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What Is and What Should Always Have Been: Long-Lived Plasma Cells Induced by T Cell–Independent Antigens

Alexandra Bortnick and David Allman

It is well accepted that Ag-induced B cell differentiation often results in the generation of exceptionally long-lived plasma cells. Much of the work supporting this viewpoint stems from studies focused on germinal center–derived plasma cells secreting high-affinity isotype-switched Abs in mice immunized with T cell–dependent Ags. In contrast, less attention has been devoted to understanding Ab responses to T cell–independent Ags and pathogens. In this study, we review recent work showing that T cell–independent Ags consisting of either polysaccharides or LPSs also induce the formation of long-lived plasma cells, despite their general inability to sustain germinal center responses. This new information provides a framework for more fully understanding the forces underlying immunity to pathogens that resist T cell recognition and the extra-cellular cues governing plasma cell longevity. The Journal of Immunology, 2013, 190: 5913–5918.

After initial expression of Ag-specific receptors, newly formed B cells depart the bone marrow and recirculate among peripheral lymphoid tissues as mature functional cells. Upon antigenic stimulation, naive B cells give rise to memory B cells and Ab-secreting plasma cells. Once generated, some plasma cells survive for months or years in mice, and perhaps even decades in people. Consequently, these long-lived plasma cells play a key role in maintaining serum Ab levels to various pathogens, and therefore they are essential components of long-lasting protective humoral immunity. Long-lived plasma cells may also function as a key source of pathogenic Abs, including self-reactive Abs in various autoimmune diseases, and for IgE Abs in type I hypersensitivity reactions. Notably, however, not all plasma cells are long-lived. Indeed, plasma cells appear to consist of at least two distinct pools characterized by key differences in half-life and physical location: short-lived cells found in extrafollicular locales such as the red pulp of the spleen or in medullary chords of lymph nodes, and long-lived cells found mainly in the bone marrow (1). Substantial numbers of IgA-secreting plasma cells can also be found within GALT (2), although the lifespan of these cells remains poorly defined. Why some plasma cells are short-lived whereas others become long-lived remains unknown.

High-affinity Ab responses to T cell–dependent Ags typically require the generation of germinal centers (GCs), unique anatomic structures in peripheral lymphoid tissues enriched for B cells undergoing somatic hypermutation, Ig class switch recombination, and Ag-mediated selection and clonal expansion (3). Because plasma cells secreting high-affinity class-switched Abs are readily found in the bone marrow, and because long-lived plasma cells are also typically found in this location, it is often assumed that long-lived bone marrow plasma cells derive mainly from GCs. However, as addressed below, recent evidence suggests that B cell responses that fail to engender bona fide GC responses, such as responses to T cell–independent Ags, also generate long-lived plasma cells.

This review charts plasma cell differentiation in T cell–dependent and T cell–independent Ab responses while also addressing current knowledge of the environmental cues governing life and death decisions in the plasma cell lineage. Along the way, we consider historical precedents driving the notion that plasma cells possess markedly distinct lifespans, as well as the idea that T cell–independent Ags are relatively ineffective at inducing the formation of long-lived plasma cells. Lastly, we discuss evidence that plasma cell longevity and the size of the overall bone marrow plasma cell pool are regulated by unique and limiting cell–cell and receptor–ligand interactions in the bone marrow.

Short- and long-lived plasma cells

Several longitudinal studies in both mice and people illustrate the advantages of inducing and maintaining effective concentrations of serum Abs. Abs generated by routine vaccinations to measles, mumps, tetanus, diphtheria, and smallpox can persist and remain protective for 25 y or longer in people (4). During the recent 2009 H1N1 pandemic, 96% of adults born between 1909 and 1919 had cross-protective Abs from persisting titers mounted during the Spanish flu pandemic. As a result, remarkably few elderly individuals suffered from H1N1 symptoms compared with the seasonal influenza virus (5, 6). However, for each example whether maintenance of serum Abs reflects the continual generation of short-lived Ab-
secreting cells, often termed plasmablasts, or the activity of long-lived plasma cells is less than immediately clear.

