cell lifespan, we also adopted an in vivo BrdU pulse-chase protocol to assess the decay rate of BrdU$^+$ NP-specific BM plasma cells without exposing mice to IR. This general approach was used previously to gauge the half-life of Ag-specific plasma cells induced by a TD Ag (5). We fed NP-LPS–immunized mice BrdU for 3 d, beginning at the time of immunization, and then assessed the fraction of BrdU$^+$ NP-specific BM plasma cells at multiple time points later by flow cytometry. As shown in Fig. 4, NP-specific BrdU$^+$ plasma cells were readily apparent in the BM between 34 and 94 d of the chase period. Therefore, many plasma cells formed within the first 3 d of the response to NP-LPS were able to enter and persist in the BM for at least 94 d. However, consistent with the data in Fig. 3, the fraction of NP-specific BM plasma cells that were BrdU$^+$ declined progressively between days 34 and 94 of the chase period, resulting in an estimated $t_{1/2}$ of 47 d (range, 41–59 d) (Fig. 4B). Of interest, although surface expression of B220 is often used to identify early plasma cells (34), many hapten-specific BrdU$^+$ plasma cells continued to exhibit low but detectable surface levels of B220, even on day 94 of the chase period (Supplemental Fig. 2), suggesting that surface levels of B220 are not consistently...
Downregulated on all plasma cells during the earliest phases of plasma cell differentiation. Altogether, these data, along with the analyses in Fig. 3, indicate that NP-LPS–induced plasma cells possess a $t_{1/2}$ of $\sim$50 d.

**Lack of evidence for induction of GCs in T cell-deficient mice**

Past work with carbohydrate-enriched (type 2) TI Ags indicate that these Ags are sufficient to induce the transient development of GC-like structures. These B cell clusters, however, resolve quickly, fail to support SHM, and require high-dose Ag for their formation (18, 19). The capacity of LPS-based Ags to induce GC formation, though, was less clear. To address whether TI GCs are induced by NP-LPS immunization, we quantified Ag-specific GCs in B6 or B6.TcRβ$^{-/-}$δ$^{-/-}$ mice immunized in parallel with this type 1 TI Ag and compared these results with those in B6 mice immunized with NP-CGG. Hapten-specific GC B cells were identified as NP-binding CD19$^+$ PNA$^{high}$ cells that lacked surface IgD expression (31, 35). Consistent with past data (36), NP-specific GC B cells were not detected in B6 mice immunized with NP-CGG until after day 5 post immunization (Fig. 5B). By contrast, low frequencies of GC B cells were identified in B6 mice inoculated with NP-LPS 3 d post immunization, but not at later time points. However, it should be noted that frequencies of NP-specific CD19$^+$ PNA$^{high}$ B cells in B6 mice immunized with NP-LPS were highly variable, perhaps owing to the inefficiency of such responses in the absence of cognate T cell help. In contrast, we were unable to detect GC B cells in NP-LPS–immunized B6.TcRβ$^{-/-}$δ$^{-/-}$ mice at all time points examined (Fig. 5A, 5B). These data, together with the data in Figs. 1, 3, and 4, establish that long-lived TI plasma cells can form independently of B cell maturation in GCs.

**Longevity of pre-GC plasma cells in TD responses**

Given that many NP-LPS–induced plasma cells become long lived without maturing in GCs, we also examined whether plasma cells induced with a TD Ag achieve longevity independently of the GC response. In this regard, several past studies suggest that B cell maturation in GCs is associated tightly with the formation of long-lived plasma cells in TD Ab responses. For instance, the BM is highly enriched for plasma cells synthesizing high-affinity isotype-switched Abs (13), and high-affinity GC B cells preferentially generate plasma cells (37). However, these studies do not exclude the possibility that low-affinity pre-GC plasma cells possess the potential to seed long-lived plasma cell pools in the BM. Furthermore, although B cells lacking the transcriptional repressor BCL6 fail to generate both GCs and long-lived BM plasma cells (38, 39), BCL6 mutation may lead to secondary effects that preempt the generation of long-lived plasma cells during the early phases of B cell differentiation.

