Long-lived plasma cells: a mechanism for maintaining persistent antibody production

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Current models suggest that continuous antigenic stimulation of memory B cells is required to maintain long-term antibody production. In view of recent developments concerning plasma cell longevity, a new model is described that incorporates the important role of long-lived plasma cells in sustaining persistent antibody responses.

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Abbreviations

ELISA	enzyme-linked immunosorbent assay
ELISPOT	enzyme-linked immunosorbent spot assay
FDC	follicular dendritic cell
LCMV	lymphocytic choriomeningitis virus

Introduction

Long-term antibody production is one of the hallmarks of effective vaccination and is an important characteristic of immunological memory. Observations of long-term antibody production have been described frequently in the literature [1•,2•]. In humans, for example, viral infections or vaccination with inert antigens such as tetanus and diptheria toxoid can elicit antibody responses that last for many years. To date, most models of long-term antibody production have focused on the role of memory B cells since plasma cells were considered too short-lived to play a significant role in prolonged antibody production [3,4•,5–7]. In this review, we will discuss some of the more well-known theories of long-term antibody production and propose a new theory which suggests that long-lived plasma cells play an important role in maintaining humoral immunity.

Mechanisms of long-term antibody production

There are many mechanisms which have been proposed by which persistent antibody production can be maintained (Table 1). These include low-grade chronic infection, repeated antigenic re-exposure, antigen–antibody complexes, idiotypic networks, and cross-reactivity to self or environmental antigens. Each of these potential mechanisms has one thing in common — continual restimulation of B cells to proliferate and differentiate into antibody-secreting plasma cells.

A common explanation for long-term antibody production is that the immune system is stimulated repeatedly by either low-grade chronic infection or intermittent re-exposure to the pathogen. Although repeated exposure to a pathogen will boost an ongoing immune response, this mechanism is not absolutely necessary to maintain antibody production. There are several documented cases of long-term humoral immunity in the absence of re-exposure to the initiating pathogen. A classic example is Panum's epidemiological study of measles infection on the Faroe Islands [8]. Following a measles epidemic in 1781,

Table 1

Models of long-term antibody production.

Proposed mechanism	Antigenic stimulation	Comments
Repeated exposure to a pathogen	Yes	
Low-grade chronic infection	Yes	
Immune complexes on follicular dendritic cells	Yes	Conventional models for maintaining humoral immunity in which short-lived plasma cells are continuously replenished by memory B cell proliferation and differentiation into plasma cells
Cross-reactivity to self or environmental antigens	Yes*	
Idiotypic networks	Yes*	
Long-lived plasma cells	No	Provide an additional mechanism for maintaining persistent antibody production, but do not necessarily represent an exclusive mechanism. Instead, plasma cells with an extended lifespan may play an important role in maintaining antibody levels in conjunction with any of the other mechanisms listed here. On the other hand, if antigen becomes limiting, long-lived plasma cells may continue to sustain ongoing antibody responses in the absence of memory B cell help.

*The stimulation of antibody production in these cases may not be due to the specific antigen that initiated the response. This table has been modified, with permission, from [1•].

island inhabitants who were immune to measles were protected against reinfection during a second epidemic in 1846 (65 years later) in the absence of re-exposure to measles during the intervening years. Other studies have also documented long-term antibody production following infection by vaccinia (15 years), polio (40 years), or yellow fever (75 years) in the absence of re-exposure [9–11]; likewise, chronic infection is not an absolute requirement for long-term antibody production since vaccination with non-replicating antigens such as tetanus and diptheria toxoid induce serum antibody responses that are maintained for at least 10 years following booster vaccination [12,13]. Together, these studies show that humoral immunity can be maintained even in the absence of continuous or intermittent re-exposure to an infectious agent.