Before 1997 it was thought that all plasma cells die within days of their generation. This viewpoint derived from studies showing that plasma cells found in peripheral lymphoid tissues soon after immunization exhibit a rapid rate of turnover (7–9), as well as other work showing that pre-existing plasma cell numbers decline rapidly after administration of hydroxyurea (10). Consequently, it was often proposed that maintenance of serum Ab concentrations required the constant replenishment of short-lived plasma cell pools by activated memory B cells engaged by persisting Ag or TLR ligands (11–13). In the late 1990s two groups revisited this question by directly monitoring numbers of Ag-induced plasma cells for hundreds of days after immunization using experimental approaches to exclude input from memory B cells (14, 15). Using in vivo BrdU pulse-chase labeling, Manz et al. (14) demonstrated that 60–70% of induced plasma cells survive for at least 120 d beginning 3 wk after secondary immunization with a hapten-protein conjugate. These researchers later showed that persisting Ab titers are maintained independently of Ag (16). In parallel, Slifka et al. (15) ablated naive and memory cells using whole-body ionizing radiation long after acute infection with lymphocytic choriomeningitis virus (LCMV). These workers detected robust LCMV-specific Ab titers and plasma cell frequencies for extended periods, even a year after ablation of LCMV-specific memory B cells. Later studies using anti-CD20 treatment in mice to deplete naive and memory B cells confirmed that loss of memory cells did not affect plasma cell pools even after 100 d (17). Similarly, people undergoing B cell ablation therapies maintain serum titers to common Ags for at least 1 y (18). Taken together, these studies show that long-lived plasma cells are essential components of sustained humoral immunity in mice and people, and they firmly established that many plasma cells persist for extended periods without input from recently activated naive or memory B cells.

However, not all Ab responses are long-lived. Indeed, whereas vaccines to T cell–dependent Ags are more likely to be long-lasting, those against certain T cell–independent Ags often elicit transient Ab responses (19). Even vaccines to T cell–dependent Ags may require boosters to maintain protective concentrations of serum Abs (20). Despite these ill-explained inconsistencies, this general dichotomy has suggested a model in which T cell–independent Ags give rise mainly to pools of short-lived extrafollicular plasma cells that die within days of their generation. In contrast, T cell–dependent Ags are thought to induce both short- and long-lived plasma cells, as well as memory B cells (Fig. 1A). As a consequence, few polysaccharide vaccines consisting of bacterial capsule Ags are currently available. One noteworthy exception is Pneumovax, which confers immunity to pneumococcal bacteria for up to 10 y in adults (21). The surprising efficacy of Pneumovax may reflect its unique high valency formulation, comprised of, in some cases, 23 different pneumococcal subtypes.

From a teleological perspective, given that early extrafollicular plasma cells are enriched for cells secreting low-affinity IgM Abs, it might be considered advantageous to preferentially limit the lifespan of extrafollicular plasma cells secreting low-affinity IgM Abs in favor of GC-derived plasma cells secreting high-affinity class-switched Abs. To this end, GC-derived Ab-secreting cells might become uniquely endowed with appropriate homing and cytokine receptors required for entry into long-lived plasma cell pools. This viewpoint is supported by work showing that plasma cells

![FIGURE 1](https://www.jimmunol.org/content/journals/s3-1675591420004392/F1.large.jpg)

**FIGURE 1.** Contrasting models for origins of long-lived plasma cells. (A) Standard model indicating that bone marrow plasma cells derive chiefly, if not exclusively, from GCs. (B) Alternative model illustrating that bone marrow plasma cells also derive from early extrafollicular plasma cell pools. Whereas GCs may serve as the chief source of bone marrow plasma cells (multiple arrows), dotted lines denote current uncertainty about the relative contribution of memory B cells and extrafollicular Ab-secreting cells to long-lived plasma cell pools.
secrating high-affinity Abs survive more easily in culture when compared with plasma cells secreting relatively low-affinity Abs (22). However, as reviewed recently by Racine and Winslow (23), low-affinity IgM Abs play unique and important roles in the clearance of many pathogens. For instance, IgM Abs play protective roles during infection with the encapsulated Gram-positive bacterium Strep_to scaus pneumoniae, as IgM-deficient mice experience higher bacterial loads compared with controls (24). Loss of B1 B cells, considered a chief source of IgM Abs, results in heightened susceptibility to S. pneumoniae (25), and IgM and B1 B cells also mediate long-term protection against the spirochete Borrelia hermsi (26, 27). Collectively, these studies indicate that the capacity to generate sustained IgM responses may be highly advantageous for protection against certain pathogens, especially those that have evolved to resist recognition by T cells.

Natural IgM, presumably derived from B1 B cells, has also been shown to be protective against viral infections, as natural Abs specific for LCMV, vesicular stomatitis virus, and vaccinia virus are found in conventional mice, and mice lacking these Abs are more likely to succumb to vesicular stomatitis virus infection. Moreover, protection against influenza requires the dual activity of low-affinity IgM and high-affinity IgG Abs (28). Taken together, these observations suggest that maintenance of IgM titers via the generation of long-lived IgM-producing cells may indeed be advantageous.