For these experiments, we sought to prevent the GC response on day 5 post immunization with NP-CGG, before the exponential increase observed in responding GC B cells (Fig. 5B), and 3 d before Ig somatic mutation events are detected in these cells (36). First, we abrogated the GC response via whole-body IR. As observed for naive B cells (Fig. 2), Ag-activated GC B cells in vivo were also sensitive to IR-induced cell death, as the NP-specific GC B cell pool was readily depleted upon exposure of NP-CGG–immunized mice to 900 R (Supplemental Fig. 3). Fig. 6A illustrates the frequency of NP-specific plasma cells in the BM of mice irradiated with 900 R and reconstituted 5 d after immunization with NP-CGG. As shown, Igκ$^+$ NP-specific plasma cells were readily detected in the BM for $\geq$105 d post IR. Consistent with the absence of GC B cell maturation and selection, NP-specific plasma cells in irradiated/reconstituted mice secreted low-affinity IgM$^+$ Abs (Fig. 6B). Notably, however, a readily detectable number of IgM$^+$ plasma cells were observed in the BM of control (nonirradiated) mice. Second, we inhibited the generation of Ag-induced GCs by blocking B cell–T cell interactions with anti-CD154 blocking Abs, beginning on day 5 post immunization. As shown, despite prevention of GC formation via this strategy (Supplemental Fig. 4A, 4B), NP-specific BM plasma cells were detected for $\geq$91 d post immunization (Fig. 6C), and these cells did not secrete high-affinity IgG1 Abs (Fig. 6D). Significantly, we did not detect NP-specific GC B cells in any mouse given anti-CD154 Abs throughout this study (Supplemental Fig. 4B). Of note, frequencies of BM $\lambda^+$ NP-specific plasma cells were decreased significantly at the earliest time point (day 14) analyzed, perhaps suggesting suboptimal clonal expansion due to CD40 blockade. Regardless, these results suggest that maturation and selection of high-affinity B cells in GCs are not requisite for propagating long-lived plasma cells in TD Ab responses. In further support of this notion, we also detected hapten-specific plasma cells in the BM of NP-CGG–immunized mice lacking activation-induced deaminase, which is strictly required for the generation of high-affinity B cells by SHM (40) (Supplemental Fig. 4C). Collectively, these data suggest that competence to enter the long-lived BM plasma cell pool occurs in early plasma cells and is not associated with maturation and affinity-based selection in the GC.

**Discussion**

TI Ags have long been deemed incapable of inducing the generation of long-lived plasma cells (1). However, our results show that many early plasma cells, including those induced with an LPS-based conjugate, persist for extended periods in the BM at fre-
The chase period. Note that BrdU+ cells were identified by comparing immunized controls that were not exposed to BrdU. The "Dump" channel includes data from an individual mouse, and the mean for each time point is indicated by a solid line. The half-life of BM plasma cells post IR was calculated as described for Fig. 3.

We used two different approaches to estimate the decay rate of hapten-specific plasma cells in the BM. We were surprised to learn that, although hapten-specific plasma cells were readily detected in the BM for >400 d following a single immunization with NP-LPS (Fig. 1A), hapten-specific BM plasma cells exhibited a t1/2 of ~50 d (Figs. 3, 4). Whereas these calculations contrast substantially with the past notion that early plasma cells in TI responses fail to survive beyond 5 d of their generation (1, 2, 41), they also suggest that the long-term maintenance of serum Abs specific for such Ags cannot be explained solely by the generation of exceptionally long-lived plasma cells. These recent studies, together with the conclusions drawn in this article, challenge this long-standing paradigm. TI Ab responses mediated by B1 B cells, for example, were sufficient to provide long-term IgM-dependent protection against B. hermsii (20, 21). However, whereas these investigators concluded that long-term protection against this spirochete was achieved through the continual induction of short-lived plasma cells, an alternative possibility supported by our data is that a significant fraction of plasma cells induced by B. hermsii infection are long lived. This perspective may apply to other TI pathogens, such as S. pneumoniae and E. muris. Indeed, recent work shows that plasma cells induced via immunization of conventional mice with S. pneumoniae or E. muris colonize and persist in the BM for extended periods (24–26). Whereas the cellular basis underlying long-term protection in many of these systems is not fully understood, our data reveal the possibility that long-lived plasma cells formed in the complete absence of T cells may play critical roles in responses to TI pathogens.