The most popular and well characterized hypothesis of long-term antibody production is that memory B cells are stimulated by persisting antigen that is retained in the form of antigen–antibody complexes on the surface of follicular dendritic cells (FDCs; [14–16]). As antigen-specific antibody levels decline, it is believed that the FDCs release more antigen in order to restimulate memory B cells and thus repopulate declining plasma cell populations. Although this theory has gained wide acceptance, there are several issues regarding antigen persistence on FDCs that remain unresolved. For instance, studies have shown that antigen may persist for several weeks or months, but antigen levels always decline. A careful study by Tew and Mandel [17] indicates that immune complexes decline with an average half-life of eight weeks. At this rate of decline, immune complexes alone cannot account for the decades of antibody production observed after vaccination with inert antigens [1•]. If one disregards the half-life of antigen itself, other issues surface; for example, what is the half-life of FDCs? Why is antigen not consumed during the restimulation of memory B cells during T-dependent antibody responses? One must also consider the process of affinity maturation; if antigen persists, then why doesn't somatic mutation and affinity maturation continue in the presence of these presumably low, but immunostimulatory, concentrations of antigen? Instead, affinity maturation occurs only during the first few weeks following vaccination with no more somatic mutations observed until after booster vaccination [15]. For these reasons, persisting antigen in the form of immune complexes does not fully explain long-term antibody production.

The development of idiotypic networks or cross-reactivity to either self or environmental antigens has been reported as potential mechanisms for prolonging antibody production [18,19]. If, however, antibody production is maintained by these mechanisms, then what would stop the potentially catastrophic increase in B cell proliferation and antibody production that would occur if there were an endless supply of stimulatory antigen? Similar to the previous discussion of the mechanism of chronic B cell stimulation, the issue of affinity maturation is raised; if B cells continue to be restimulated by idiotypic networks or by cross-reactivity, then it is difficult to understand why affinity maturation ceases after a few weeks following vaccination and is resumed only after booster vaccination [15]. The role of these mechanisms in maintaining antibody responses after natural infection or vaccination therefore remains unresolved.

A mechanism that does not exclude these previously described mechanisms is the prolonged antibody production that may be maintained by long-lived plasma cells. Plasma cells may be less responsive to changes in antigen levels since these cells down-regulate surface immunoglobulin and MHC Class II molecules [20,21]. Therefore the continued presence (or loss) of persisting antigen should not have an impact on their longevity.

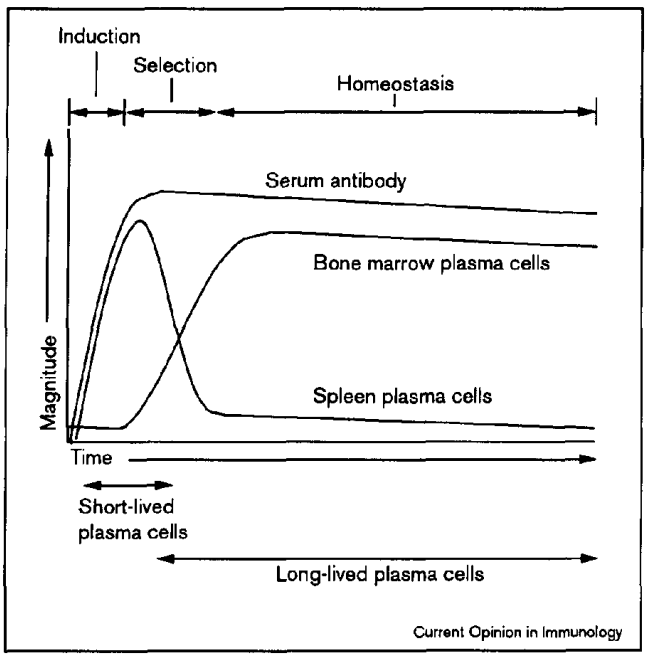
Plasma cell lifespan: short- or long-lived?