Nonetheless, given that Ag-specific plasma cell frequencies drop exponentially early in primary responses (29, 30), it appears relatively safe to conclude that many newly generated plasma cells are short-lived. But why? One possibility is that many early plasma cells may fail to express key receptors responsible for transmitting antiapoptotic signals such as the receptors for IL-6 or the BlyS family cytokines APRIL and/or BlyS. Another and potentially related possibility is that early plasma cells have yet to express sufficient levels of the transcription factors required for optimal expression of these plasma cell pools. The latter scenario is consistent with work from Sze et al. (30) showing that whereas elevating frequencies of hapten-specific B cells by transferring increasing numbers of BCR transgenic B cells into adoptive hosts before immunization enhances numbers of induced plasma cells in the spleen, this strategy fails to increase numbers of hapten-specific plasma cells in the bone marrow. A third and non–mutually exclusive possibility is that early plasma cells may exhibit a suboptimal unfolded protein response, the physiologic process that allows plasma cells to cope with the metabolic “stress” of continual high-titer Ab synthesis (32). Clearly, why many early plasma cells are short-lived remains an open question.

Past work on T cell–independent plasma cell responses

The widely held view that T cell–independent Ab responses are short-lived is underpinned by a collection of studies mostly performed before 1990. After transferring wild-type B cells from LPS-immunized mice into LPS nonresponder B10.Cr mice, Freitas and colleagues (33) observed a rapid decline in Ab titers after transfer, suggesting that any plasma cells transferred were short-lived. However, in these studies survival of the transferred cells was not monitored directly. In vivo BrdU labeling studies have shown that cellular turnover within the splenic plasma cell pool in rats and immunized mice is exceptionally high, with an ∼50% renewal rate of 1–2 d (30, 34). However, these data alone do not reveal whether cellular exit from splenic plasma cell pools reflects death versus migration of cells to alternate sites such as the bone marrow. Indeed, nondividing pre-B cells in the bone marrow exhibit a similarly rapid renewal rate, and yet many pre-B cells yield immature B cells (35). In this regard, attempts to identify apoptotic cells directly in extrafollicular plasma cell pools failed to detect substantial numbers of dying cells (36); however, such studies are technically demanding because dying cells are likely to be cleared rapidly by macrophages. Without direct and accurate measurements of the numbers of dead and dying cells among extrafollicular plasma cells throughout the course of an early immune response, the fraction of recently formed extrafollicular plasma cells that are indeed short-lived will remain difficult to determine.

Even before 1980 there were hints that T cell–independent Abs can induce the generation of long-lived plasma cells. For instance, Fidler (29) quantified numbers of plasma cells after immunization with trinitrophenol–LPS out to 4 wk, reporting a sharp reduction in the number of splenic plasma cells between days 4 and 8 after immunization. However, plasma cell numbers in this study never dropped to background, and the potential for cells to migrate elsewhere, particularly the bone marrow, was unexplored. Even earlier, while studying LPS-induced Ab-secreting cells, Möller (37) noted: “After the peak there was a rapid decline, but background level usually was not reached even after 16 days after immunization.” Therefore, although many early extrafollicular plasma cells in these responses appear to die, the failure of induced plasma cell numbers to return to background is consistent with the possibility that some of these cells subsequently enter long-lived plasma cell pools.

