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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 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 T 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 t½ 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...
pennsylvania Office of Regulatory Affairs.

(Jackson Laboratories). All animal procedures were approved by the University of Pennsylvania Office of Regulatory Affairs.

Materials and Methods

Mice

C57BL/6 (B6), B6.TcR^−/− B cells (31). We immunized C57BL/6 (B6) or T cell-deficient B6.TcR^−/− B6.TcR^−/− mice with NP-LPS. Because the Ab response to NP-conjugates in B6-background mice is dominated by λ B cells (30, 32), we assayed frequencies of plasma cells secreting λ NP-binding Abs. Although, as expected, splenic NP-specific plasma cell frequencies were significantly increased and then decreased exponentially between days 1 and 5 (15), NP-specific plasma cells were readily observed in the spleen and BM for ≥200 d in B6 mice and ≥91 d in B6.TcR^−/− mice (Fig. 1A, 1B). 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 λ 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^−/− mice than in B6 mice, although whether this

CD40-CD154 blockade

On days 5, 7, and 9 post immunization, NP-CGG/alum–immunized mice were given i.v. inoculations (300 μg 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^−/− adults with the hapten (4-hydroxy-3-nitrophenyl)acetetyl (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^−/− mice with NP-LPS. Because the Ab response to NP-conjugates in B6-background mice is dominated by λ B cells (30, 32), we assayed frequencies of plasma cells secreting λ 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 ≥200 d in B6 mice and ≥91 d in B6.TcR^−/− mice (Fig. 1A, 1B). 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 λ 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^−/− mice than in B6 mice, although whether this
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 naïve 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 naïve 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
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+ PNAhigh 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+ PNAhigh 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, IgMNP-specific plasma cells were readily detected in the BM for ≥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 ≥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 λ* 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-
FIGURE 4. Pulse-chase kinetics for hapten-specific NP-LPS–induced plasma cells. NP-LPS–immunized B6 adults were fed BrdU in the drinking water for 3 d post immunization. (A) The frequency of NP-binding splenic plasma cells that were BrdU+ on day 3 post immunization is shown. Data are the mean of four mice. (B) Frequencies of NP-binding BM plasma cells that were BrdU+ on days 34, 64, and 94 d of the chase are shown. Each circle represents 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. (C) Representative flow cytometric data illustrating the identification of NP-binding BrdU-positive plasma cells in the BM at the indicated time points of the chase period. Note that BrdU+ cells were identified by comparing immunized controls that were not exposed to BrdU. The “Dump” channel includes Abs to CD4, CD8, F4/80, TER-119, and Gr-1. Each plot was derived from files containing 7–9 × 10^6 events.

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quencies that mirror TD responses, despite the significant contrac-
tion in Ag-specific plasma cell frequencies observed early in such responses (Fig. 1A) (15). Thus, many early plasma cells achieve competence to enter long-lived BM plasma cell pools early in TI responses and in the absence of T cell-derived signals associated with the generation of long-lived immunity. These findings contrast with the recently proposed “imprinting model” positing that TD Ags are unique in their capacity to induce B cells to yield long-lived plasma cells (12). Indeed, our findings suggest that TLR4 and T cell-derived signals such as those provided by CD40 activation on B cells may provide comparable signals required for inducing plasma cell longevity.

We used two different approaches to estimate the decay rate of hapten-specific NP-LPS–induced 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. 1), hapten-specific BM plasma cells exhibited a t_1/2 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 generated early in such responses. Accordingly, we favor the notion that the BM plasma cell pool consists of both short- and long-lived cells. This viewpoint is supported by recent work from Racine et al. (24), who characterized a surface IgM+ BM plasmablast population with a potential role in maintaining Ab titers to the intracellular bacterium E. muris. Further evidence for heterogeneous within the BM plasma cell pool stems from our analysis of BrdU-retaining hapten-specific BM plasma cells. Thus, although surface B220 expression is typically considered a trait restricted to recently formed plasma cells (34), we found that many long-lived BM plasma cells exhibited a bimodal pattern for surface B220 expression (Supplemental Fig. 2). Together, these findings underscore the need for increased understanding of the cellular dynamics of BM plasma cells and the potential influence of Ag and other factors on maintenance of the plasma cell pool.

Although TI pathogens were previously thought to be ineffectual inducers of long-term protective immunity, recent results, including those presented in this article, challenge this long-standing para-
digm. 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 con-
cluded 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 un-
derstood, 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 \( \lambda^+\) 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\(_{2}\)-BSA–coated plates evaluated with anti-IgG1–specific Abs. Differences in \( \lambda^+\) 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 \( \lambda^+\) 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\(_{2}\)-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.
the majority of early plasma cells induced by either TI or TD Ags may die before exiting the spleen (2, 41, 43, 44), only 9% of early splenic plasma cells in a TD response show clear signs of apoptosis (43). By quantifying frequencies of hapten-specific plasma cells in the BM following immunization with LPS or protein-based hapten conjugates, we have found that plasma cells persist in the BM for extended periods in response to both TI and TD Ags and without undergoing GC maturation and selection.