Several early studies determined the lifespan of plasma cells in order to distinguish whether there was a correlation with the longevity of this particular cell type and the persistence of specific serum antibody levels. During the first one or two weeks after vaccination, it was apparent that most plasma cells observed in either mice [22] or rats [23–25] were short-lived and had a half-life of only a few days. These studies determined the lifespan of plasma cells during the early stages of the immune response when B cells are rapidly dividing and being deleted during selection and affinity maturation [26•]; therefore, these results cannot be directly extrapolated to the lifespan of plasma cells that may be observed later in the immune response, after cellular proliferation has subsided and homeostasis has been re-established. By monitoring plasma cell numbers in rats for several months following vaccination, a biphasic plasma cell response was observed [27]. Similar to the other studies [22–25], ^{3}H-labeled plasma cells disappeared rapidly from the draining lymph nodes during the first few weeks following vaccination. At later timepoints, however, numbers of plasma cells declined more slowly, with a substantial number still present six months after vaccination. Although a specific half-life could not be determined, the existence of long-lived plasma cells was thus documented.

A common feature of each of these early studies is that plasma cell longevity was only determined in the spleen or in lymph nodes. The bone marrow was not examined even though this is the major site of antibody production after plasma cell numbers in the periphery decline ([1•,28]; Figure 1). In a slightly different approach the continuous infusion of rats with ^{3}H-thymidine was used to determine the number of plasma cells generated (i.e. radiolabeled) during a period of up to ten days in the absence of specific vaccination [29]. Based on these renewal rates, the potential lifespan of plasma cells in different anatomical sites was indirectly extrapolated. The

data suggested that plasma cells in the bone marrow had a half-life of three–four weeks whereas plasma cells in the spleen had a median lifespan of less than three days. It should be be noted that since the antigen specificity of the plasma cells was not determined the presence of long-lived plasma cells in this study was probably overlooked due to the ^{3}H-labeling of new and unrelated plasma cells.

Figure 1



Kinetics of antibody production following vaccination. The y-axis shows the relative magnitude of the antibody response. Plasma cell numbers were determined by ELISPOT. Serum antibody is initially produced by antibody-secreting cells in the draining lymph nodes (not shown) and in the spleen. The antibody response in these organs transiently peaks and then declines within a few weeks of antigenic stimulation. The decline in these plasma cells may be due to selection for higher-affinity plasma cells and apoptotic loss of low-affinity cells. As splenic plasma cell populations decline, antigen-specific plasma cells begin to migrate and accumulate in the bone marrow compartment. After the germinal center reaction subsides, the bone marrow remains the predominant site of antibody production with 80–90% of the host's antibody-secreting cells located here [28,32]. Plasma cell longevity studies have indicated that the initial antibody response is produced by short-lived plasma cells whereas long-term antibody production is maintained by long-lived plasma cells.

Recently, two independent studies on the longevity of plasma cells have provided new and compelling evidence that plasma cells can survive for several months in the absence of repopulation by memory B cells. In a study by Manz and colleagues [30••], ovalbumin-specific plasma cells were labeled with bromodeoxyuridine during vaccination and the number of labeled plasma cells was monitored for up to 120 days. The results of this study demonstrated that over 60% of ovalbumin-specific plasma cells in the bone marrow survived for at least 90 days in the absence of cell division. In addition, long-term labeling experiments (administering bromodeoxyuridine from day 19 to day 120 after vaccination) indicated that very few antigen-specific plasma cells were generated after 60 days following vaccination. Together, these data suggest that the majority of plasma cells localized in the bone marrow was not maintained by continuous proliferation of memory B cells. This study did not quantitate a specific plasma cell half-life, but similar to Miller [27], the existence of long-lived antigen-specific plasma cells was documented following use of metabolic labeling techniques.