Lasting plasma cells for T cell–independent Ags

Several groups, including ours, recently revisited these issues by testing directly whether plasma cells induced by classic T cell–independent Ags can become long-lived. For responses to polysaccharides, classically referred to as type 2 T cell–independent Ags, Taillardet et al. (38) demonstrated that S. pneumoniae immunization induced the formation of bone marrow plasma cells that persisted for 180 d after B cell depletion. With another approach, Foote et al. (39) showed that the response to the type 2 T cell–dependent pathogen Enterobacter cloacae engenders the formation of BrdU-retaining, Ag-specific long-lived plasma cells in the bone marrow that resist depletion by cyclophosphamide and anti-CD20 treatment, in contrast to splenic plasma cell pools. Likewise, immunization with haptenated Ficoll also induces enduring plasma cell responses despite subsequent elimination of peripheral B cells with whole-body irradiation (40). Our laboratory focused on responses to haptenated LPS, often termed a type 1 T cell–independent Ag. By quantifying and comparing frequencies of hapten-specific plasma cells in the
bone marrow following immunization of T cell–deficient mice, we found that hapten-specific plasma cells were readily detected in the bone marrow >200 d later, and that such cells possess a half-life of ~50 d (41). Furthermore, we found that T cell–deficient mice, unlike their T cell–sufficient counterparts, failed to generate short-lived GCs, suggesting that occupancy in GCs is not requisite for induction of long-lived plasma cells. In separate experiments, we also found that prevention of the GC response after immunizing with a T cell–dependent haptenated protein conjugate, although perturbing affinity maturation and class switch recombination as expected, did not affect the formation of long-lived plasma cells (41). Collectively, these results contrast substantially with past notions that all early plasma cells in T cell–independent responses fail to survive beyond 5 d of their generation, while also indicating that B cell maturation and selection in the GC is not required for lasting Ab responses to occur. These results also suggest that whereas many or perhaps most bone marrow plasma cells arise in GCs, extrafollicular responses are also likely to contribute to marrow plasma cell pools (Fig. 1B).

The bone marrow plasma cell niche

Shortly after their generation in peripheral lymphoid tissues, plasma cells begin to express the chemokine receptor CXCR4, which mediates their migration into the bone marrow (42). Similar to hematopoietic stem cells (43, 44), once in the bone marrow plasma cells are thought to occupy limited niches uniquely suited for their survival and function (45). Occupancy within such microenvironments may provide plasma cells with life-sustaining signals required for long-term maintenance of serum Ab titers, and failure to enter and/or persist in such niches may compromise long-lived humoral immunity. This viewpoint is supported by studies showing that plasma cells are readily identified in the bone marrow in association with stromal cells expressing the CXCR4 ligand CXCL12 (42, 46). The effective generation and survival of plasma cells also requires APRIL and BlyS and is compromised dramatically in mice lacking the APRIL/BlyS receptor BCMA (47). Attempts to define the cell types comprising the bone marrow plasma cell niche have identified several potential sources of these cytokines, including megakaryocytes, eosinophils, and basophils (48–50). Currently, the extent to which each of these cell types contributes to plasma cell longevity remains unclear, although it is likely that these and additional cell types each produce relevant cytokines such as APRIL under various circumstances (51). However, given that mice lacking the APRIL receptor BCMA exhibit depressed early and late plasma cell responses (47), the extent to which BCMA signaling mediates maintenance versus production of plasma cells remains uncertain. Moreover, although it is likely that plasma cells are actively retained in the bone marrow by CXCR4–CXCL12 interactions as well as the integrin receptors LFA-1 and VLA-4 (52, 53), whether retention of Ab-secreting cells in the bone marrow is strictly required for plasma cell longevity is questionable.

Notably, Ag-induced plasma cells are also often found in the spleen long after immunization (15). These data raise important questions about whether the spleen and other sites might also provide supportive niches that sustain plasma cells. This possibility is supported by recent characterization of splenic plasma cells in patients given B cell ablation therapy to treat immunothrombocytopenia (54). Alternatively, bone marrow–resident plasma cells might re-enter the circulation under certain circumstances, perhaps in response to inflammatory signals known to induce other cells, including B lineage precursors to exit the bone marrow (55). This possibility is supported by experiments suggesting that vaccination prompts pre-existing bone marrow plasma cells to enter the blood (56). Furthermore, there is little information currently about the recirculation dynamics of plasma cells once they first enter the marrow or other supportive niches and what specific signals might enact or otherwise affect certain migratory properties.

The notion that the bone marrow provides uniquely supportive niches for plasma cells raises several additional questions concerning the nature and potential limitations of the bone marrow microenvironment. One important question concerns how newly formed plasma cells gain access to such niches. Although it is frequently assumed that all or most bone marrow plasma cells are long-lived (48, 57), recent work suggests otherwise. As mentioned briefly above, Nuss and colleagues (31) employed a GFP reporter gene for Blimp-1 to infer bone marrow plasma cell maturity, as relative expression levels for Blimp-1 increase as early plasmablasts mature into bona fide plasma cells. This strategy revealed that some 20% of bone marrow plasma cells express relatively low levels of Blimp-1, suggesting that a sizeable fraction of the bone marrow plasma cell pool consists of recently formed and potentially short-lived cells. Thus, many newly formed plasma cells may reach full maturity and longevity after entering the bone marrow. Also consistent with this possibility, Racine et al. (58) described IgM-secreting plasmablasts in the bone marrow of mice exposed to the intracellular bacterium Ehrlichia muris, further showing that these cells persist for >300 d postinfection. Collectively, these observations suggest that bone marrow plasma cell pools consist of both short- and long-lived cells, and they raise questions about the degree of functional heterogeneity among bone marrow plasma cells and its relevance to achieving and maintaining long-lived Ab responses.