These recent studies, together with the conclusions drawn in this article, contrast substantially with the currently dominant model, which posits that T cell-regulated GC maturation and selection events are associated with the propagation of long-lived plasma cells (4, 10, 12). Why, then, were TI Ags thought to induce only
short-lived plasma cells? As reported by Fidler (15), following a robust clonal burst the number of hapten-specific NP-LPS–induced plasma cells in the spleen declines by \( \sim 90\% \) by day 5 in this response (see Fig. 1A), suggesting that the vast majority of early NP-LPS–induced plasma cells are indeed short-lived. However, this observation does not exclude the possibility that a meaningful number of TI plasma cells enter the BM and persist for extended periods. Indeed, other past studies have documented small numbers of hapten-specific BM plasma cells 3 wk after immunization with haptenated LPS, but these workers did not address whether the plasma cells were maintained beyond 21 d, nor did they seek to measure the half-life of these cells (42). Furthermore, although

FIGURE 5. Failure to detect GCs in immunized T cell-deficient mice. B6 or B6.TcR\(^{β^{-/-}\,δ^{-/-}}\) adults were immunized with either NP-LPS/PBS or NP-CGG/alum, and frequencies of NP-binding CD19\(^{+}\) PNA\(^{high}\) GC B cells in the spleen were determined by flow cytometry. (A) Flow cytometric data for the indicated mice, representative of three to six mice per group. Left-most plots are pregated on viable (DAPI\(^{+}\)) IgD\(^{-}\) cells. A total of 10\(^6\) events were collected for each sample. Abs used for the “Dump” channel are as described for Fig. 4. (B) Numbers of NP-specific GC B cells at 3, 5, or 7 d were calculated by multiplying the frequency of hapten-binding GC B cells, using the gating strategy shown in (A) by the total number of splenocytes harvested in each mouse. Results are expressed as means \( \pm \) SEM of three to six mice per group performed over two separate experiments. Absolute numbers for days 3, 5, and 7 are graphed separately to better reveal the small number of PNA-binding B cells in NP-LPS–immunized mice at early time points, which fell below detection levels in all mice after day 5.

FIGURE 6. Long-lived TD plasma cells (PCs) without maturation in GCs. (A) Frequencies of NP-specific \( λ^{+}\) PCs post IR in the BM of B6 adults immunized with NP-CGG/alum and then left untouched (closed) or irradiated and reconstituted (open) on day 5 post immunization (see Materials and Methods). (B) BM cells from the mice in (A) on day 105 post IR were assayed by ELISPOT, using either NP\(^{33}\)-BSA–coated plates evaluated with anti-IgM specific Abs (left) or NP\(^{4}\)-BSA–coated plates evaluated with anti-IgG1–specific Abs. Differences in \( λ^{+}\) and IgM\(^{+}\) PC frequencies between irradiated and control groups were not significant. The \( p \) value for differences in frequencies of NP-specific IgG1\(^{+}\) PCs is shown on the graph. (C and D) NP-CGG/alum–immunized B6 adults were given three injections of anti-CD154 (MR-1) Abs (open) or hamster IgG (closed), beginning 5 d post immunization (see Materials and Methods). (C) Shown are BM NP-specific \( λ^{+}\) PCs assayed with NP\(^{33}\)-BSA. Differences between MR1-treated and control mice were significant on day 14 (\( p = 0.02 \)) and day 190 (\( p = 0.04 \)). At all other time points, no significant differences were found between these groups. (D) NP-specific IgM\(^{+}\) PCs were assayed with NP\(^{33}\)-BSA (left) or NP-specific IgG1\(^{+}\) PCs were evaluated with NP\(^{4}\)-BSA (right). The \( p \) values for statistically significant differences are shown on graphs. All results are expressed as means \( \pm \) SEM of three to four mice per group.
References