Our data also suggest that autoantibodies to TI self-antigens, such as rheumatoid factor Abs induced by DNA-chromatin complexes (45), might arise from long-lived plasma cells despite a fully tolerant T cell compartment. This notion emphasizes the importance of central and peripheral B cell tolerance mechanisms, especially in scenarios in which such self-antigens are sufficient to induce somatic hypermutation without engaging Th cells (46). The notion that most Ags are capable of inducing long-lived plasma cells may also apply to TI self-antigens underlying the formation of polyreactive natural Abs, which have been shown to be protective against several pathogens (47).

We used resistance to radiation-induced cell death as a tool to investigate plasma cell longevity in the context of TI and TD immune responses. In this context, we were surprised to find that radiosensitivity in plasma cells is established exceptionally early during these responses. It should be noted that, at present, the underlying mechanisms allowing plasma cells to avoid death despite widespread DNA damage are not understood. However, other studies have shown that antiapoptotic DNA repair pathways are induced by TLR signaling in memory CD4+ T cells (48). Hence, TLR signaling may also play a similar role in activated B cells.

In sum, we propose that LPS-based Ags readily elicit the formation of a long-standing pool of plasma cells secreting low-affinity but high-avidity IgM Abs that may play fundamental roles in combating certain pathogens. Accordingly, we suggest that the role of such plasma cells in host protection be re-evaluated. In addition, the control of gene expression patterns in short- and long-lived plasma cells, the signals required to induce the formation of long-lived cells, and, conversely, the mechanisms underlying the apparent loss of many plasma cells during early phases of Ab responses all remain important areas of investigation.

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Disclosures

The authors have no financial conflicts of interest.

References


Supporting on-line material for:

Long-lived plasma cells are induced early in response to T-cell independent and T-cell dependent antigens

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This PDF file contains four supplemental figures:
Supplemental Figure 1. Detection of NP-LPS induced plasma cells long after immunization. These data are an extension of the experiment illustrated in Figure 1A. NP-specific $\lambda^+$ plasma cells (PCs) in the BM were quantified by ELISPOT after B6 adults were immunized once with NP-LPS in PBS. Each symbol represents data from an individual mouse.
Supplemental Figure 2. B220 expression on persisting NP-LPS induced plasma cells. BM cells from a mouse immunized with NP-LPS in PBS 97 days previously and given BrdU for three days immediately after immunization were stained with antibodies to the indicated surface antigens, fixed, permabilized, treated with DNase, and then stained with NP-APC and anti-BrdU antibodies. The “Dump” channel includes antibodies to CD4, CD8, F4/80, Gr-1, and TER-119. B220 levels on mature B220 expression on surface IgD⁺ BM B cells is shown for comparison.
Supplemental Figure 3. Germinal center B cells are radiosensitive. B6 adults were immunized with NP-CGG/alum. 168 hours (7 days) later cohorts of immunized mice were exposed to 900R (bottom), and 9 hours later (177 hours post-immunization) splenocytes were prepared for analysis compared to mice immunized precisely 168 hours previously. Splenocytes from mice in each group were stained with the indicated reagents before analysis of $10^6$ events on an LSR2 flow cytometer. Representative of three separate experiments.
Supplemental Figure 4. Disruption of germinal B cell differentiation or selection. (A, B) Early CD40-CD154 blockade prevents germinal center B cell differentiation. NP-CGG/alum immunized mice were given anti-CD154 (MR-1) or hamster IgG intravenously in three doses (days 5, 7, and 9 post-immunization) at a dose of 300μg/injection. GC B cell differentiation was measured by flow cytometry of splenocytes on days 14, 34, 61, and 91 post-immunization. (A) Representative analysis of splenocytes on day 14 post-immunization. (B) Quantification of viable (DAPI-) Dump-IgD-NP-binding CD19+ PNA<sup>high</sup> germinal center B cells at the indicated time points post-immunization. Dump antibodies were CD4, CD8, Gr-1, TER119, and F4/80. Symbols are means of 3 mice per group per time point. Error bars represent the SEM of each group. (C) BM plasma cells in AID-deficient mice. 8-week old littermate control (wild type) or AID<sup>−/−</sup> mice were immunized with NP-CGG/alum. ELISPOT assays for plasma cells secreting NP-specific antibodies were performed on the spleen on day 7 using NP<sub>33</sub>-BSA coated plates with anti-λ antibodies or with BM cells on day 30 post-immunization. BM analyses were performed using NP<sub>33</sub>-BSA (left) or NP<sub>4</sub>-BSA (right) coated plates that were developed with anti-λ or anti-IgG1 antibodies, respectively. Wild type (closed), AID<sup>−/−</sup> (open). Columns represent means of 3 mice per group. Error bars represent the SEM for each group.