Are there constraints on the size of long-lived plasma cell pools? If so, do these constraints reflect limiting access of plasma cells to key cells and ligands that promote their survival? One prediction of this scenario is that entry of newly formed plasma cells into the bone marrow might displace pre-existing plasma cells from the bone marrow, potentially resulting in their demise. Consistent with this possibility, booster vaccination with tetanus toxin has been shown to induce the emergence into the blood of mature plasma cells secreting Abs specific for alternative unknown Abs (56). Although the ultimate fate of the latter cells was difficult to establish, these observations suggest that, to become long-lived, newly formed Ab-secreting cells must compete successfully with pre-existing plasma cells for entry into supportive yet size-limited niches in the bone marrow. This view was supported by experiments in mice showing that frequencies of previously induced Ag-specific bone marrow plasma cells declined upon subsequent repeated immunizations with several different Ags (59).

The capacity of T cell–independent Ags to induce long-lived plasma cell formation may reveal another layer of complexity concerning the mechanisms underlying plasma cell longevity. It is generally thought that Ab responses to T cell–independent Ags, including both LPS- and polysaccharide-based Ags, are
dominated by specialized naive B cells within the marginal zone and B1 subpopulations. Interestingly, in striking contrast to other naive B cells, the survival of B1 B cells does not require the canonical BlyS receptor BR3 (60). Given that many B1 B cells arise early in life from distinct B-lineage precursors (61, 62), it is tempting to speculate that plasma cells derived from B1 B cells might also use novel survival mechanisms. Alternatively, the absence of helper T cell–derived signals such as those induced by CD40 ligation on activated B cells might lead to the expression of alternative growth receptors or functionally relevant differences in the timing with which receptors such as BCMA are expressed, regardless of the developmental origins or previous selection history of responding B cells. In this regard, TLRs may also deliver signals that induce the expression of prosurvival receptors important for inducing long-lasting Ab responses. This possibility is in line with the capacity of TLR agonists such as CpG-enriched oligonucleotides to enhance durable plasma cell responses (63), as well as our capacity to induce long-lived responses with haptenated LPS without additional adjuvants (41).

Memory B cells and persisting Ab titers

The capacity of many plasma cells to persist for extended periods has also altered views on the role of memory B cells in long-lived humoral immunity. Most notably, continual stimulation of memory B cells may not be a prerequisite for maintaining Ab titers. This point of view is also consistent with work showing that memory B cell survival is not mediated by persisting Ag (64), as well as with other studies challenging the notion that memory B cells are routinely or readily engaged by TLR agonists in vivo (65). Nonetheless, memory B cells certainly play an essential role in mounting rapid robust secondary responses to cognate Ag, a viewpoint consistent with the capacity of memory B cells to rapidly generate plasma cells upon encountering Ag (65, 66). Interestingly, although conventional models hold that GC and memory B cell differentiation are associated intimately, recent work suggests that many memory B cells are generated independently of GCs (67, 68). These latter observations, together with our work showing that long-lived plasma cells can also form without GCs (41), further emphasize that the cells responsible for mediating long-term humoral immunity can arise from several alternative pathways.

Conclusions

The quality and quantity of Ab responses to T cell–dependent versus T cell–independent Ags are thought to differ dramatically. We suggest that the effective control of pathogens that avoid T cell recognition is mediated by a combination of B-lineage effector modalities. These include the exceptionally rapid production of large numbers of short-lived plasmablasts, which is likely to continue throughout infection, and the generation of long-lived plasma cells. We further suggest that the polyvalent low-affinity IgM Abs produced in response to these pathogens are often able to evoke effector activities such as the complement cascade to effectively control such infections. Consequently, the failure of immunization regimens for T cell–independent pathogens to elicit protective immunity may reflect an inability to generate high-affinity, class-switched Abs rather than long-lived Ab-secreting cells. These ideas may also be relevant for IgA responses to T cell–independent Ags. Indeed, it is clear that B cells in the gut can undergo class switching to IgA without T cell help (69, 70). However, to our knowledge whether T cell–independent Ags evoke long-lived IgA-producing plasma cells, and whether such cells remain in the gut or home to the bone marrow, is currently unknown.

Disclosures

The authors have no financial conflicts of interest.

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