Plasma cell longevity was also studied *in vivo* following acute viral infection [31••]. In these experiments, mice with steady-state antibody titers against lymphocytic choriomeningitis virus (LCMV) were depleted of memory B cells by irradiation *in vivo*. The persistence of virus-specific antibody levels and plasma cell numbers was monitored: following depletion of host B cells (identified by the IgH^{a} allotype), mice were reconstituted with naive allotypic B cells (identified by the IgH^{b} allotype) to distinguish between antibody production by pre-existing host cells (IgH^{a}) and by donor cells (IgH^{b}) by allotype-specific ELISA and ELISPOT. Although the irradiated mice became fully reconstituted with donor B lymphocytes, no donor-derived virus-specific serum antibody production was observed. This indicated that naive donor-derived B cells were not stimulated to produce antibody and did not participate in the ongoing antibody response even if host memory B cells were depleted. Naive B cells were also not stimulated into becoming memory B cells, since LCMV-specific B cell memory (IgH^{a} or IgH^{b}) remained below detection levels for at least eight months after irradiation, as determined by limiting dilution analysis and adoptive transfer challenge experiments. In the same series of experiments plasma cell longevity was examined with the aid of mathematical modeling, and the rate of serum antibody decline following memory B cell depletion indicated that murine plasma cells have an average half-life of 138 days (Figure 2). Plasma cells secreting IgG1 or IgG2a antibodies had very similar half-lives (140 and 128 days, respectively) indicating that IgG isotypes indicative of either Th1 or Th2 cytokine responses were both equally long-lived. In addition to the irradiation studies, adoptive transfer of virus-specific plasma cells into naive (unimmunized) mice was used to monitor plasma cell survival. Following adoptive transfer, LCMV-specific antibody titers in the recipient mice reached equilibrium in approximately 15 days. Virus-specific antibody was then monitored over a four month period and the slow decline in antibody titers indicated that long-lived plasma cells were responsible for sustaining the prolonged antibody response.

To address the issue of plasma cell longevity in specific anatomical locations, the ELISPOT assay was used to quantitate individual plasma cells in the spleen and bone marrow. In contrast to an earlier study in which

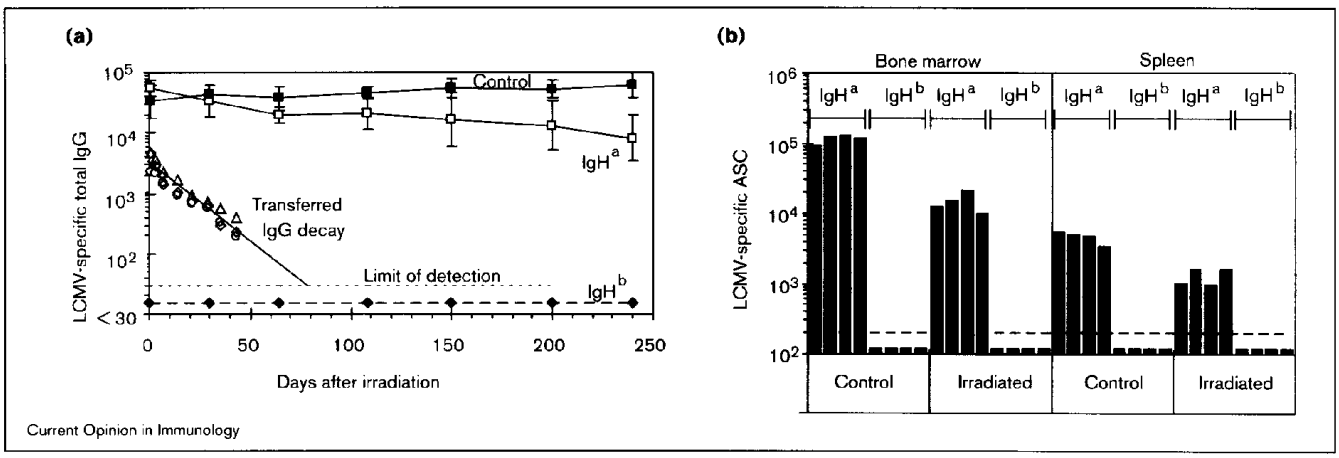
plasma cells in the bone marrow appeared to have a much longer lifespan than those found in the spleen [29], it was shown that plasma cells in both spleen and bone marrow were long-lived. This study [31••] thus documented the existence of long-lived antigen-specific plasma cells, quantitated plasma cell survival rates in different anatomical compartments and demonstrated that antibody production could be maintained for more than one year in mice without plasma cell repopulation by memory B cells.

Implications of generating plasma cells with an extended lifespan

It is apparent that the early antibody response mounted against a foreign antigen is provided by short-lived plasma cells whereas, later, the humoral response is maintained primarily by long-lived plasma cells. Intuitively, this makes sense because it would not be advantageous to have a long-lived antibody response of poor specificity and/or low affinity. If the first plasma cells generated during an immune response had an extended lifespan, then isotype switching and affinity maturation would be less likely to occur. By initially generating ASC with a short lifespan, one is able to mount a rapid, yet transient response (mostly comprising IgM) that can later be replaced by a more selective, affinity-matured antibody response. This may explain why there is a lag period between antibody production in the spleen (or in draining lymph nodes) and long-term antibody production in the bone marrow ([32]; Figure 1). Following acute viral infection, the virus-specific IgG subclasses produced in the bone marrow differ from the initial IgG subclasses produced during the earlier peak antibody response in the spleen [33]. This finding suggests that selection may be occurring before plasma cells accumulate in the bone marrow. Convincing evidence supporting the hypothesis that selection occurs before plasma cell migration and/or accumulation in the bone marrow has been provided by a study that characterized the degree of affinity maturation between the spleen and bone marrow compartments following a primary antibody response [34••]. In this study the distribution of somatic mutations and the production of high-affinity antibody by plasma cells in the bone marrow indicated that the majority of plasma cells persisting in this compartment appear to be selected on the basis of affinity.

Long-lived plasma cells may play a critical role in maintaining homeostasis as well as long-term immune surveillance. If antibody responses were sustained primarily by short-lived plasma cells, then a substantial fraction of memory B cells (and CD4+ T cells) must constantly be actively engaged in the maintenance of antigen-specific plasma cell populations. On the other hand, if long-lived plasma cells are generated, then the degree of regeneration

Figure 2

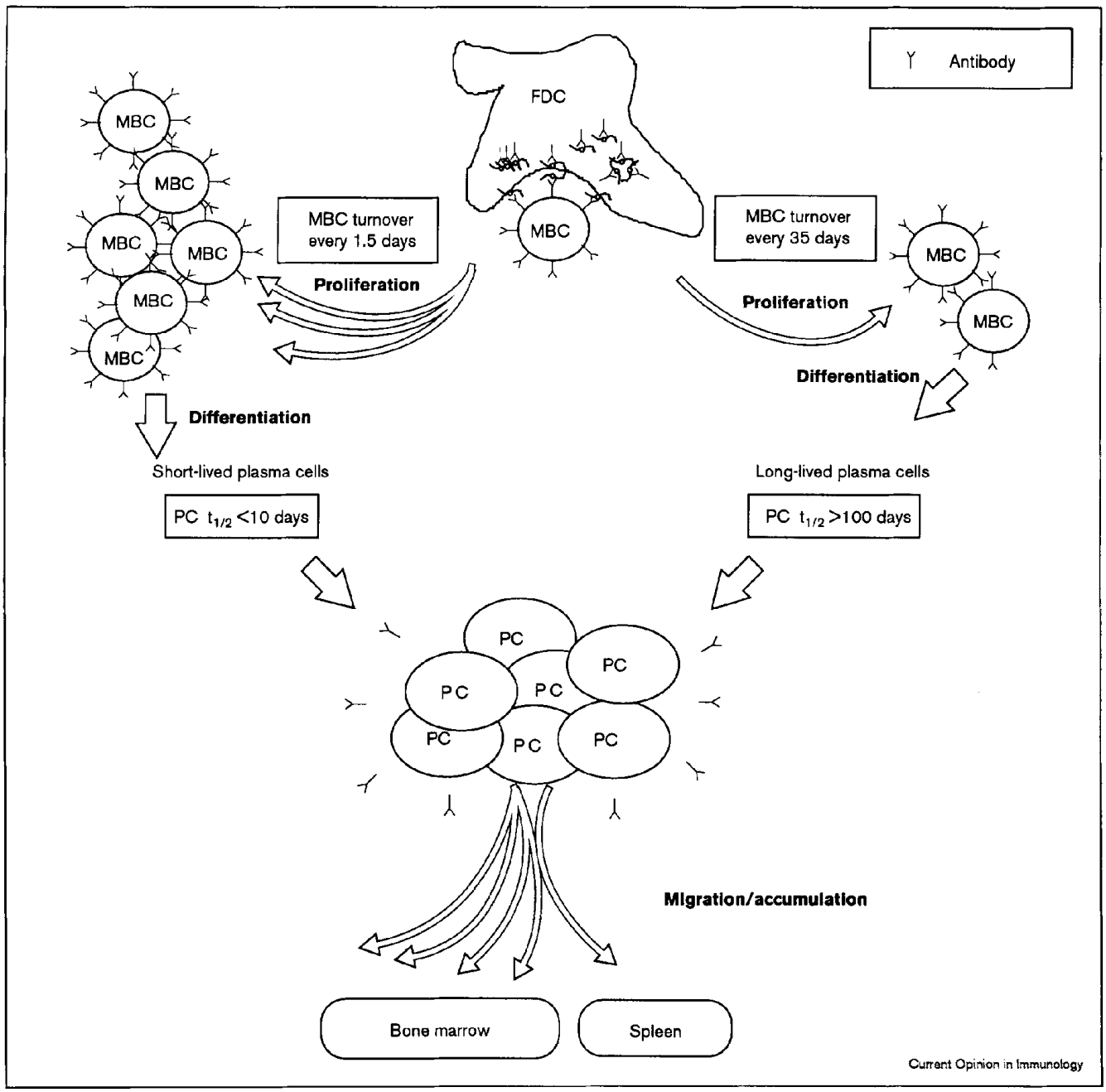


Prolonged virus-specific antibody production after memory B cell depletion. Sixty days after infection, LCMV-immune BALB/c IgH^a mice were irradiated to deplete memory B cells and then reconstituted with naive BALB/c IgH^b B cells. **(a)** The level of LCMV-specific IgG (in arbitrary units) was determined by ELISA using sera from control (nonirradiated) mice (black squares) or irradiated/reconstituted mice with both host-derived IgH^a (open squares) or donor-derived IgH^b (black diamonds) antibody levels shown. LCMV-specific antibody consisted entirely of the host IgH^a allotype and no donor IgH^b antibody responses were detected. The half-life of plasma cells (138 days) was obtained by fitting the data for antibody concentration in individual mice to an equation of bi-exponential decay [31••]. The decay rate of free IgG was determined by passively transferring LCMV-immune serum into naive recipients and monitoring the decline in serum antibody levels by ELISA (triangles, circles and open diamonds represent three individual recipient mice). The mean value for the half-life of transferred IgG was 12 days. **(b)** The total number of virus-specific plasma cells in the spleen and bone marrow of irradiated/reconstituted LCMV-immune BALB/c mice was compared to nonirradiated controls at 240 days after irradiation using the ELISPOT assay. The dashed line indicates the limit of detection. Each bar in the panel represents the number of virus-specific plasma cells of either IgH^a or IgH^b allotype that secrete IgG in the spleen or bone marrow of each of four individual mice. No LCMV-specific IgH^b-secreting plasma cells were detected in the spleens or bone marrow of irradiated mice, indicating that antibody production was sustained only by pre-existing host-derived plasma cells. This figure has been adapted, with permission, from reference [31••]. ASC, antibody-secreting cell.

required to maintain persistent antibody levels is greatly diminished ([31••]; Figure 3).

During the aging process, the ability to mount new immune responses is often impaired [35,36]. It has, however, been shown that the half-life of plasma cells is similar between young mice two–four months of age) and old mice (12–26 months of age), indicating that plasma cell longevity is not greatly influenced by age [31••]. This finding suggests that, in aged individuals, a dichotomy

Figure 3



Memory B cell (MBC) turnover in relation to plasma cell (PC) longevity. The existence of long-lived PCs has profound effects on the relationship between antigen-specific MBC and PC populations during an ongoing antibody response. By estimating the number of antigen-specific MBCs present following vaccination and by quantitating the lifespan of antigen-specific PCs, one can estimate the relative rate of MBC turnover required to maintain antibody production [31••]. If PCs are short-lived then MBCs must divide every 1.5 days in order to maintain PC numbers as well as regenerate MBC numbers. If PCs are long-lived, the rate of MBC turnover is greatly reduced and is more consistent with published observations of MBC turnover [37,40]. This model illustrates how critical PC longevity is in maintaining immunological homeostasis as well as persistent antibody production. The number of arrows gives an approximation of relative proportions. $t_{1/2}$, half-life.

exists between the ability to maintain pre-existing plasma cells and the ability to generate new ones. This also implies that effective vaccination at an early age may provide protection later in life when the capacity to mount new immune responses has diminished.

Conclusions and future directions

Long-term antibody production provides the host with the first line of defense against reinfection and is important for protection against a range of pathogens [1•,2•]. Theories regarding the mechanisms underlying the persistence of specific antibody production have focused primarily on the memory B cell component of humoral immunity [3,4•,5–7]; however, the recent studies on plasma cell longevity [30••,31••] have renewed the interest in the role of plasma cells in the maintenance of long-term antibody synthesis. Not surprisingly, several aspects of plasma cell immunology have yet to be described. For instance, what are the mechanisms that regulate plasma cell longevity? This may be determined by the local microenvironment, since mucosal antibody responses are relatively short-lived in comparison to serum antibody responses that are maintained primarily by long-lived plasma cells in the bone marrow and spleen [1•]. Short-lived mucosal antibody responses may be due to either migration of plasma cells out of the mucosa (possibly homing to the bone marrow) or due to plasma cells in these sites being short-lived. The role of plasma cells in maintaining mucosal antibody responses therefore requires further examination. Another question of interest is whether accessory cells are required to maintain plasma cell survival. CD4+ T cells are, by definition, required for the induction and/or restimulation of T-dependent antibody responses [37], but do they play a role in regulating plasma cell longevity? Since long-lived plasma cells down-regulate MHC Class II molecules [31••], it is unlikely that they interact with CD4+ cells directly, but this remains to be formally proven. Another unresolved issue is whether disruption in the selective mechanisms that generate long-lived plasma cells might contribute to autoimmune disorders. This has been discussed in a recent review on rheumatoid arthritis which speculates that plasma cells found in the synovial fluid of inflamed joints may be long-lived [38]. It is possible that there are phenotypic differences between short-lived and long-lived plasma cells. Early in an immune response, antibody-secreting cells express surface immunoglobulin and MHC class II molecules [39•] whereas long-lived plasma cells have down-regulated these surface markers [31••]. What role this process plays in plasma cell survival remains to be determined. A critical parameter that has yet to be examined is how the form of the antigen, the types of adjuvant, and the routes of immunization determine whether or not long-lived plasma cells are induced. It is possible that by developing new adjuvants and vaccines that are more effective at eliciting an antibody response composed of long-lived plasma cells, one may confer a more stable and long-lasting humoral response.

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